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Preface

This manual describes the sequence handling and analysis software developed at the Medical Research Council Laboratory of Molecular Biology, Cambridge, UK, which has come to be known as the Staden Package. Besides the current group, a number of people have made important contributions to earlier versions of the package, notably Tim Gleeson and Simon Dear; and others, including David Judge and several past and present workers at the Sanger Centre, have helped with comments and suggestions. John Taylor made everything work on Windows9X/NT.

The focus of the group since 1990 has been to produce improved methods for processing the data for large scale sequencing projects, and this is reflected in the scope of the package: the most advanced components (trev, prefinish, pregap4 and gap4) are those used in that area. Nevertheless the package also contains a program (spin) for the analysis and comparison of finished sequences. The latter also provides a graphical user interface to EMBOSS.

Gap4 performs sequence assembly, contig ordering based on read pair data, contig joining based on sequence comparisons, assembly checking, repeat searching, experiment suggestion, read pair analysis and contig editing. It has graphical views of contigs, templates, readings and traces which all scroll in register. Contig editor searches and experiment suggestion routines use confidence values to calculate the confidence of the consensus sequence and hence identify only places requiring visual trace inspection or extra data. The result is extremely rapid finishing and a consensus of known accuracy.

Pregap4 provides a graphical user interface to set up the processing required to prepare trace data for assembly or analysis. It also automates these processes. The possible processes which can be set up and automated include trace format conversion, quality analysis, vector clipping, contaminant screening, repeat searching and mutation detection.

Trev is a rapid and flexible viewer and editor for ABI, ALF, SCF and ZTR trace files.

Prefinish analyses partially completed sequence assemblies and suggests the most efficient set of experiments to help finish the project.

Tracediff and hetscan automatically locate mutations by comparing trace data against reference traces. They annotate the mutations found ready for viewing in gap4.

Spin analyses nucleotide sequences to find genes, restriction sites, motifs, etc. It can perform translations, find open reading frames, count codons, etc. Many results are presented graphically and a sliding sequence window is linked to the graphics cursor. Spin also compares pairs of sequences in many ways. It has very rapid dot matrix analysis, global and local alignment algorithms, plus a sliding sequence window linked to the graphical plots. It can compare nucleic acid against nucleic acid, protein against protein, and protein against nucleic acid.

The manual describes, in turn, each of the main programs in the package: gap4, and then pregap4 and its associated programs such as trev, and then spin. This is followed by a description of the graphical user interface, the ZTR, SCF and Experiment file formats used by our software, UNIX manpages for several of the smaller programs, and finally a list of papers published about the software. The description for each of the programs includes an introductory section which is intended to be sufficient to enable people to start using them, although in order to get the most from the programs, and to find the most efficient ways of using them we recommend that the whole manual is read once. The mini-manual is made up from the introductory sections for each of the main programs.
Chapter 1: Sequence assembly and finishing using Gap4

1 Sequence assembly and finishing using Gap4

1.1 Organisation of the gap4 Manual

The main body of the gap4 manual is divided, where possible, into sections covering related topics. If appropriate, these sections commence with an overview of the functions they contain. After the Introduction, the manual contains chapters on some important components of the user interface: the Contig Selector (see Section 1.3 [Contig Selector], page 31), the Contig Comparator (see Section 1.4 [Contig Comparator], page 34), and then, in the chapter on Contig Overviews (see Section 1.5 [Contig Overviews], page 37) we describe the Template Display (see Section 1.5.1 [Template Display], page 37), and its subcomponents the Stop Codon Plot (see Section 1.5.4 [Stop Codon Map], page 54), and the Restriction Enzyme Plot (see Section 1.5.5 [Restriction Enzyme Search], page 55).

Then there is a long chapter on the powerful Contig Editor (see Section 1.6 [Editor introduction], page 58), followed by a chapter describing the many assembly engines and assembly modes which gap4 can offer (see Section 1.7 [Assembly Introduction], page 94).

Gap4 contains functions to use the data in an assembly database to find the left to right order of contigs, and to compare their consensus sequences to look for joins that may have been missed during assembly. A "read-pair" is obtained by sequencing a DNA template (or "insert") from both ends: we then know the relative orientations of the two readings, and if we know the approximate template length, we know how far apart they should be after assembly. The next chapter is on the use of read-pair data for ordering contigs and checking assemblies and on the use of consensus comparisons for finding joins (see Section 1.8 [Ordering and Joining Contigs], page 105).

The next chapter is on checking assemblies and removing readings (see Section 1.9 [Checking Assemblies and Removing Readings], page 120). The following chapter describes gap4’s methods for suggesting experiments for helping to finish a sequencing project (see Section 1.10 [Finishing Experiments], page 126). Then we describe the various consensus calculation algorithms, and the options for creating consensus sequence files (see Section 1.11.5 [The Consensus Calculation], page 140). Next is the description of a set of miscellaneous functions (see Section 1.12 [Miscellaneous functions], page 147), followed by chapters on the Results Manager (see Section 1.13 [Results Manager], page 155), Lists (see Section 1.14 [Lists Introduction], page 156), Notes (see Section 1.15 [Notes], page 159), Configuring gap4 (see Section 1.20.1 [Options Menu], page 176), gap4 Database Files (see Section 1.16 [Gap Database Files], page 162), Checking Databases for corruptions (see Section 1.18 [Check Database], page 168) and Doctoring corrupted databases (see Section 1.19 [Doctor database], page 171).

1.2 Introduction

Gap4 is a Genome Assembly Program. The program contains all the tools that would be expected from an assembly program plus many unique features and a very easily used interface. The original version was described in Bonfield, J. K., Smith, K. F. and Staden, R. A new DNA sequence assembly program. Nucleic Acids Res. 24, 4992–4999 (1995)

Gap4 is very big and powerful. Everybody employs a subset of options and has their favourite way of accessing and using them. Although there is a lot of it, users are encouraged to go through the whole of the documentation once, just to discover what is possible, and the way that best suits their own work. At the very least, the whole of this introductory chapter should be read, as in the long run, it will save time.

This chapter serves as a cross reference point, to give an overview of the program and to introduce some of the important ideas which it uses. The main topics that are introduced are listed in the
current section. We introduced the use of base call accuracy values for speeding up sequencing projects (see Section 1.2.5 [The use of numerical estimates of base calling accuracy], page 26). The ability to annotate segments of readings and the consensus can be very convenient (see Section 1.2.7 [Annotating and masking readings and contigs], page 29). Generally the 3' ends of readings from sequencing instruments are of too low a quality to be used to create reliable consensus, but they can be useful, for example, for finding joins between contigs (see Section 1.2.6 [Use of the "hidden" poor quality data], page 28).

One of the most powerful features of gap4 is its graphical user interface which enables the data to be viewed and manipulated at several levels of resolution. The displays which provide these different views are introduced, with several screenshots (see Section 1.2.3 [Introduction to the gap4 User Interface], page 9).

It is important to understand the different files used by our sequence assembly software, and how the data is processed before it reaches gap4 (see Section 1.2.1 [Summary of the Files used and the Preprocessing Steps], page 5).

Note that gap4 is a very flexible program, and is designed so that it can easily be configured to suit different purposes and ways of working. For example it is easy to create a beginners version of gap4 which has only a subset of functions. What is described in this manual is the full version, and so is likely to contain some perhaps more esoteric options that few people will need to use. This introductory section also contains a complete list of the options in the gap4 main menus (see Section 1.2.4 [Gap4 Menus], page 23).

In addition to sequence assembly, gap4 can be used for managing mutation study data and for helping to discover and check for mutations (see Section 2.1 [Introduction to Searching for Mutations], page 185).

Two further useful facilities of gap4 are "Lists" and "Notes". For many operations it is convenient to be able to process sets of data together - for example to calculate a consensus sequence for a subset of the contigs. To facilitate this gap4 uses lists (see Section 1.14 [Lists Introduction], page 156) A ‘Note’ (see Section 1.15 [Notes], page 159) is an arbitrary piece of text which can be attached to any reading, any contig, or to the database in general.
1.2.1 Summary of the Files used and the Preprocessing Steps

Gap4 stores the data for an assembly project in a gap4 database. Before being entered into the gap4 database the data must be passed through several preassembly steps, usually via pregap4 (see Section 3.2 [Pregap4 introduction], page 202). These steps are outlined below.

The programs can handle data produced by a variety of sequencing instruments. They can also handle data entered using digitisers or that has been typed in by hand. Usually the trace files in proprietary format, such as those of ABI, are converted to SCF files (see Section 10.1 [SCF introduction], page 383) or ZTR files. As originally put forward in Bonfield, J. K. and Staden, R. The application of numerical estimates of base calling accuracy to DNA sequencing projects. Nucleic Acids Research 23, 1406-1410 (1995). gap4 makes important use of basecall confidence values, (see Section 1.2.5 [The use of numerical estimates of base calling accuracy], page 26) which are normally stored in the reading’s SCF file.

One of the first steps in the preprocessing is to copy the base calls from the trace files to text files known as Experiment files (see Section 10.3 [Experiment files], page 401). All the subsequent processes operate on the Experiment files. Other preassembly steps include quality and vector clipping. Each step is performed by a specific program controlled by the program pregap4 (see Section 3.2 [Pregap4 introduction], page 202).

Experiment file format is similar to that of EMBL sequence entries in that each record starts with a two letter identifier, but we have invented new records specific to sequencing experiments. One of pregap4’s tasks is to augment the Experiment files to include data about the vectors, primers and templates used in the production of each reading, and if necessary it can extract this information from external databases. Some of the information is needed by pregap4 and some by gap4. (Note that in order to get the most from gap4 it is essential to make sure that it is supplied, via the Experiment files, with all the information it needs.)

The trace files are not altered, but are kept as archival data so that it is always possible to check the original base calls and traces. Any changes to the data prior to assembly (and we recommend that none are made until readings can be viewed aligned with others) are made to the copy of the sequence in the Experiment file.

The reading data, in Experiment file format, is entered into the project database (see Section 1.16 [Gap Database Files], page 162), usually via one of the assembly engines. Because Experiment file format was based on EMBL file format, EMBL files can also be entered and their feature tables will be converted to tags. There is no limit to the length of readings which can be entered.

All the changes to the data made by gap4 are made to the copies of the data in the project database. Once the data has been copied into the gap4 database the Experiment files are no longer required.

Gap4 uses the trace files to display the traces (see Section 1.6.11 [Traces], page 81), and to compare the edited bases with the original base calls (see Section 1.6.6.11 [Search by Evidence for Edit (1)], page 70), (see Section 1.6.6.12 [Search by Evidence for Edit (2)], page 70). However gap4 databases do not store trace files: they record only the names of the trace files (which are copied from the readings’ Experiment files). This means that if the trace files for a project are not in the same directory/folder as the gap4 database, gap4 needs to be told where they are, otherwise it cannot use them. Ideally, all the trace files for a project should be stored in one directory. To tell gap4 where they are the “Trace file location” command in the Options menu should be used (see Section 1.20.8 [Trace File Location], page 179).

Gap4 databases have a number of size constraints, some of which can be altered by users and others which are fixed.

While gap4 is running it often needs to calculate a consensus. The maximum size of this sequence is controlled by a variable "maxseq". Most routines are able to automatically increase the value
of maxseq while they are running, but some of the older functions, including some of the original assembly engines, are not. This means that it is important for users to set maxseq to a sufficiently high value before running these elderly routines. By default maxseq is currently set to 100000, but users can set it on the command line or from within the Options menu.

Gap4 databases contain one record for each reading and one for each contig. The sum of these two sets of records is the "database size", and the maximum value that database size is permitted to reach is "maxdb". When databases are initialised maxdb is set, by default, to 8000. Users can alter this value on the command line or from within the Options menu of gap4.

Gap4 databases also limit the number and names of readings so that various output routines know how many character positions are required: the maximum number imposed in this way is 99,999,999, and the maximum reading name length is 40.

Currently we have sites with single gap4 databases containing over 200,000 readings with consensus sequences in excess of 7,000,000 bases.

A gap4 database can be used by several users simultaneously, but only one is allowed to change the contents of the database, and the others are given "readonly" access. As part of its mechanism to prevent more than one person editing a database at once gap4 uses a "BUSY" file to signify that the database is opened for writing. Before opening a database for writing, gap4 checks to see if the BUSY file for that database exists. If it does, the database is opened only for reading, if not it creates the file, so that any additional attempts to open the database for writing will be blocked. When the user with write access closes the database, the BUSY file is deleted, hence re-enabling its ability to be opened for changes. It is worth remembering that a side effect of this mechanism, is that in the event of a program or system crash the BUSY file will be left on the disk, even though the database is not being used. In this case users must remove the BUSY file before using the database (see Section 1.16 [Gap4 Database Files], page 162).

The final result from a sequencing project is a consensus sequence (see Section 1.11.5 [The Consensus Calculation], page 140) and gap4 can write these in Experiment file format, fasta format or staden format. Of course the whole database and all the trace files are also useful for future reference as they allow any queries about the accuracy of the sequence to be answered.
1.2.2 Summary of Gap4’s Functions

The tasks which gap4 can perform can be roughly divided into assembly (see Section 1.7 [Assembly Introduction], page 94), finishing (see Section 1.10 [Finishing Experiments], page 126), and editing (see Section 1.6 [Editor introduction], page 58). But gap4 contains many other functions which can help to complete a sequencing project with the minimum amount of effort, and some of these are listed below.

Readings are entered into the gap4 database using the assembly algorithms (see Section 1.7 [Assembly Introduction], page 94). In general these algorithms will build the largest contigs they can by finding overlaps between the readings, however some, perhaps more doubtful, joins between contigs may be missed, and these can be discovered, checked and made using Find Internal Joins (see Section 1.8.3 [Find Internal Joins], page 114), Find repeats (see Section 1.8.4 [Find repeats], page 118) and Join Contigs (see Section 1.6.14 [The Join Editor], page 87). Find Internal Joins compares the ends of contigs to see if there are possible overlaps and then presents the overlap in the Contig Joining Editor, from where the user can view the traces, make edits and join the contigs. Find Repeats can be used in a similar way, but unlike Find Internal Joins it does not require the matches it finds to continue to the ends of contigs.

Read-pair data can be used to automatically put contigs into the correct order (see Section 1.8.1 [Ordering Contigs], page 107), and information about contigs which share templates can be plotted out (see Section 1.8.2 [Find Read Pairs], page 110). The relationships of readings and templates, within and between contigs can also be shown by the Template Display (see Section 1.5.1 [Template Display], page 37) which has a wide selection of display modes and uses.

Problems with the assembly can be revealed by use of Check Assembly (see Section 1.9 [Checking Assemblies], page 121), Find repeats (see Section 1.8.4 [Find repeats], page 118), and Restriction Enzyme mapping (see Section 1.5.5 [Plotting Restriction Enzymes], page 55). Check Assembly compares every reading with the segment of the consensus it overlaps to see how well it aligns. Those that align poorly are plotted out in the Contig Comparator. Find Repeats also presents its results in the Contig Comparator, so if used in conjunction with Check Assembly, it can show cases where readings have been assembled into the wrong copy of a repeated element. At the end of a project the Restriction Enzyme map function can be used to compare the consensus sequence with a restriction digest of the target sequence. Problems can also be found by use of the various Coverage Plots available in the Consistency Display (see Section 1.5.2 [Consistency Display], page 46). These plots will show regions of low or high reading coverage (see Section 1.5.2.2 [Reading Coverage Histogram], page 48), places with data for only one strand (see Section 1.5.2.4 [Strand Coverage], page 50), or where there is no read-pair coverage (see Section 1.5.2.3 [Read-Pair Coverage Histogram], page 49). Errors can be corrected by Disassemble Readings (see Section 1.9.1.2 [Disassembling Readings], page 125) and Break Contig (see Section 1.9.1.1 [Breaking Contigs], page 124) which can remove readings from contigs or databases or can break contigs.

The general level of completeness of the consensus sequence can be seen diagrammatically using the Quality Plot (see Section 1.5.1.5 [Quality Plot], page 44), and the confidence values for each base in the consensus sequence can be plotted (see Section 1.5.2.1 [Confidence Values Graph], page 48).

The most powerful component of gap4 is its Contig Editor (see Section 1.6 [Editor introduction], page 58), which has many display modes and search facilities to enable very rapid discovery and fixing of base call errors.

If working on a protein coding sequence, the consensus can be analysed using the Stop Codon Map (see Section 1.5.4 [Stop Codon Map], page 54), and its translation viewed using the Contig Editor (see Section 1.6.8.1 [Status Line], page 74).

The final result from a sequencing project is a consensus sequence (see Section 1.11.5 [The Consensus Calculation], page 140).
1.2.3 Introduction to the gap4 User Interface

Gap4 has a main window from which all the main options are selected from menus. When a database is open it also has a Contig Selector which will transform into a Contig Comparator whenever needed. In addition many of the gap4 functions, such as the Contig Editor or the Template Display will create their own windows when they are activated. All the graphical displays and the Contig Editor can be scrolled in register. The base of the graphical display windows usually contains an Information Line for showing short textual data about results or items touched by the mouse cursor. Gap4 is best operated using a three button mouse, but alternative keybindings are available. Full details of the user interface are described elsewhere (see [User Interface], page 373), and here we give an introduction based around a series of screenshots.

The main window (shown below) contains an Output window for textual results, an Error window for error messages, and a series of menus arranged along the top. The contents of the two text windows can be searched, edited and saved. Each set of results is preceded by a header containing the time and date when it was generated.

Some of the text will be underlined and shaded differently. These are hyperlinks which perform an operation when clicked (with the left mouse button) on, typically invoking a graphical display such as the contig editor. Clicking on these with the right mouse button will bring up a menu of additional operations. At present only a few commands (Show Relationships and the Search functions) produce hypertext, but if there is sufficient interest this may be expanded on.
1.2.3.1 Introduction to the Contig Selector

The gap4 Contig Selector is used to display, select and reorder contigs. In the Contig Selector all contigs are shown as colinear horizontal lines separated by short vertical lines. The length of the horizontal lines is proportional to the length of the contigs and their left to right order represents the current ordering of the contigs. Users can change the contig order by dragging the lines representing the contigs. This is done by clicking and holding the middle mouse button, or Alt left mouse button, on a line and then moving the mouse cursor. The Contig Selector can also be used to select contigs for processing. For example, clicking with the right mouse button on the line representing a contig will invoke a menu containing the commands which can be performed on that contig. There are several alternative ways of specifying which contig an operation should be performed on. Contigs are identified by the name or number of any reading they contain. When a dialogue is requesting a contig name, using the left mouse button to click on the contig in the Contig Selector will transfer its name to the dialogue box. Other methods are available (see Section 1.3.1 [Selecting Contigs], page 31).

As the mouse is moved over a contig, it is highlighted and the contig name (left most reading name) and length are displayed in the Information Line. The number in brackets is the contig number (actually the number of its leftmost reading). Tags or annotations (see Section 1.2.7 [Annotating and masking readings and contigs], page 29) can also be displayed in the Contig Selector window.

The figure shows a typical display from the Contig Selector. At the top are the File, View and Results menus. Below that are buttons for zooming and for displaying the crosshair. The four boxes to the right are used to display the X and Y coordinates of the crosshair. The rightmost two display the Y coordinates when the contig selector is transformed into the Contig Comparator. The two leftmost boxes display the X coordinates: the leftmost is the position in the contig and the other is the position in the overall consensus. The crosshair is the vertical line spanning the panel below. Tags are shown as coloured rectangles above and below the lines (see Section 1.3 [Contig Selector], page 31).
1.2.3.2 Introduction to the Contig Comparator

Gap4 commands such as Find Internal Joins (see Section 1.8.3 [Find Internal Joins], page 114), Find Repeats (see Section 1.8.4 [Find Repeats], page 118), Check Assembly (see Section 1.9 [Check Assembly], page 121), and Find Read Pairs (see Section 1.8.2 [Find Read Pairs], page 110) automatically transform the Contig Selector (see Section 1.3 [Contig Selector], page 31) to produce the Contig Comparator. To produce this transformation a copy of the Contig Selector is added at right angles to the original window to create a two dimensional rectangular surface on which to display the results of comparing or checking contigs.

Each of the functions plots its results as diagonal lines of different colours. In general, if the plotted points are close to the main diagonal they represent results from pairs of contigs that are in the correct relative order. Lines parallel to the main diagonal represent contigs that are in the correct relative orientation to one another. Those perpendicular to the main diagonal show results for which one contig would need to be reversed before the pair could be joined. The manual contig dragging procedure can be used to change the relative positions of contigs. See Section 1.3.2 [Changing the Contig Order], page 32. As the contigs are dragged the plotted results will automatically be moved to their corresponding new positions. This means that, in general, if users drag the contigs to move their plotted results close to the main diagonal they will simultaneously be putting their contigs into the correct relative positions.

This plot can simultaneously show the results of independent types of search, making it easy for users to see if different analyses produce corroborating evidence for the ordering of contigs. Indications that a reading may have been assembled in an incorrect position can also be seen - if for example a result from Check Assembly lies on the same horizontal or vertical projection as a result from Find Repeats, users can see the alternative position to place the doubtful reading.

The plotted results can be used to invoke a subset of commands by the use of pop-up menus. For example if the user clicks the right mouse button over a result from Find Internal Joins a menu containing Invoke Join Editor (see Section 1.6.14 [The Join Editor], page 87) and Invoke Contig Editors (see Section 1.6 [Editing in gap4], page 58) will pop up. If the user selects Invoke Join Editor the Join Editor will be started with the two contigs aligned at the match position contained in the result. If required one of the contigs will be complemented to allow their alignment.
A typical display from the Contig Comparator is shown above. It includes results for Find Internal Joins in black, Find Repeats in red, Check Assembly in green, and Find Read Pairs in blue. Notice that there are several internal joins, read pairs and repeats close to the main diagonal near the top left of the display. This indicates that the contigs represented in that area are likely to be in the correct positions relative to one another. In the middle of the bottom right quadrant there is a blue diagonal line perpendicular to the main diagonal. This indicates a pair of contigs that are in the wrong relative orientation. The crosshairs show the positions for a pair of contigs. The vertical line continues into the Contig Selector part of the display, and the position represented by the horizontal line is also duplicated there (see Section 1.4 [Contig Comparator], page 34).
1.2.3.3 Introduction to the Template Display

The Template Display can show schematic plots of readings, templates, tags, restriction enzyme sites and the consensus quality. Colour coding distinguishes reading, primer and template types. The Template Display can also be used to reorder contigs and to invoke the Contig Editor.

An example showing all these information types can be seen in the Figure below.

![Template Display](image_url)

The large top section contains lines and arrows representing readings and templates. Beneath this are rulers; one for each contig, and below those is the quality plot. The template and reading section of the display is in two parts. The top part contains the templates which have been sequenced from both ends but which are in some way inconsistent - for example given the current relative positions of their readings, they may have a length that is larger or greater than that expected, or the two readings may, as it were, face away from one another. Colour coding is used to distinguish between different types of inconsistency, and whether or not the inconsistency involves readings within or between contigs. For example, most of the problems shown in the screendump above are coloured dark yellow, indicating an inconsistency between a pair of contigs. The rest of the data, (mostly dark blue indicating templates sequenced from only one end), is plotted below the data.
for the inconsistent templates. Forward readings are light blue and reverse readings are orange. Templates in bright yellow have been sequenced from both ends, are consistent and span a pair of contigs (and so indicating the relative orientation and separation of the contigs).

At the bottom is the restriction enzyme plot. The coloured blocks immediately above and below the ruler are tags. Those above the ruler can also be seen on their corresponding readings in the large top section. The display can be zoomed. The position of a crosshair is shown in the two leftmost boxes in the top right hand corner. The leftmost shows the distance in bases between the crosshair and the start of the contig underneath the crosshair. The middle box shows the distance between the crosshair and the start of the first contig. The right box shows the distance between two selected cut sites in the restriction enzyme plots (see Section 1.5.1 [Template Display], page 37).
1.2.3.4 Introduction to the Consistency Display

The Consistency Display provides plots designed to highlight potential problems in contigs. It is invoked from the main gap4 View menu by selecting any of its plots. Once a plot has been displayed, any of the other types of consistency plot can be displayed within the same frame from the View menu of the Consistency Display.

An example showing the Confidence Values Graph and the corresponding Reading Coverage Histogram, Read-Pair Coverage Histogram and Strand Coverage is shown below.
If more than one contig is displayed, the contigs are drawn immediately after one another but are staggered in the y direction.

The ruler ticks can be turned on or off from the View menu of the consistency display. The plots can be enlarged or reduced using the standard zooming mechanism. See Section 9.4.1 [Zooming], page 378.

The crosshair toggle button controls whether the crosshair is visible. This is shown as a black vertical and horizontal line. The position of the crosshair is shown in the 3 boxes to the right of the crosshair toggle. The first box indicates the cursor position in the current contig. The second box indicates the overall position of the cursor in the consensus. The last box shows the y position of the crosshair. (see Section 1.5.2 [Consistency Display], page 46).
1.2.3.5 Introduction to the Restriction Enzyme Map

The restriction enzyme map function finds and displays restriction sites within a specified region of a contig. Users can select the enzyme types to search for and can save the sites found as tags within the database.

This figure shows a typical view of the Restriction Enzyme Map in which the results for each enzyme type have been configured by the user to be drawn in different colours. On the left of the display the enzyme names are shown adjacent to their rows of plotted results. If no result is found for any particular enzyme eg here APAI, the row will still be shown so that zero cutters can be identified. Three of the enzymes types have been selected and are shown highlighted. The results can be scrolled vertically (and horizontally if the plot is zoomed in). A ruler is shown along the base and the current cursor position (the vertical black line) is shown in the left hand box near the top right of the display. If the user clicks, in turn, on two restriction sites their separation in base pairs will appear in the top right hand box. Information about the last site touched is shown in the Information line at the bottom of the display. At the top the edit menu is shown and can be used to create tags for highlighted enzyme types (see Section 1.5.5 [Restriction Enzyme Search], page 55).
1.2.3.6 Introduction to the Stop Codon Map

The Stop Codon Map plots the positions of all the stop codons on one or both strands of a contig consensus sequence. If the Contig Editor is being used on the same contig, the Refresh button will be enabled, and if used, will fetch the current consensus from the editor, repeat the search and replot the stop codons.

The figure shows a typical zoomed in view of the Stop Codon Map display. The positions for the stop codons in each reading frame (here all six frames are shown) are displayed in horizontal strips. Along the top are buttons for zooming, the crosshair toggle, a refresh button and two boxes for showing the crosshair position. The left box shows the current position and the right-hand box the separation of the last two stop codons selected by the user. Below the display of stop codons is a ruler and a horizontal scrollbar. The information line is showing the data for the last stop codon the user has touched with the cursor. Also shown on the left is the View menu which is used to select the reading frames to display (see Section 1.5.4 [Stop Codon Map], page 54).
1.2.3.7 Introduction to the Contig Editor

The gap4 Contig Editor is designed to allow rapid checking and editing of characters in assembled readings. Very large savings in time can be achieved by its sophisticated problem finding procedures which automatically direct the user only to the bases that require attention. The following is a selection of screenshots to give an overview of its use.

The figure above shows a screendump from the Contig Editor which contains segments of aligned readings, their consensus and a six phase translation. The Commands menu is also shown. The main components are: the controls at the top; reading names on the left; sequences to their right; and status lines at the bottom. Some of the reading names are written in light grey which indicates that their traces/chromatograms are being displayed (in another window, see below).

One reading name is written with inverse colours, which indicates that it has been selected by the user. To the left of each reading name is the reading number, which is negative for readings which have been reversed and complemented. The first of the status lines, labelled "Strands", is showing a summary of strand coverage. The left half of the segment of sequence being displayed is covered only by readings from one strand of the DNA, but the right half contains data from both strands.

Along the top of the editor window is a row of command buttons and menus. The rightmost pair of buttons provide help and exit. To their left are two menus, one of which is currently in use. To the left of this is a button which initially displays a search dialogue, and then pressing it again, will perform the selected search. Further left is the undo button: each time the user clicks on this box the program reverses the previous edit command. The next button, labelled "Cutoffs", is used to toggle between showing or hiding the reading data that is of poor quality or is vector sequence. In this figure it has been activated, showing the poor quality data in light grey. Within this, sequencing vector is displayed in lilac. The next button to the left is the Edit Modes menu which allows users to select which editing commands are enabled. The next command toggles between insert and replace and so governs the effect of typing in the edit window.

One of the readings contains a yellow tag, and elsewhere some bases are coloured red, which indicates they are of poor quality. The Information Line at the bottom of the window can show
information about readings, annotations and base calls. In this case it is showing information about
the reliability of the base beneath the editing cursor.

A better way of displaying the accuracy of bases is to shade their surroundings so that the
lighter the background the better the data. In the figure above, this grey scale encoding of the
base accuracy or confidence has been activated for bases in the readings and the consensus. This
screenshot also shows the Contig Editor displaying disagreements and edits. Disagreements between
the consensus and individual base calls are shown in dark green. Notice that these disagreements are
in poor quality base calls. Edits (here they are all pads) are shown with a light green background.
When they are present, replacements/insertions are shown in pink, deletions in red and confidence
value changes in purple. The consensus confidence takes into account several factors, including
individual base confidences, sequencing chemistry, and strand coverage. It can be seen that the
consensus for the section covered by data from only one strand has been calculated to be of lower
confidence than the rest. The Status Line includes two positions marked with exclamation marks
(!) which means that the sequence is covered by data from both strands, but that the consensus for
each of the two strands is different. The Information Line at the bottom of the window is showing
information about the reading under the cursor: its name, number, clipped length, full length,
sequencing vector and BAC clone name.

The Contig Editor can rapidly display the traces for any reading or set of readings. The number
of rows and columns of traces displayed can be set by the user. The traces scroll in register with
one another, and with the cursor in the Contig Editor. Conversely, the Contig Editor cursor can be scrolled by the trace cursor. A typical view is shown above.

This figure is an example of the Trace Display showing three traces from readings in the previous two Contig Editor screen dumps. These are the best two traces from each strand plus a trace from a reading which contains a disagreement with the consensus. The program can be configured to automatically bring up this combination of traces for each problem located by the "Next search" option. The histogram or vertical bars plotted top down show the confidence value for each base call. The reading number, together with the direction of the reading (+ or -) and the chemistry by which it was determined, is given at the top left of each sub window. There are three buttons ('Info', 'Diff', and 'Quit') arranged vertically with X and Y scale bars to their right. The Info button produces a window like the one shown in the bottom right hand corner. The Diff button is mostly used for mutation detection, and causes a pair of traces to be subtracted from one another and the result plotted, hence revealing their differences. (see Section 1.6.11 [Traces], page 81).
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1.2.3.8 Introduction to the Contig Joining Editor

Contigs are joined interactively using the Join Editor. This is simply a pair of contig editor displays stacked one above the other with a "differences" line in between. The Contig Join Editor is usually invoked by clicking on a Find Internal Joins, or Find Repeats result in the Contig Comparator. In which case the two contigs will appear with the match found by these searches displayed.

The few differences between the Join Editor and the Contig Editor can be seen in the figure below. Otherwise all the commands and operations are the same as those for the Contig Editor.

In this figure the Cutoff or Hidden data is being displayed for the right hand contig. One difference between the Contig Editor and the Join Editor is the Lock button. When set (as it is in the illustration) the two contigs scroll in register, otherwise they can be scrolled independently.

The Align button aligns the overlapping consensus sequences (see Section 1.6.14 [Editor joining], page 87).
1.2.4 Gap4 Menus

The main window for gap4 contains File, Edit, View, Options, Experiments, Lists and Assembly menus.

1.2.4.1 Gap4 File menu

The File menu includes database opening and copying functions and consensus calculation options.

- Change Directory (see Section 1.16.1 [Directories], page 162)
- Check Database (see Section 1.18 [Check Database], page 168)
- New (see Section 1.16.2 [Opening a New Database], page 163)
- Open (see Section 1.16.3 [Opening an Existing Database], page 163)
- Copy Database (see Section 1.16.4 [Making Backups of Databases], page 163)
- Copy Readings (see Section 1.17.1 [Copying Readings], page 165)
- Save Consensus (see Section 1.11.5 [The Consensus Calculation], page 140)
- Extract Readings (see Section 1.12.5 [Extract Readings], page 152)

1.2.4.2 Gap4 Edit menu

The Edit menu contains options that alter the contents of the database.

- Edit Contig (see Section 1.6 [Editor introduction], page 58)
- Join Contigs (see Section 1.6.14 [Editor joining], page 87)
- Save Contig Order (see Section 1.8.1 [Order Contigs], page 107)
- Break Contig (see Section 1.9.1.1 [Break Contig], page 124)
- Complement a Contig (see Section 1.12.1 [Complement a Contig], page 147)
- Order Contigs (see Section 1.8.1 [Order Contigs], page 107)
- Quality Clip (see Section 1.12.6.2 [Quality Clipping], page 154)
- Difference Clip (see Section 1.12.6.1 [Difference Clipping], page 153)
- Double Strand (see Section 1.10.1 [Double Strand], page 126)
- Disassemble Readings (see Section 1.9.1.1 [Break Contig], page 124)
- Enter Tags (see Section 1.12.2 [Enter Tags], page 147)
- Edit Notebooks (see Section 1.15 [Notes], page 159)
- Doctor Database (see Section 1.19 [Doctor database], page 171)

1.2.4.3 Gap4 View menu

The View menu contains options to look at the data at several levels of detail, and analytic functions which present their results graphically.

- Contig Selector (see Section 1.3 [Contig Selector], page 31)
- ResultsManager (see Section 1.13 [Results Manager], page 155)
- Find Internal Joins (see Section 1.8.3 [Find Internal Joins], page 114)
- Find Read Pairs (see Section 1.8.2 [Find Read Pairs], page 110)
- Find Repeats (see Section 1.8.4 [Find repeats], page 118)
- Check Assembly (see Section 1.9 [Check Assembly], page 121)
- Sequence Search (see Section 1.12.4 [Find Oligos], page 150)
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- Template Display (see Section 1.5.1 [Template Display], page 37)
- Show Relationships (see Section 1.12.3 [Show Relationships], page 148)
- Restriction Enzyme map (see Section 1.5.5 [Restriction Enzyme Search], page 55)
- Stop Codon Map (see Section 1.5.4 [Stop Codon Map], page 54)
- Quality Plot (see Section 1.5.1.5 [Quality Plot], page 44)
- List Confidence (see Section 1.11.6 [List Confidence], page 145)
- Reading Coverage Histogram (see Section 1.5.2.2 [Reading Coverage Histogram], page 48)
- Read-Pair Coverage Histogram (see Section 1.5.2.3 [Read-Pair Coverage Histogram], page 49)
- Strand Coverage (see Section 1.5.2.4 [Strand Coverage], page 50)
- Confidence Values Graph (see Section 1.5.2.1 [Confidence Values Graph], page 48)

1.2.4.4 Gap4 Options menu

The Options menu contains options for configuring gap4.
- Consensus Algorithm (see Section 1.20.2 [Consensus Algorithm], page 177)
- Set Maxseq (see Section 1.20.3 [Set Maxseq], page 177)
- Set Fonts (see Section 1.20.4 [Set Fonts], page 177)
- Configure Menus (see Section 1.20.5 [Configuring Menus], page 177)
- Set Genetic Code (see Section 1.20.6 [Set Genetic Code], page 178)
- Alignment Scores (see Section 1.20.7 [Alignment Scores], page 179)
- Trace File Location (see Section 1.20.8 [Trace File Location], page 179)

1.2.4.5 Gap4 Experiments menu

The Experiments menu contains options to analyse the contigs and to suggest experimental solutions to problems.
- Suggest Long Readings (see Section 1.10.3 [Suggest Long Readings], page 130)
- Suggest Primers (see Section 1.10.2 [Suggest Primers], page 128)
- Compressions and Stops (see Section 1.10.4 [Compressions and Stops], page 132)
- Suggest Probes (see Section 1.10.5 [Suggest Probes], page 134)

1.2.4.6 Gap4 Lists menu

The Lists menu contains a set of options for creating and editing lists for use in various parts of the program.
- Creation and Editing (see Section 1.14 [Lists Introduction], page 156)
- Contigs To Readings (see Section 1.14.3 [Contigs To Readings Command], page 156)
- Minimal Coverage (see Section 1.14.4 [Lists Minimum Coverage], page 157)
- Unattached Readings (see Section 1.14.5 [Lists Unattached Readings], page 157)

1.2.4.7 Gap4 Assembly menu

The Assembly menu contains various assembly and data entry methods.
- Normal Shotgun Assembly (see Section 1.7.1 [Normal Shotgun Assembly], page 94)
- Directed Assembly (see Section 1.7.2 [Directed Assembly], page 99)
- Screen Only (see Section 1.7.3 [Assembly Screen Only], page 101)
- Assembly Independently (see Section 1.7.1.1 [Assembly Independently], page 97)
1.2.5 The use of numerical estimates of base calling accuracy

In this section we give an overview of our use, when available, of base call accuracy estimates or confidence values. We also explain the importance of the consensus calculations used by gap4, and their role in minimising the work needed to complete sequencing projects.


In Bonfield and Staden (1995), we stated "...the most useful outcome of having a sequence reading determined by a computer-controlled instrument would be that each base was assigned a numerical estimate of its probability of having been called correctly... having numerical estimates of base accuracy is the key to further automation of data handling for sequencing projects. ... The simple procedure we propose in this paper is a method of using the numerical estimates of base calling accuracy to obviate much of the tedious and time consuming trace checking currently performed during a sequencing project. In summary we propose that the numerical estimates of base accuracy should be used by software to decide if conflicts between readings require human expertise to help adjudicate. We argue that if the accuracy estimates are reasonably reliable then the majority of conflicts can be ignored... and so the time taken to check and edit a contig will be greatly reduced."

This has been achieved by making the consensus calculations (see Section 1.11.5 [The Consensus Calculation], page 140) central to gap4, and by providing calculations which make use of base call accuracy estimates to give each consensus base a quality measure. The consensus is not stored in the gap4 database but is calculated when required by each function that needs it, and hence always takes into account the current data. In the Contig Editor the consensus is updated instantly to reflect any change made by the user.

In 1998 the first useable probability values became available through the program Phred (Ewing, B. and Green, P. Base-Calling of Automated Sequencer Traces Using Phred. II. Error Probabilities. Genome Research. Vol 8 no 3. 186-194 (1998)). Phred produces a confidence value that defines the probability that the base call is correct. This was an important step forward and these values are widely used and have defined a decibel type scale for base call confidence values. Gap4 is currently set to use confidence values defined on this scale.

The confidence value is given by the formula

\[
C_{\text{value}} = -10 \times \log_{10}(\text{probability of error})
\]

A confidence value of 10 corresponds to an error rate of 1/10; 20 to 1/100; 30 to 1/1000; and so on. Using the main gap4 consensus algorithm they enable the production of a consensus sequence for which the expected error rate for each base is known.

As is described elsewhere (see Section 1.11.6 [List Consensus Confidence], page 145) being able to calculate the confidence for each base in the consensus sequence makes it possible to estimate the number of errors it contains, and hence the number of errors that will be removed if particular bases are checked and, if necessary, edited. For example, if 1000 bases in the consensus had confidence 20, we would expect those 1000 bases (with an error rate of 1/100) to contain 10 errors.

Another program which produces decibel scale confidence values for ABI 377 data is ATQA Daniel H. Wagner, Associates, at http://www.wagner.com/.

For gap4 the confidence values are expected to lie in the range 1 to 99, with 0 and 100 having special meanings to the program.
The confidence values are stored in SCF or Experiment files and copied into gap4 databases during assembly or data entry.

The searches provided by the Contig Editor (see Section 1.6.6 [Searching], page 68) are one of gap4’s most important time saving features. The user selects a search type, for example to find places where the confidence for the consensus falls below a given threshold, and the search automatically moves the cursor to the next such position in the consensus. The Contig Editor locates the next problem by applying the consensus calculation to the contig. To edit a contig the user selects "Search" repeatedly, knowing that it will only move to places where there is a conflict between good data or where the data is poor. Note that the program is usually configured to automatically display the relevant traces for each position located by the search option.

The main result is that far fewer disagreements between data are brought to the attention of the user and fewer traces have to be inspected by eye, and so the whole process is faster. Another consequence of the strategy is that, as fewer bases need changing to produce the correct consensus, most of what appears on the screen will be the original base calls. Indeed we have taken this a step further and suggest that if a base needs changing because it has a high accuracy estimate, and is conflicting with other good data, then rather than change the character shown on the screen, the user should lower its accuracy value. By so doing more of the original base calls are left unchanged and hence are visible to the user. There is a function within the contig editor to reset the accuracy value for the current base to 0. Alternatively the accuracy value for the base that is thought to be correct can be set within the contig editor to 100.
1.2.6 Use of the "hidden" poor quality data

In general sequences obtained from machines contain segments such as vector sequence and poor quality data that need either to be removed or ignored during assembly and editing. In our package we do not remove such segments but instead we mark them so that the programs can deal with them appropriately. In gap4 such data is referred to as "hidden". The positions to hide are determined initially by preprocessing programs such as vector.clip (see Chapter 5 [Screening Against Vector Sequences], page 267) and qclip (see Section 11.13 [qclip], page 436).

The hidden data can be revealed in the Contig Editor by toggling the Cutoffs button (see Section 1.6.3.4 [Adjusting the Cutoff data], page 65); can be used to search for possible joins between contigs (see Section 1.8.3 [Find Internal Joins], page 114), and can be included in the consensus sequence (see Section 1.11.2 [Extended consensus], page 138) to be used by external screening programs. For these cases the program can distinguish data that is hidden because it is vector and data that is hidden because it is of poor quality: only poor quality data is included.

The position of hidden data can be changed interactively in the Contig Editor. In addition the Double Strand function (see Section 1.10.1 [Double stranding], page 126) will reduce the amount of hidden data for readings that cover single stranded regions of contigs, if the data aligns well with that on the other strand.
1.2.7 Annotating and masking readings and contigs

Gap4 can label segments of readings and contigs using "tags" (see Section 1.6.5 [Create Tag], page 66). The program recognises a set of standard tags types and users can also invent their own. Each tag type has a unique four character identifier, a name, a direction, a colour and a text string for recording notes. Tags can be created, edited and removed by users and by internal routines. Tags can also be input along with readings. This is important when reference sequences are used during mutation detection (see Section 2.1.3 [Reference sequences], page 190).

1.2.7.1 Standard tag types

The standard tag types include those shown below plus the FT records from EMBL sequence file entries. Users can also invent their own and add them to their personal GTAGDB. This is a file that describes the available tag types and their colours (see Section 1.20.10 [Configure the tag database], page 181).

<table>
<thead>
<tr>
<th>Code</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMM</td>
<td>Comment</td>
</tr>
<tr>
<td>COMP</td>
<td>Compression</td>
</tr>
<tr>
<td>RCMP</td>
<td>Resolved compression</td>
</tr>
<tr>
<td>STOP</td>
<td>Stop</td>
</tr>
<tr>
<td>OLIG</td>
<td>Oligo (primer)</td>
</tr>
<tr>
<td>REPT</td>
<td>Repeat</td>
</tr>
<tr>
<td>ALUS</td>
<td>Alu sequence</td>
</tr>
<tr>
<td>SVEC</td>
<td>Sequencing vector</td>
</tr>
<tr>
<td>CVEC</td>
<td>Cloning vector</td>
</tr>
<tr>
<td>MASK</td>
<td>Mask me</td>
</tr>
<tr>
<td>FNSH</td>
<td>Finished segment</td>
</tr>
<tr>
<td>ENZ0</td>
<td>Restriction enzyme 0</td>
</tr>
<tr>
<td>ENZ9</td>
<td>Restriction enzyme 9</td>
</tr>
<tr>
<td>MUTN</td>
<td>Mutation</td>
</tr>
<tr>
<td>DIFF</td>
<td>Sequence different to consensus</td>
</tr>
<tr>
<td>HET+</td>
<td>Heterozygous mutation False +ve</td>
</tr>
<tr>
<td>HET-</td>
<td>Heterozygous mutation False -ve</td>
</tr>
<tr>
<td>HOM+</td>
<td>Homozygous mutation False +ve</td>
</tr>
<tr>
<td>HOM-</td>
<td>Homozygous mutation False -ve</td>
</tr>
<tr>
<td>FCDS</td>
<td>FEATURE: CDS</td>
</tr>
<tr>
<td>F***</td>
<td>All other (60) EMBL FT record types</td>
</tr>
</tbody>
</table>

1.2.7.2 Active tags and masking

Tags are used for a variety of purposes and for each function in the program the user can choose which tag types are currently "active". Where they are being used to provide visual clues this will determine which tag types appear in the displays, but for other functions they can be used to control which parts of the sequence are omitted from processing. This mode of tag use is called "masking". For example the program contains a routine to search for repeats, and if any are found, the user needs to know if such sequence duplications are caused by incorrect assembly or are genuine repeats. Once the user has checked a duplication reported by the program and found it to be a repeat, it can be labelled with a REPT tag. If the repeat routine is run in masking mode and with REPT tags active, any segment covered by a REPT tag will not be reported as a match. So once the "problem" has been dealt with it can be labelled so it is not reported on subsequent searches.
In addition the tag is available to provide annotation for the completed sequence when it is sent to the data libraries.

A more complicated application of masking is available for two of the other search procedures in the program: (see Section 1.7.1 [Shotgun assembly], page 94) and (see Section 1.8.3 [Find Internal Joins], page 114). The former is the general assembly function and the latter is used to find potential joins between contigs in the database. Below we describe how masking can be used during assembly and similar comments apply to Find Internal Joins.

In the assembly function the user can choose to employ masking and then select the types of tags to be used as masks. Readings are compared in two stages: first the program looks for exact matches of some minimum length and then for each possible overlap it performs an alignment. If the masking mode is selected the masked regions are not used during the search for exact matches, but they are used during alignment. The effect of this is that new readings that would lie entirely inside masked regions will not produce exact matches and so will not be entered. However readings that have sufficient data outside of masked segments can produce matches and will be correctly aligned even if they overlap the masked data. A common use for masking during assembly or Find Internal Joins is to avoid finding matches that are entirely contained in Alu segments.

A further mode related to masking is "marking". Marking is available for the consensus calculation (see Section 1.11 [Consensus calculation], page 136) and for Find Internal Joins (see Section 1.8.3 [Find Internal Joins], page 114). Instead of masking the regions covered by active tags these routines simply write these sections of the consensus sequence in lowercase letters. That is they make it easy for users to see where the tagged segments are. Marking has no other effect.
1.3 Contig Selector

The gap4 Contig Selector is used to display, select and reorder contigs. It can be invoked from the gap4 View menu, but will automatically appear when a database is opened. In the Contig Selector all contigs are shown as colinear horizontal lines separated by short vertical lines. The length of the horizontal lines is proportional to the length of the contigs and their left to right order represents the current ordering of the contigs. This Contig Order is stored in the gap database and users can change it by dragging the lines representing the contigs in the display. The Contig Selector can also be used to select contigs for processing. Tags (see Section 1.2.7 [Annotating and masking readings and contigs], page 29) can also be displayed in the Contig Selector window. As the mouse is moved over a contig, it is highlighted and the contig name (left most reading name) and length are displayed in the status line. The number in brackets is the contig number.

The figure shows a typical display from the Contig Selector. At the top are the File, View and Results menus. Below that are buttons for zooming and for displaying the crosshair. The four boxes to the right are used to display the X and Y coordinates of the crosshair. The rightmost two display the Y coordinates when the contig selector is transformed into the contig comparator (see Section 1.4 [Contig Comparator], page 34). The two leftmost boxes display the X coordinates: the leftmost is the position in the contig and the other is the position in the overall consensus. The crosshair is the vertical line spanning the panel below.

This panel shows the lines that represent the contigs and the currently active tags. Those tags shown above the contig lines are on readings and those below are on the consensus.

The information line is showing data for the contig that is currently under the crosshair.

1.3.1 Selecting Contigs

Contigs can be selected by either clicking with the left mouse button on the line representing the required contig in the contig selector window or alternatively by choosing the "List contigs" option from the "View" menu. This option invokes a "Contig List" list box where the contig names and numbers are listed in the same order as they appear in the contig selector window.
Within this list box the contig names can be sorted alphabetically on contig name or numerically on contig number. This is done by selecting the corresponding item from the sort menu at the top of the list box. Clicking on a name within the list box is equivalent to clicking on the corresponding contig in the contig selector. More than one contig can be selected by dragging out a region with the left mouse button. Dragging the mouse off the bottom of the list will scroll it to allow selection of a range larger than the displayed section of the list. When the left button is pressed any existing selection is cleared. To select several disjoint entries in the list press control and the left mouse button.

Most commands require a contig identifier (which can be the name or number of any reading on the contig) and gap4 contains several mechanisms for obtaining this information from users. The names or numbers can be typed or cut and pasted into dialogue boxes (note that a reading number must be preceded by a # character, e.g. "#102" means reading number 102 but "102" means the reading with name 102). Also any currently active dialogue boxes that require a contig to be selected can be updated simply by clicking on a contig in the contig selector or clicking on an entry in the "Contig Names" list box. For example, if the Edit contig command is selected from the Edit menu it will bring up a dialogue requesting the identity of the contig to edit. If the user clicks the left mouse button on a contig in the contig selector window, the contig editor dialogue will automatically change to contain the name of the selected contig. Some commands, such as the Contig Editor, can be selected from a popup menu that is activated by clicking the right mouse button on the contig line in the Contig Selector or clicking the right mouse button on the corresponding name within the "Contig List" list box. This simultaneously defines the contig to operate on and so the command starts up without dialogue.

Several contigs can be selected at once by either clicking on each contig with the left mouse button or dragging out a selection rectangle by holding the left mouse button down. Contigs which are entirely enclosed within the rectangle will be selected. Alternatively, selecting several contigs from the "Contig Names" list box will also result in each contig being selected. Selected contigs are highlighted in bold. Selecting the same contig again will unselect it.

The currently selected contigs are also kept in a 'list' named contigs.

1.3.2 Changing the Contig Order

The order of contigs is shown by the order of the lines representing them within the Contig Selector. The order of contigs can be changed by moving these lines using the middle mouse button, or Alt left mouse button. Several contigs may be moved at once by selecting several contigs using the above method. After selection, move the contigs with the middle mouse button, or Alt left mouse button, and position the mouse cursor where you want the selection to be moved to. Upon release of the mouse button the contigs will be shuffled to reflect their new order. The separator line at the point the contig was moved from increases in height.
The contig order is saved automatically whenever a contig is created or removed (eg auto assemble), including operations like disassemble which temporarily create contigs. The order can be saved manually using the Save Contig Order option on the File menu.

1.3.3 The Contig Selector Menus

The File menu contains only one command; "Exit". This simply quits the contig selector display.

The View menu gives access to the Results Manager (see Section 1.13 [Results Manager], page 155), allows contigs to be selected using a list box containing the contig names (See Section 1.3.1 [Selecting Contigs], page 31), allows active tags (see Section 1.20.9 [TagSelector], page 181) to be selected, and the list of selected contigs to be cleared.

The Results menu is updated on the fly to contain cascading menus for each of the plots shown when the contig selector is in its 2D Contig Comparator mode (see Section 1.4 [Contig Comparator], page 34). The contents of these cascading menus are identical to the pulldown menus available from within the Results Manager.
1.4 Contig Comparator

gap4 commands such as Find Internal Joins (see Section 1.8.3 [Find Internal Joins], page 114), Find Repeats (see Section 1.8.4 [Find Repeats], page 118), Check Assembly (see Section 1.9 [Check Assembly], page 121), and Find Read Pairs (see Section 1.8.2 [Find Read Pairs], page 110) automatically transform the Contig Selector (see Section 1.3 [Contig Selector], page 31) to produce the Contig Comparator. To produce this transformation a copy of the Contig Selector is added at right angles to the original window to create a two dimensional rectangular surface on which to display the results of comparing or checking contigs. Each of the functions plots its results as diagonal lines of different colours. If the plotted points are close to the main diagonal they represent results from pairs of contigs that are in the correct relative order. Lines parallel to the main diagonal represent contigs that are in the correct relative orientation to one another. Those perpendicular to the main diagonal show results for which one contig would need to be reversed before the pair could be joined. The manual contig dragging procedure can be used to change the relative positions of contigs. See Section 1.3.2 [Changing the Contig Order], page 32. As the contigs are dragged the plotted results will be automatically moved to their corresponding new positions. This means that if users drag the contigs to move their plotted results close to the main diagonal they will be simultaneously putting their contigs into the correct relative positions.

Because this plot can simultaneously show the results of independent types of search, users can see if different analyses produce corroborating evidence for the ordering of contigs. Also, if for example, a result from Check Assembly lies on the same horizontal or vertical projection as a result from Find Repeats, users can see the alternative position to place the doubtful reading. I.e this is an indication that a reading may have been assembled in an incorrect position.

By use of popup menus the plotted results can be used to invoke a subset of commands. For example if the user clicks the right mouse button over a result from Find Internal Joins a menu containing Invoke Join Editor (see Section 1.6.14 [The Join Editor], page 87) and Invoke Contig Editors (see Section 1.6 [Editing in gap4], page 58) will pop up. If the user selects Invoke Join Editor the Join Editor will be started with the two contigs aligned at the match position contained in the result. If required one of the contigs will be complemented to allow their alignment.

A typical display from the Contig Comparator is shown below. It includes results for Find Internal Joins in black, Find Repeats in red, Check Assembly in green, and Find Read Pairs in blue. Notice that there are several Find Internal Joins, Find Read Pairs and Find Repeats results close to the main diagonal near the top left of the display, indicating that the contigs represented in that area are likely to be in the correct relative positions to one another. In the middle of the bottom right quadrant there is a blue diagonal line perpendicular to the main diagonal which indicates a pair of contigs that are in the wrong relative orientation. The crosshairs show the positions for a pair of contigs. The vertical line continues into the Contig Selector part of the display, and the position represented by the horizontal line is also duplicated there.
1.4.1 Examining Results and Using Them to Select Commands

Moving the cursor over plotted results highlights them, and the information line gives a brief description of the currently highlighted match. This is in the form:

\[ \text{match name: contig1\_number@position\_in\_contig1, with contig2\_number@position\_in\_contig2, length\_of\_the\_match} \]

For Find Internal Joins the percentage mismatch is also displayed.

Several operations can be performed on each match. Pressing the right mouse button over a match invokes a popup menu. This menu will contain a set of options which depends on the type of result to which the match corresponds. The following is a complete list, but not all will appear for each type of result.

**Information**

Sends a textual description of the match to the Output Window.

**Hide**

Removes the match from the Contig Comparator. The match can be revealed again by using "Reveal all" within the Results Manager.
Invoke contig editors
Invoke join editors
Invoke template display

When invoked these options bring up their respective displays to show the match in greater detail.

Remove

Removes the match from the Contig Comparator. The match cannot be revealed again by using "Reveal all" within the Results Manager.

One of the items in the popup menu may have an asterisk next to it. This is the default operation which can also be performed by double clicking the left mouse button on the match. For Repeat or Find Internal Joins matches this will normally be the Join Editor, or two Contig Editors when the match is between two points in the same contig. For Read Pairs two Template Displays are shown.

The crosshairs can be toggled on and off and a diagonal line going from top left to bottom right of the plot can also be displayed if required. This is useful as a guide for moving the contigs such that their matches lie upon the diagonal line.

The "Results" menu on the contig selector window provides a similar mechanism of accessing results, but at the level of all matches in a particular search. This is simply a menu driven interface to the Results Manager window (see Section 1.13 [Results Manager], page 155), but containing only the results relevant to the contig comparator window.

1.4.2 Automatic Match Navigation

The "Next" button of the contig comparator window automatically invokes the default operation on the next match from the current active result. This provides a mechanism to step through each match in turn ensuring that no matches have been missed.

With a single result (set of matches) plotted, the "Next" button simply steps through each match in turn until all have been seen. Moving the mouse above the "Next" button, without pressing it, highlights the next match and displays brief information about it in the status line at the bottom of the window. To step through the matches in "best first" order, select the "Sort Matches" option from the relevant name in the Results menu. The exact order is dependent on the result in question, but is generally arranged to be the most interesting ones first. For example, Find Internal Joins shows the lowest mismatch first whilst Check Assembly shows the highest mismatches first.

Bringing up another result now directs "Next" to step through each of the new matches. To change the result that "Next" operates on, use the Result menu to select the "Use for 'Next'" option in the desired result. Alternatively, double clicking on a match also causes "Next" to process the list starting from the selected result.

The "Next" scheme remembers any matches that have been previously examined either by itself or by manually double clicking, and will skip these. To clear this 'visited' information select "Reset 'Next'" in the Results Manager.
1.5 Contig Overviews

Gap4 provides views of the data for an assembly project at 3 levels of resolution: the whole project can be seen from the Contig Selector (see Section 1.3 [Contig Selector], page 31), the most detail from the Contig Editor (see Section 1.6 [Editing in gap4], page 58), and the Contig Overview Displays, described in this section, provide an intermediate level of information and data manipulation. They are available from the main gap4 View menu.

These middle level resolution displays provide graphical overviews of individual contigs or sets of contigs. The possible information shown includes readings, templates, tags, restriction enzyme sites, stop codons, plots of the consensus quality, read coverage, read-pair coverage, strand coverage and consensus confidence. The displays of readings, templates, tags, restriction enzyme sites and plots of the consensus quality can be shown in a single window called the Template Display (see Section 1.5.1 [Template Display], page 37). The plots of reading coverage, read-pair coverage, strand coverage and consensus confidence can be shown in a single display called the Consistency Display (see Section 1.5.2 [Consistency Display], page 46), or as separate plots. The Stop Codon Plot (see Section 1.5.4 [Plotting Stop Codons], page 54) and a more informative version of the Restriction Enzyme Plot (see Section 1.5.5 [Plotting Restriction Enzymes], page 55) can be shown in separate windows.

1.5.1 Template Display

The Template Display can show schematic plots of readings, templates, tags, restriction enzyme sites and the consensus quality. It can be used to reorder contigs, create tags and invoke the Contig Editor. It is invoked from the main gap4 View menu.

An example showing all these information types can be seen in the Figure below.
The large top section contains lines and arrows representing readings and templates. Beneath this are rulers; one for each contig, and below those is the quality plot. The template and reading section of the display is in two parts. The top part contains the templates which have been sequenced from both ends but which are in some way inconsistent - for example given the current relative positions of their readings, they may have a length that is larger or greater than that expected, or the two readings may, as it were, face away from one another. Colour coding is used to distinguish between different types of inconsistency, and whether or not the inconsistency involves readings within or between contigs. For example, most of the problems shown in the screendump above are coloured dark yellow, indicating an inconsistency between a pair of contigs. The rest of the data, (mostly dark blue indicating templates sequenced from only one end), is plotted below the data for the inconsistent templates. Forward readings are blue and reverse readings are orange. Templates in bright yellow have been sequenced from both ends, are consistent and span a pair of contigs (and so indicate the relative orientation and separation of the contigs).

The coloured blocks immediately above and below the ruler are tags. Those above the ruler can also be seen on their corresponding readings in the large top section. Zooming is available. The position of a crosshair is shown in the two left most boxes in the top right hand corner. The leftmost shows the distance in bases between the crosshair and the start of the contig underneath the crosshair. The middle box shows the distance between the crosshair and the start of the first
contig. The right box shows the distance between two selected cut sites in the restriction enzyme plots.

![Image of Gap4 interface]

As seen in the dialogue above, users can choose to display a single contig, all contigs, or a subset of contigs from a file of filenames ("file") or a list ("list"). If either the file or list options are chosen, the "browse" button will be activated and can be used to call up a file or list browser dialogue.

The items to be shown in the initial template display can be selected from the list of checkboxes. The default is to display all templates and readings. However, it is possible to display only templates with more than one reading ("Ignore 'single' templates") or templates with both forward and reverse readings ("Show only read pairs"). These latter two options may be beneficial if the database is very large.

In the section below we give details about the individual components of the overall Template Display.

1.5.1.1 Reading and Template Plot

The Reading and Template Plot shows templates and readings. The following sections describe the display, its options, and the operations which it can be used to perform. It is invoked from the main gap4 View menu.

1.5.1.2 Reading and Template Plot Display

The Reading and Template Plot shows templates and readings. Colour is used to provide additional information. The reading colour is used to convey the primer information. The default colours are:

- **red**: primer unknown
- **green**: forwards primer
- **orange**: reverse primer
- **dark_cyan**: custom forward primer
- **orange-red**: custom reverse primer

Colour is used to distinguish the number and the location of the readings derived from each template. Templates with readings derived from only one end are drawn in blue. Those with readings from both ends are pink when both ends are contained within the same contig. Those
with readings from both ends are green when the readings are in different contigs and one of the contigs is not being plotted.

For each template gap4 stores an expected length, as a range between two values. From an assembly it is often possible to work out the actual length of a template based upon the positions within a contig of readings sequenced using the forward and reverse primers. The forward and reverse readings on a single template (called a read pair) are considered to be inconsistent if this observed distance is outside of the range of acceptable sizes and then the template is drawn in black. Alternatively it may be possible that both forward and reverse readings are assembled on the same strand (in which case both arrows will point in the same direction). This too is a problem and hence the templates are drawn in black.

If more than one contig is displayed then the distance between adjacent contigs is determined from any read pair information. If there are spanning templates between two adjacent contigs and the readings on that template are consistent, i.e. are in the correct orientation, the template is coloured yellow. Templates which span non-adjacent contigs in the display or contain inconsistent readings are coloured dark yellow.

A summary of the default template colours follows.

- **blue**: the template contains only readings from one end
- **pink**: the template contains both forward and reverse readings in the same contig
- **green**: the template contains both forward and reverse readings, but they are in separate contigs, and one of the contigs is not being displayed.
- **black**: the readings on the template are within the same contig but are in contradictory orientations or are an unexpected distance apart
- **yellow**: the readings on the template are within different contigs (both of which are being displayed) and are consistent
- **dark yellow**: the readings on the template are within different contigs (both of which are being displayed) and are inconsistent

If more than one contig is displayed, the contigs are positioned in the same left to right order as the input contig list, (which need not necessarily be in the same order as the contig selector). Overlapping contigs are drawn as staggered lines. If the user selects the "Calculate contig positions" option from the menu the horizontal distance between adjacent contigs is determined from any available read pair information. Otherwise, or in the absence of any read pair information, the second contig is positioned immediately following the first contig, but will be drawn staggered in the vertical direction. If the readings on a template spanning two contigs are consistent, the distance between the contigs is determined using the template’s mean length. If there are several templates spanning a pair of contigs an average distance is calculated and used as the final offset between the contigs. Templates which span non-adjacent contigs or contain inconsistent readings are not used in the calculation of the contig offsets. It is possible that data in the database is inconsistent to such an extent that, although spanning templates have consistent readings, the averaging can lead to a display which shows the templates to have inconsistent readings, e.g. the readings are pointing in opposite directions.

A summary of the templates and readings used to calculate the distance between two contigs is displayed in the output window. An example is given below:

```
Wed 02 Apr 10:35:51 1997: template display

Contig zf98g12.r1(651) and Contig zf23d2.s1(348)
```
The contig names and numbers are given in the top line. Below this, the spanning template name, number and length is displayed. Below this the reading name, whether the reading has been complemented (+: original -: complemented), number, primer information, starting position, length and contig number. This is of similar format to that displayed by the read pairs output. See Section 1.8.2.2 [Find Read Pairs], page 112. The average gap between the contigs is given and finally the distance in bases between the start of the second contig and the start of the left most contig in the display.
1.5.1.3 Reading and Template Plot Options

Within the figure shown above the contents of the View menu are visible. The "Templates", "Readings", "Quality Plot" and "Restriction Enzyme Plot" commands control which attributes are displayed. The graphics are always scaled to fit the information within the window size, subject to the current zoom level. This means that turning off templates, but leaving readings displayed, will improve visibility of the reading information.

The "Ruler ticks" checkbox determines whether to draw numerical ticks on the contigs. The number of ticks is defined in the .gaprc (see Section 1.20.1 [Options Menu], page 176) file as NUM.TICKS although the actual number of ticks per contig that will be displayed also depends on the space available on the screen.

The "ignore 'single' templates" toggle controls whether to display all templates or only those containing more than one reading. The "show only read pairs" toggle controls whether all templates or only those containing both forward and reverse readings are displayed. Hence when set the templates displayed are those with a known (observed) length. The "Show only spanning read
pairs" toggle controls whether to display all templates or only those containing forward and reverse readings which are in different contigs.

The plot can be enlarged or reduced using the standard zooming mechanism. See Section 9.4.1 [Zooming], page 378.

The crosshair toggle button controls whether the cursor is visible. This is shown as a black vertical line. The position of the crosshair is displayed in the two boxes to the right of the crosshair toggle. The first box indicates the cursor position in the current contig. The second box indicates the overall position of the cursor in the consensus. The third box is used to show the distance between restriction enzyme cut sites. See Section 1.5.1.6 [Restriction Enzyme Plot], page 46.

Tags that are on the consensus can only be seen on the ruler. These are marked beneath the ruler line. Tags on readings can be seen both on the ruler (above the line) and on their appropriate readings within the template window. To configure the tag types that are shown use the "select Tags" command in the View menu. This brings up the usual tag selection dialog box. See Section 1.20.9 [Tag Selector], page 181.

1.5.1.4 Reading and Template Plot Operations

The contig editor can be invoked by double clicking the middle mouse button, or Alt the left mouse button, in any of the displays, ie template, ruler, quality or restriction enzyme plots. The editor will start up with the editing cursor on the base that corresponds to the position clicked on in the Template Display. If more than one contig is currently being displayed the editor decides which contig to show using the following rules. If the user clicks on the Quality Plot, the contig lines or the Restriction Enzyme Display, the corresponding contigs will be shown. If the user clicks on a gap between these displays the nearest contig will be selected. If the user clicks on the template or reading lines, the editor will show the contig whose left end is to the left of and closest to the cursor.

The order of the contigs can be changed within the Template Display by clicking with the middle mouse button, or Alt left mouse button, on a contig line and dragging the line to the new position. The Template Display will update automatically once the mouse button is released. The change of a dark yellow template to bright yellow is indicative that the two contigs are now in consistent positions and orientations. The order of the contigs in the gap4 database, as displayed in the contig selector, can be updated by selecting the "Update contig order" command in the Edit menu.

By clicking on any of the contig lines in the ruler a popup menu is invoked. From this, information on the contig can be obtained, the contig editor can be started, the contig can be complemented, and the templates within the contig can be highlighted (shown by changing their line width).

A list named readings always exists. It contains the list of readings that are highlighted in all the currently shown template displays. See Section 1.14 [Lists], page 156. The highlighting mechanism used is to draw the readings as thicker, bolder, lines. The "clear Active Readings" command from the View menu clears this list. The "highlight reading list" command loads a new set of readings to use for the "readings" list and then highlights these.

To interactively add and remove readings from the active list use the left mouse button. Clicking on an individual reading will toggle its state from active to non active and back again. Pressing and holding the left mouse button, and moving the mouse, will drag out a bounding box. When the button is released all readings that are contained entirely within the bounding box will be toggled.
Activating a reading (using any of the above methods) when an editor is running, will also highlight the reading within the editor. Similarly, highlighting the reading in the editor activates it within the template display and adds it to the active reading list.

### 1.5.1.5 Quality Plot

This option can be invoked from the main gap4 View menu, in which case it appears as a single plot, or from the View menu of the Template Display, in which case it will appear as part of the Template Display.

This display provides an overview of the quality of the consensus. The Contig Editor can be used to examine the problems revealed. A typical plot is displayed below.

For each base in the consensus a quality is computed based on the accuracy of the data on each strand. As can be seen in the Figure above, this information is then plotted using colour and height to distinguish between the different quality assignments. The colour and height codes are explained below.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Height</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>grey</td>
<td>0 to 0</td>
<td>OK on both strands, both agree</td>
</tr>
<tr>
<td>blue</td>
<td>0 to 1</td>
<td>OK on plus strand only</td>
</tr>
<tr>
<td>green</td>
<td>-1 to 0</td>
<td>OK on minus strand only</td>
</tr>
<tr>
<td>red</td>
<td>-1 to 1</td>
<td>Bad on both strands</td>
</tr>
<tr>
<td>black</td>
<td>-2 to 2</td>
<td>OK on both strands but they disagree</td>
</tr>
</tbody>
</table>

For example, in the figure we see that the first four hundred or so bases are mostly only well determined on the forward strand.

Note that when a large number of bases are being displayed the limited screen resolution causes the quality codes for adjacent bases to be drawn as single pixels. However the use of varying heights ensures that all problematic bases will be visible. Hence when the quality plot consists of a single grey line all known quality problems have been resolved, at the current consensus and quality cutoffs.

To check problems the contig editor can be invoked by double clicking on the middle mouse button, or Alt left mouse button. It will appear centred on the base corresponding to the position on which the mouse was clicked.

The quality plot appears as "Calculate quality" in the Results Manager window (see Section 1.13 [Results Manager], page 155).

Within the Results Manager commands available, using the right mouse button, include "Information", which lists a summary of the distribution of quality types to the output window, and
"List" which lists the actual quality values for each base to the output window. These quality values are written in a textual form of single letters per base and are listed below.

<table>
<thead>
<tr>
<th>Strand</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Good</td>
</tr>
<tr>
<td>-</td>
<td>Good</td>
</tr>
<tr>
<td>a</td>
<td>Good</td>
</tr>
<tr>
<td>b</td>
<td>Good</td>
</tr>
<tr>
<td>c</td>
<td>Bad</td>
</tr>
<tr>
<td>d</td>
<td>Good</td>
</tr>
<tr>
<td>e</td>
<td>None</td>
</tr>
<tr>
<td>f</td>
<td>Bad</td>
</tr>
<tr>
<td>g</td>
<td>None</td>
</tr>
<tr>
<td>h</td>
<td>Bad</td>
</tr>
<tr>
<td>i</td>
<td>None</td>
</tr>
<tr>
<td>j</td>
<td>None</td>
</tr>
</tbody>
</table>

An example of the output using "Information" and "List" follows.

```
+Strand -Strand
a    Good Good (in agreement)
b    Good Bad
c    Bad Good
d    Good None
e    None Good
f    Bad Bad
g    Bad None
h    None Bad
i    Good Good (disagree)
j    None None
```

An example of the output using "Information" and "List" follows.

```
Wed 02 Apr 12:14:06 1997: quality summary

Contig xb56b6.s1 (#11)
  81.00 OK on both strands and they agree (a)
  3.94 OK on plus strand only (b, d)
  11.98 OK on minus strand only (c, e)
  1.85 Bad on both strands (f, g, h, j)
  1.22 OK on both strands but they disagree (i)
```

```
Wed 02 Apr 12:14:09 1997: quality listing

Contig xb56b6.s1 (#11)

```
10 20 30 40 50 60

[ Output removed for brevity ]
```
1.5.1.6 Restriction Enzyme Plot

The restriction enzyme plot within the template display is a reduced version of the main Restriction Enzyme Map function. The dialogue used for choosing the restriction enzymes is identical and is described with the main function. See Section 1.5.5 [Plotting Restriction Enzymes], page 55. It is invoked from the Template Display View menu. An example plot from the template display can be seen below.

<table>
<thead>
<tr>
<th>2000</th>
<th>4000</th>
<th>6000</th>
<th>8000</th>
<th>10000</th>
<th>12000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Here we see the searches for two restriction enzymes. Each vertical line is drawn at the cut position of the matched restriction site. Unlike the main restriction enzyme plot here all matches are plotted on a single horizontal plot. Initially all sites are drawn in black. To distinguish one site from another either touch the site with the mouse cursor and read the template display information line, or place the mouse cursor above a site and press the right mouse button. This pops up a menu containing "Information" and "Configure". The "Configure" option can be used to change the colour of all matches found for this enzyme. In the figure above we have changed the initial colours for both of the restriction enzymes searched for. The "Information" command displays information for all sites found in the text output window.

As with the main Restriction Enzyme Map function, clicking the left mouse button on two restriction sites in turn displays the distance between the chosen sites in the information line. This figure is also displayed in the box at the top right hand corner of the template display.

1.5.2 Consistency Display

The Consistency Display provides plots designed to highlight potential problems in contigs. It is invoked from the main gap4 View menu by selecting any of its plots. Once a plot has been displayed, any of the other types of consistency plot can be displayed within the same frame from the View menu of the Consistency Display.

An example showing the Confidence Values Graph and the corresponding Reading Coverage Histogram, Read-Pair Coverage Histogram and Strand Coverage is shown below.
One or more contigs can be displayed and are drawn in the same order at the input contig list (which need not necessarily be in the same order as the contig selector). If more than one contig is displayed, the contigs are drawn immediately after one another but are staggered in the y direction.

The ruler ticks can be turned on or off from the View menu of the consistency display.

The plots can be enlarged or reduced using the standard zooming mechanism. See Section 9.4.1 [Zooming], page 378.

The crosshair toggle button controls whether the crosshair is visible. This is shown as a black vertical and horizontal line. The position of the crosshair is shown in the 3 boxes to the right of the crosshair toggle. The first box indicates the cursor position in the current contig. The second
box indicates the overall position of the cursor in the consensus. The last box shows the y position of the crosshair.

### 1.5.2.1 Confidence Values Graph

This option can be invoked from the main gap4 View menu, in which case it appears as a single plot, or from the View menu of the Consistency Display in which case it appear part of the Consistency Display.

The confidence values are determined from the current consensus algorithm (see Section 1.11.5 [The Consensus Algorithms], page 140).

![Confidence Values Graph](image)

Please note that this plot can be very slow for long contigs. This is caused by the large number of points (not the calculation) and we hope to speed it up in a future release.

### 1.5.2.2 Reading Coverage Histogram

This option can be invoked from the main gap4 View menu, in which case it appears as a single plot, or from the View menu of the Consistency Display in which case it will appear as part of the Consistency Display.

The number of readings which cover each base position along the contig are plotted as a histogram.
As can be seen in the dialogue below, the user can select the contigs(s) to display, and whether to plot: Forward strand only, Reverse strand only, Both strands or the Summation of both strands. In the example shown above both strands have been plotted: forward in red and reverse in black.

1.5.2.3 Read-Pair Coverage Histogram

This option can be invoked from the main gap4 View menu, in which case it appears as a single plot, or from the View menu of the Consistency Display in which case it will appear as part of the Consistency Display.

The number of read-pairs which cover each base position along the contig are plotted as a histogram.
1.5.2.4 Strand Coverage

This option can be invoked from the main gap4 View menu, in which case it appears as a single plot, or from the View menu of the Consistency Display in which case it will appear as part of the Consistency Display.

The display is used to show which regions of the data are covered by readings from each of the two strands of the DNA. A separate line is drawn for each strand: forward in red and reverse in black. The function works in two complementary modes: it can plot the positions which are covered, or the positions which are not. The latter is probably the most useful as it directs users to the places requiring further data.

The figure below shows the covered positions, and the figure below that shows the uncovered positions for the same contig.
The plot can be regarded as a coarse version of the Quality Plot (see Section 1.5.1.5 [Quality Plot], page 44), in that it shows the strand coverage using the Quality Calculation (see Section 1.11.5.4 [The Quality Calculation], page 144), but does not reveal problems with individual base positions.

The dialogue allows user to select the contig(s) and strands to analyse and whether to plot Coverage or Problems.
1.5.3 Plotting Consensus Quality

This option can be invoked from the main gap4 View menu, in which case it appears as a single plot, or from the View menu of the Template Display, in which case it will appear as part of the Template Display.

For each base in the consensus a "quality" code is computed based on the accuracy of the data on each strand and whether or not the two strands agree. In a future release it will be renamed the "Strand Comparison Plot." This "quality" is then plotted using colour and height to distinguish the quality codes shown below.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Height</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>grey</td>
<td>0 to 0</td>
<td>OK on both strands, both agree</td>
</tr>
<tr>
<td>blue</td>
<td>0 to 1</td>
<td>OK on plus strand only</td>
</tr>
<tr>
<td>green</td>
<td>-1 to 0</td>
<td>OK on minus strand only</td>
</tr>
<tr>
<td>red</td>
<td>-1 to 1</td>
<td>Bad on both strands</td>
</tr>
<tr>
<td>black</td>
<td>-2 to 2</td>
<td>OK on both strands but they disagree</td>
</tr>
</tbody>
</table>

For example, in the figure we see that the first four hundred or so bases are mostly only well determined on the forward strand.

1.5.3.1 Examining the Quality Plot

Note that when displaying many bases the screen resolution implies that the quality codes for many bases will appear in the same screen pixel. However the use of varying heights ensures that all problematic regions will be visible, even when the problem is only with a single base position. Hence when the quality plot consists of a single grey line all known quality problems have been resolved, at the current consensus and quality cutoffs.

The quality plot appears as "Calculate quality" in the Results Manager window (see Section 1.13 [Results Manager], page 155).

Within the Results Manager commands available, using the right mouse button, include "Information", which lists a summary of the distribution of quality types to the output window, and "List" which lists the actual quality values for each base to the output window. These quality values are written in a textual form of single letters per base and are listed below.

+Strand -Strand

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Good Good (in agreement)</td>
</tr>
<tr>
<td>b</td>
<td>Good Bad</td>
</tr>
<tr>
<td>c</td>
<td>Bad Good</td>
</tr>
</tbody>
</table>
Chapter 1: Sequence assembly and finishing using Gap4

An example of the output using "Information" and "List" follows.

Wed 02 Apr 12:14:06 1997: quality summary

Contig xb56b6.s1 (#11)
81.00 OK on both strands and they agree(a)
3.94 OK on plus strand only(b,d)
11.98 OK on minus strand only(c,e)
1.85 Bad on both strands(f,g,h,j)
1.22 OK on both strands but they disagree(i)

Wed 02 Apr 12:14:09 1997: quality listing

Contig xb56b6.s1 (#11)

[ output removed for brevity ]
1.5.4 Plotting Stop Codons

The Stop Codon Map plots the positions of all the stop codons on one or both strands of a contig consensus sequence. It can be invoked from the gap4 View menu. If the Contig Editor is being used on the same contig, the Refresh button will be enabled and if used will fetch the current consensus from the editor, repeat the search and replot the stop codons.

1.5.4.1 Examining the Plot

Positioning the cursor over a plotted point will cause its codon and position to appear in the information line.

It is possible to find the distance between any two stop codons. Pressing the left mouse button on a plotted point will display "Select another codon" at the bottom of the window. Then, pressing the left button on another plotted point will display the distance, in bases, between the two sites. This is shown in the box located at the top right corner of the window.

1.5.4.2 Updating the Plot

If the Contig Editor (see Section 1.6 [Editing in gap4], page 58) is currently running on the same contig as is being displayed as a Stop Codon Map, the Refresh button will be shown in bold lettering and hence be active, otherwise it will be greyed out. Pressing the button will fetch the current consensus from the Contig Editor and replot its stop codons. Hence the plot can be kept current with the changes being made in the editor.
1.5.5 Plotting Restriction Enzymes

The restriction enzyme map function finds and displays restriction sites within a specified region of a contig. It is invoked from the gap4 View menu. Users can select the enzyme types to search for and can save the sites found as tags within the database.

This figure shows a typical view of the Restriction Enzyme Map in which the results for each enzyme type have been configured by the user to be drawn in different colours. On the left of the display the enzyme names are shown adjacent to the lines of plotted results. If no result is found for any particular enzyme eg here APAI, the line will still be drawn so that zero cutters can be identified. Three of the enzymes types have been selected and are shown highlighted. The results can be scrolled vertically (and horizontally if the plot is zoomed in). A ruler is shown along the base and the current cursor position (the vertical black line) is shown in the left hand box near the top right of the display. If the user clicks, in turn, on two restriction sites their separation in base pairs will appear in the top right hand box. Information about the last site touched is shown in the Information line at the bottom of the display. At the top the edit menu is shown torn off and can be used to create tags for highlighted enzyme types.

1.5.5.1 Selecting Enzymes

Files of restriction enzyme names and their cut sites are stored in disk files. For the format of these files and notes about creating new ones see Section 10.4 [Restriction enzyme files], page 414.

When the file is read, the list of enzymes is displayed in a scrolling window. To select enzymes press and drag the left mouse button within the list. Dragging the mouse off the bottom of the list will scroll it to allow selection of a range larger than the displayed section of the list. When the left button is pressed any existing selection is cleared. To select several disjoint entries in the list press control and the left mouse button. Once the enzymes have been chosen, pressing OK will create the plot.

1.5.5.2 Examining the Plot

Positioning the cursor over a match will cause its name and cut position to appear in the information line. If the right mouse button is pressed over a match, a popup menu containing Information and Configure will appear. The Information function in this menu will display the data for this cut site and enzyme in the Output Window.
It is possible to find the distance between any two cut sites. Pressing the left mouse button on a match will display "Select another cut" at the bottom of the window. Then, pressing the left button on another match will display the distance, in bases, between the two sites. This is shown in a box located at the top right corner of the window.

1.5.5.3 Reconfiguring the Plot

The plot displays the results for each restriction enzyme on a separate line. Enzymes with no sites are also shown. The order of these lines may be changed by pressing and dragging the middle mouse button or alt + left mouse button on one of the displayed names at the left side of the screen.

The results are plotted as black lines but users can select colours for each enzyme type by pressing the right button on any of its matches. A menu containing Information and Configure will pop up. Configure will display a colour selection dialogue. Adjusting the colour here will adjust the colour for all matches for this restriction enzyme.

1.5.5.4 Creating Tags for Cut Sites

Clicking the left mouse button on an enzyme name at the left of the display toggles a highlight. The Create tags command from the Edit menu will add tags to the database for all the matches whose enzyme names are highlighted. The command displays a dialogue box listing the enzyme names on the left, and the tag type to create for that enzyme on the right. Tag types must be chosen for all the listed restriction enzyme types before the tags can be created. Suitable tag types to choose are the ENZ0, ENZ1 (etc) tags.

1.5.5.5 Textual Outputs

The Results menu of the plot contains options to list the restriction enzyme sites found. One option sorts the results by enzyme name and the other by the positions of the matches.

The output below shows the textual output from "Output enzyme by enzyme". The Fragment column gives the size of the fragments between each of the cut sites. The Lengths column contains the fragment sizes sorted on size.

The output below shows the textual output from "Output ordered on position".

Contig zf98g12.r1 (#801)
Number of enzymes = 3
Number of matches = 7
Matches found = 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
<th>Fragment lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 AATII</td>
<td>GACGT'C</td>
<td>7130 7129</td>
<td>556 556 7129</td>
</tr>
</tbody>
</table>

Matches found = 5

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
<th>Fragment lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ACCI</td>
<td>GT'CGAC</td>
<td>414 413</td>
<td>189 413</td>
</tr>
<tr>
<td>2 ACCI</td>
<td>GT'CTAC</td>
<td>1296 882</td>
<td>413 882</td>
</tr>
<tr>
<td>3 ACCI</td>
<td>GT'CTAC</td>
<td>3871 2575</td>
<td>882 2575</td>
</tr>
<tr>
<td>4 ACCI</td>
<td>GT'CTAC</td>
<td>5816 1945</td>
<td>1681 1945</td>
</tr>
<tr>
<td>5 ACCI</td>
<td>GT'CGAC</td>
<td>7497 1681</td>
<td>1945 1681</td>
</tr>
</tbody>
</table>

The output below shows the textual output from "Output ordered on position".
Contig zf98g12.r1 (#S01)
Number of enzymes = 3
Number of matches = 7

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
<th>Fragment lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACCI</td>
<td>GT’CGAC</td>
<td>414 413 3</td>
</tr>
<tr>
<td>2</td>
<td>ACCI</td>
<td>GT’CTAC</td>
<td>1296 882 189</td>
</tr>
<tr>
<td>3</td>
<td>ACCI</td>
<td>GT’CTAC</td>
<td>3871 2575 367</td>
</tr>
<tr>
<td>4</td>
<td>ACCI</td>
<td>GT’CTAC</td>
<td>5816 1945 413</td>
</tr>
<tr>
<td>5</td>
<td>AHAII</td>
<td>GA’CGTC</td>
<td>7127 1311 882</td>
</tr>
<tr>
<td>6</td>
<td>AATII</td>
<td>GACGT’C</td>
<td>7130 3 1311</td>
</tr>
<tr>
<td>7</td>
<td>ACCI</td>
<td>GT’CGAC</td>
<td>7497 367 1945</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th>189 2575</th>
</tr>
</thead>
</table>

1.6 Editing in Gap4

The gap4 Contig Editor is designed to allow rapid checking and editing of characters in assembled readings. Very large savings in time can be achieved by its sophisticated problem finding procedures which automatically direct the user only to the bases that require attention. The following is a selection of screenshots to give an overview of its use.

The figure above shows a screendump from the Contig Editor which contains segments of aligned readings, their consensus and a six phase translation. The Commands menu is also shown. The main components are: the controls at the top; reading names on the left; sequences to their right; and status lines at the bottom. Some of the reading names are written in light grey which indicates that their traces/chromatograms are being displayed (in another window, see below).

One reading name is written with inverse colours, which indicates that it has been selected by the user. To the left of each reading name is the reading number, which is negative for readings which have been reversed and complemented. The first of the status lines, labelled "Strands", is showing a summary of strand coverage. The left half of the segment of sequence being displayed is covered only by readings from one strand of the DNA, but the right half contains data from both strands.

Along the top of the editor window is a row of command buttons and menus. The rightmost pair of buttons provide help and exit. To their left are two menus, one of which is currently in use. To the left of this is a button which initially displays a search dialogue, and then pressing it again, will perform the selected search. Further left is the undo button: each time the user clicks on this box the program reverses the previous edit command. The next button, labelled "Cutoffs" is used to toggle between showing or hiding the reading data that is of poor quality or is vector sequence. In this figure it has been activated, revealing the poor quality data in light grey. Within this, sequencing vector is displayed in lilac. The next button to the left is the Edit Modes menu which allows users to select which editing commands are enabled. The next command toggles between insert and replace and so governs the effect of typing in the edit window. The 2 entryboxes on the left hand side labelled C and Q set the consensus and quality cutoff values (see Section 1.6.17.1 [Consensus and Quality Cutoffs], page 88).
One of the readings contains a yellow tag, and elsewhere some bases are coloured red, which indicates they are of poor quality. The Information Line at the bottom of the window can show information about readings, annotations and base calls. In this case it is showing information about the reliability of the base beneath the editing cursor.

A better way of displaying the accuracy of bases is to shade their surroundings so that the lighter the background the better the data. In the figure above, this grey scale encoding of the base accuracy or confidence has been activated for bases in the readings and the consensus. This screenshot also shows the Contig Editor displaying disagreements and edits. Disagreements between the consensus and individual base calls are shown in dark green. Notice that these disagreements are in poor quality base calls. Edits (here they are all pads) are shown with a light green background.

When they are present, replacements/insertions are shown in pink, deletions in red and confidence value changes in purple. The consensus confidence takes into account several factors, including individual base confidences, sequencing chemistry, and strand coverage. It can be seen that the consensus for the section covered by data from only one strand has been calculated to be of lower confidence than the rest. The Status Line includes two positions marked with exclamation marks (!) which means that the sequence is covered by data from both strands, but that the consensus for each of the two strands is different. The Information Line at the bottom of the window is showing information about the reading under the cursor: its name, number, clipped length, full length, sequencing vector and BAC clone name.

The Contig Editor can rapidly display the traces for any reading or set of readings. The number of rows and columns of traces displayed can be set by the user. The traces scroll in register with one another, and with the cursor in the Contig Editor. Conversely, the Contig Editor cursor can be scrolled by the trace cursor. A typical view is shown below.
This figure is an example of the Trace Display showing three traces from readings in the previous two Contig Editor screen dumps. These are the best two traces from each strand plus a trace from a reading which contains a disagreement with the consensus. The program can be configured to automatically bring up this combination of traces for each problem located by the "Next search" option. The histogram or vertical bars plotted top down show the confidence value for each base call. The reading number, together with the direction of the reading (+ or -) and the chemistry by which it was determined, is given at the top left of each sub window. There are three buttons ('Info', 'Diff', and 'Quit') arranged vertically with X and Y scale bars to their right. The Info button produces a window like the one shown in the bottom right hand corner. The Diff button is mostly used for mutation detection, and causes a pair of traces to be subtracted from one another and the result plotted, hence revealing their differences. (see Section 1.6.11 [Traces], page 81).

1.6.1 Moving the visible segment of the contig

The contig editor displays only one segment of the entire contig, although several contig editors can be in use at once. Above the sequence display is a "scrollbar". This line represents the entire contig, with a greyed section representing the currently displayed segment. To change the displayed segment put the mouse cursor in the scrollbar and use the mouse buttons. The available controls are:

- **Middle Mouse Button** Set displayed section
- **Alt Left Mouse Button** Set displayed section
- **Left Mouse Button** Scroll left or right one screenful

On the far right side of the contig is a vertically oriented scrollbar. Typically the editor will be showing all available data, in which case the vertical scrollbar cannot be scrolled. In regions of exceptionally deep coverage, the editor makes sure that the controls, the consensus, and any status lines are visible. The remaining space is taken up with however many sequences fit. The vertical scrollbar can then be used, using the mouse buttons listed above, to scroll through the sequences.

In addition to the scrollbars there are four buttons on the left hand side for scrolling by fixed amounts.

- **<<** Scroll left half a screenful
- **<** Scroll left one base
- **>** Scroll right one base
- **>>** Scroll right half a screenful
Within the editor window itself two more key combinations can be used for scrolling forwards and backwards an entire screenful. These, and several others, are modelled after the Emacs key bindings.

- Control v: Scroll right one screenful
- Meta v: Scroll left one screenful

Finally, moving the editing cursor will always adjust the displayed section so that the editing cursor is visible. Hence this can also be used to scroll around the editor in both horizontal and vertical fashions.

1.6.2 Names

At the left side of the editor window is a display containing the reading names and numbers. Each line consists of its number (negative numbers represent complemented readings) and its name. The bottom line is always CONSENSUS. Also on the bottom line is the current edit status. This is modelled on Emacs, and consists of one of ----, -%%- and -***, to symbolise "No unsaved edits made", "No edits made - editor is in read only mode", and "Unsaved edits made".

Pressing the right mouse button on a name will popup a menu containing operations to perform for that specific reading. These include Remove Reading (see Section 1.6.7.13 [Remove Reading], page 73), Set as reference sequence (see Section 1.6.12.1 [Reference sequences], page 83), Set as reference trace (see Section 1.6.12.2 [Reference traces], page 84), and List Notes (see Section 1.15.1 [Selecting Notes], page 159). Similar menus exist for the CONSENSUS name and for any editor status lines displayed.

The maximum length of a reading name is 40 characters. Additionally there are 7 characters taken up with the direction and number of a reading. By default the names display only shows 23 characters (enough to show 16 letters of a reading name). A horizontal scrollbar just above the reading names can be used to scroll the reading names. Note that the numbers and orientation are always visible. To change the width of the editor names display set the CONTIG_EDITOR.NAMES_WIDTH setting in your '.gaprc'. For example:

```bash
set_def CONTIG_EDITOR.NAMES_WIDTH 23
```

The foreground colour for the text reveals whether the trace for this reading is shown - a grey foreground indicates that the trace is visible. The background colour represents a user highlight and the disassembly mode. The default background colour is light grey (the same colour as the general editor background). Clicking the left mouse button on a reading name toggles the background of the name component of number-name pair to black. This is particularly useful for keeping track of an individual reading whilst scrolling the editor. As the editor scrolls an individual reading will move up and down the editor display. By highlighting this reading it becomes easy to track. The number component of the number-name pair is used to highlight readings that are to be disassembled. See Section 1.9.1.2 [Disassemble Readings], page 125. In this case the background is dark grey.

If the template display is in use, highlighting a reading name in the editor will select this reading in the template display (by marking it as bold). Similarly selecting a reading in the template display (left mouse button) will highlight the reading in the contig editor. Additionally the contig editor cursor is visible within the template display allowing the position of the editor to be controllable from the template display and connected plots (such as the quality plot). See Section 1.5.1 [Template Display], page 37.

The readings contained within the "readings" list are automatically highlighted when the editor starts. Toggling the highlighted names in the editor updates the "readings" list accordingly. See Section 1.14.1 [Special List Names], page 156.

Once an output list for the editor has been set, pressing the middle mouse button, or Alt left mouse button, on the names display has the same effect as the using the left button, except that it
adds (and never removes) the reading name to the specified list. See Section 1.6.8.13 [Set Output List], page 79. This is similar to using the left mouse button to add names to the "readings" list, except that it allows for multiple lists to be built up.

1.6.3 Editing

Editing can take up a significant portion of the time taken to finish a sequencing project. Gap4 has a selection of searches (see Section 1.6.6 [Searching], page 68) designed to speed up this process. The problems that require most attention are conflicts between good bases. Where base confidence values are present it should be unnecessary to edit all conflicting bases as, in general, this will amount to adjusting poor quality data to agree with good quality data, in which case the consensus sequence should be correct anyway.

Pads in the consensus should not be considered a problem requiring edits because it is possible to output the consensus sequence (from the main Gap4 File menu) with pads stripped out. Obviously poorly defined pads (a mixture of several pads and real bases) require checking in the same manner as other poorly defined consensus bases.

If you wish to check all base conflicts set the consensus algorithm to Frequency (see Section 1.11.5 [The Consensus Algorithms], page 140) and the consensus cutoff to 100. The consensus will then be a dash in all places where there is not a 100% agreement in the sequences. The "Next Problem" editor button will then step one at a time through each conflict.

1.6.3.1 Moving the editing cursor

Nearly all editing operations happen at the location of the editing cursor. This cursor appears as a solid block. The simplest mechanism of moving the cursor is simply use the left mouse button. Alternatively the following keys can be used.

<table>
<thead>
<tr>
<th>Key Sequence</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left arrow or Control b</td>
<td>Move left one base</td>
</tr>
<tr>
<td>Right arrow or Control f</td>
<td>Move right one base</td>
</tr>
<tr>
<td>Up arrow or Control p</td>
<td>Move up one base</td>
</tr>
<tr>
<td>Down arrow or Control n</td>
<td>Move down one base</td>
</tr>
<tr>
<td>Control a</td>
<td>Move editing cursor to start of used</td>
</tr>
<tr>
<td>Control e</td>
<td>Move editing cursor to end of used</td>
</tr>
<tr>
<td>Meta a</td>
<td>Move editing cursor to start of cutoff</td>
</tr>
<tr>
<td>Meta e</td>
<td>Move editing cursor to end of cutoff</td>
</tr>
<tr>
<td>Meta &lt;</td>
<td>Move editing cursor to start of contig</td>
</tr>
<tr>
<td>Meta &gt;</td>
<td>Move editing cursor to end of contig</td>
</tr>
</tbody>
</table>

The difference between the last four Control and Meta key combinations depends on whether "Cutoffs" is set. If it is, then "Control a" will move to the start of the used data for this reading and "Meta a" will move to the start of the cutoff data for this reading. Otherwise they both move to the same point (the used data start). Similarly for "Control e" and "Meta e". The action of these four key presses in the consensus line is simply to move to the start or end of the entire consensus sequence.

The cursor can be placed on any sequence data shown in the editor.

1.6.3.2 Editing Modes

The editor operates in two main edit modes - Replace and Insert. Replace allows a character to be replaced by another and Insert allows characters to be inserted. Replace is the default mode. The mode can be changed by pressing the button marked "Insert". The checkbox next to the button will be set (filled by a dark colour) when the mode is "Insert". By default these modes are restricted until the Edit Modes menu is used to change them.
The Edit Modes menu consists of a series of checkboxes and radiobuttons which control which editing options are enabled.

**Allow insert in read**
**Allow del in read**

Insertion or deletion within a reading will shift the sequence characters and so will alter their alignment. This is acceptable provided action is taken to correct it, by either shifting the reading or by inserting or deleting a base elsewhere. This functionality is disabled by default and is enabled by checking the appropriate checkbox. Note though that insertion and deletion of bases within the cutoff data will shuffle the cutoff data rather than the reading itself and hence will not break alignment. However this operation still requires the edit mode to be enabled.

**Allow insert any in cons**
**Allow del dash in cons**
**Allow del any in cons**

These operations control the editing actions allowed for the consensus. By default the only operations allowed are insertion and deletion of pads. This is because consensus editing is typically used for removing columns of pads where a single reading has been overcalled.

When editing at 100% disagreement, such cases will be dashes in the consensus, so "Allow del dash in cons" enables deletion of both dash and pads.

"Allow insert any in cons" and "Allow del any in cons" allow any column to be completely inserted or deleted. These are potentially dangerous actions, however the "Evidence for edits" options can detect such edits.

**Allow replace in cons**
Replacing a base in the consensus changes all of the bases in readings at this point that disagree with the typed base. The actual edit performed depends upon the "Edit by base type" and "Edit by confidence" radiobuttons.

**Allow reading shift**
To shift a reading place the cursor at the far left end of the reading. If cutoffs is set this should be the far left end of the cutoff data. Then typing space or delete will move the reading right or left respectively by one position. This operation is disabled by default.

**Allow transpose any**
Moving pads within a reading is often a useful procedure, and the 'movement' of a pad alone will not break the alignment. For this reason it is possible to move pads around without using insert/delete. Placing the cursor over a pad in a reading and pressing "Control l" or "Control r" will move that pad left or right one base. This operation will not work with the cursor on the consensus. Pad movement is allowed at all times. The selection of "Allow transpose any" allows any pair of adjacent characters to be swapped.

**Allow uppercase**
A rule often followed by users is to type all modifications in lower case which makes edited characters easier to see. The "Allow uppercase" checkbox controls whether this rule is enforced or not. By default "Allow uppercase" is checked which means that the rule is not enforced.
Edit by base type
Edit by confidence

These two selections are radiobuttons, and are mutually exclusive. They control the outcome when replacing bases in the consensus. When editing the consensus "Edit by base type" changes bases that disagree with the consensus to the base typed. "Edit by confidence" changes the confidence of disagreeing bases to 0. If the consensus quality cutoff value is greater than or equal to zero, characters with an accuracy value of 0 are ignored in the consensus calculation. That is, although the characters still appear in the reading, they are not used to calculate the consensus. In this way it is possible to maintain the original base calls for visual inspection, but get the correct consensus. Note that "Edit by confidence" will not work if the "frequency" consensus algorithm is in use (see Section 1.11.5 [The Consensus Calculation], page 140). If you wish to use "Edit by confidence", make sure that the quality cutoff is zero or higher, otherwise the frequency consensus algorithm will be used instead.

Mode set 1
Mode set 2

To make it easier to set the editing modes two user definable sets are available. By default these are as follows.

Mode set 1:
- Disallow insert in read
- Disallow del in read
- Disallow insert any in cons
- Allow del dash in cons
- Disallow del any in cons
- Disallow replace in cons
- Disallow reading shift
- Disallow transpose any
- Allow uppercase
- Edit by confidence

Mode set 2:
- Allow insert in read
- Allow del in read
- Allow insert any in cons
- Allow del dash in cons
- Disallow del any in cons
- Allow replace in cons
- Allow reading shift
- Disallow transpose any
- Allow uppercase
- Edit by confidence

Currently the only way of redefining these sets is to add lines to your '.gaprc' file. See Section 1.20.1 [Options Menu], page 176. The method is to define a list of 1s and 0s to specify the states in the order listed above. The two default sets are defined as follows.

```
set_def CONTIG_EDITOR.SE_SET.1 { 0 0 0 1 0 0 0 0 1 1 }
set_def CONTIG_EDITOR.SE_SET.2 { 1 1 1 1 0 1 1 0 1 1 }
```
1.6.3.3 Adjusting the Quality Values

Each base has its own quality value. Assembly will allow only values between 1 and 99 inclusive. A quality value of 0 means that this base should be ignored. A quality value of 100 means that this base is definitely correct and the consensus will be forced to be the same base type and will be given a consensus confidence of 100. If two conflicting bases both have a quality of 100 the consensus will be a dash with a confidence of 0.

Newly added bases or replaced bases are assigned their own quality values. By default these are both 100. The "Set Default Confidence" option in the settings menu allows these values to be changed.

Several keyboard commands are available to edit the quality value of an individual base. The '[]' and ']' keys set the quality to 0 and 100 respectively. To increment or decrement the confidence of a base by 1 use Shift plus the Up and Down arrow keys. To increment or decrement by 10 use Control plus the Up and Down arrow keys. The editor will beep if you reach quality 0 or 100. Finally note that quality values can also be made visible by the use of grey scales for the sequence background colour. See Section 1.6.8.9 [Show Quality], page 77.

1.6.3.4 Adjusting the Cutoff Data

The cutoff data is displayed by pressing the "Cutoffs" toggle at the top of the editor. The cutoff sequence will be displayed in grey. We call the boundary between the cutoff data and the used data the cutoff position. These positions can be shifted left or right for each end of the reading using the Meta Left-arrow and Meta Right-arrow keys respectively. As keyboards may not have a meta key, Control Left-arrow and Control Right-arrow also have the same effect. These key combinations adjust the cutoff positions by a single base at a time. They only work when the cursor is on the very first or very last "used" base, depending on which cutoff you wish to adjust.

If large changes are required the cutoffs can be "zapped" to new positions using the "<" and ">" keys. To use these, place the editing cursor to the position required (which may be within the cutoff data or the used data) and press the "<" key to set the left cutoff to the base between the cursor and the base leftwards of the cursor. Similarly ">" sets the right cutoff to the base between the cursor and the base rightwards of the cursor. Note that many keyboards have "<" and ">" above the "," and "." keys. In this case you will need to press Shift in conjunction with "," and "." to perform the operations.

1.6.3.5 Summary of Editing Commands

A brief summary of these editing operations and which (if any) edit modes are required can be seen below:

<table>
<thead>
<tr>
<th>Key</th>
<th>Location</th>
<th>Ins/Rep</th>
<th>Edit Mode</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>base/*</td>
<td>Reading</td>
<td>Replace</td>
<td>any</td>
<td>Change base</td>
</tr>
<tr>
<td>base/*</td>
<td>Reading</td>
<td>Insert</td>
<td>Insert in read</td>
<td>Change base</td>
</tr>
<tr>
<td>delete</td>
<td>Reading</td>
<td>both</td>
<td>Delete in read</td>
<td>Del base left &amp; move</td>
</tr>
<tr>
<td>Ctrl d</td>
<td>Reading</td>
<td>both</td>
<td>Delete in read</td>
<td>Delete base to left</td>
</tr>
<tr>
<td>delete</td>
<td>Read start</td>
<td>both</td>
<td>Readint shift</td>
<td>Shift left</td>
</tr>
<tr>
<td>space</td>
<td>Read start</td>
<td>both</td>
<td>Reading shift</td>
<td>Shift right</td>
</tr>
<tr>
<td>Ctrl l</td>
<td>Reading</td>
<td>both</td>
<td>any</td>
<td>Move pad left</td>
</tr>
<tr>
<td>Ctrl r</td>
<td>Reading</td>
<td>both</td>
<td>any</td>
<td>Move pad right</td>
</tr>
<tr>
<td>Ctrl l</td>
<td>Reading</td>
<td>both</td>
<td>Transpose any</td>
<td>Move base left</td>
</tr>
<tr>
<td>Ctrl r</td>
<td>Reading</td>
<td>both</td>
<td>Transpose any</td>
<td>Move base left</td>
</tr>
</tbody>
</table>
[ Reading both any Set quality to 0
]
Shift Up Reading both any Incr. quality by 1
Shift Down Reading both any Decr. quality by 1
Ctrl Up Reading both any Incr. quality by 10
Ctrl Down Reading both any Decr. quality by 10
< Reading both any Set left cutoff
> Reading both any Set right cutoff
Meta left Reading both any Adjust left cutoff
Meta right Reading both any Adjust right cutoff
*
Consensus both any Insert pad column
base Consensus Insert Insert any in cons Insert column
base Consensus Replace any Replace column
delete *
Consensus both any Delete column
delete-
Consensus both Del dash in con Delete column
delete any
Consensus both Del any in cons Delete column
Ctrl d
Consensus both Del dash/any Delete column

1.6.4 Selections

It is possible to highlight an area of a reading or the consensus sequence in preparation for performing some further action upon it. Such examples of actions are: creating annotations and aligning sequence. We call these highlighted areas "selections". They will be displayed as an underlined region.

The simplest way to make a selection is using the left mouse button. Pressing the mouse button marks the base beneath the cursor as the start of the selection. Then, without releasing the button, moving the mouse cursor adjusts the end of the selection. Finally releasing the button will allow normal use of the mouse again.

Sometimes we may wish to make a selection longer than is visible on the screen, or to extend our current selection. This can be done by using shift left mouse button to adjust the end of the selection. Hence we can mark the start of the selection using the left button, scroll along the contig to the desired position, and set the end using the shift left button.

The selection is stored in a "cut buffer". This allows for the usual "cut and paste" operations between applications, although the contig editor only supports this in one direction (as it is not possible to "paste" into the window). The mechanism employed for this follows the usual X Windows standard of using the middle mouse button (or Alt left mouse button). For example, to send a piece of sequence to a text editor (e.g. Emacs) mark the desired region using the left mouse button in the editor window and then press the middle button, or Alt left mouse button, whilst the mouse cursor is in the text editor window. The sequence will then be inserted into the text editor.

A quick summary of the mouse commands follows.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left button</td>
<td>Position editing cursor to mouse cursor</td>
</tr>
<tr>
<td>Left button (drag)</td>
<td>Mark start and end of selection</td>
</tr>
<tr>
<td>Shift left button</td>
<td>Adjust end of selection</td>
</tr>
<tr>
<td>Middle button (another window)</td>
<td>Copy selected sequence</td>
</tr>
<tr>
<td>Alt left button (another window)</td>
<td>Copy selected sequence</td>
</tr>
</tbody>
</table>

1.6.5 Annotations

Annotations (or tags) can be placed at any position on readings or on the consensus. They are usually used to record positions of primers for walking, or to mark sites, such as repeats or com-
pressions, that have caused problems during sequencing. They can also be used to contain feature table data as read from an EMBL format sequence file (see Section 2.1.3 [Reference sequences], page 190). Each annotation has a type such as "primer", a position, a length, a strand (forward, reverse or both) and an optional comment. Each type and strand has an associated colour that will be shown on the display. For information on searching for annotations see Section 1.6.6.4 [Searching by Tag Type], page 69, and Section 1.6.6.3 [Searching by Annotation Comments], page 69.

To create an annotation, make a selection and then select "Create Tag" from the contig editor commands menu. See Section 1.6.7 [The Commands Menu], page 71. This will bring up a further window; the "tag editor" (shown above). The "Type:" button at the top of the editor invokes a selectable list from which tag types can be chosen. See below.

Use this to select the desired type of annotation.

Next the strand of the annotation can be selected. This will be displayed as one of "<-——>", "<——" and "——>". The comment (the box beneath the buttons) can be edited using the usual combination of keyboard input and arrow keys. The "Save" button will exit the tag editor and create the annotation. To abandon editing without creating the annotation use the "Cancel" button.

To edit an existing annotation, position the editing cursor within a annotation and select "Edit Tag" from the commands menu. Once again the tag editor will be invoked and operates as before. Here "Save" will save the edited changes and "Cancel" will abandon changes.
Removing a annotation involves positioning the editing cursor within an annotation and selecting "Delete Tag" from the commands menu.

As usual, "undo" can be used to undo any of these annotation creations, edits and removals.

The Control-Q key sequence may be used to toggle the displaying of tags. Pressing it once will prevent all tags from being displayed in the editor. This is sometimes useful to see any colouring information underneath the tag. Pressing Control-Q once more will redisplay them.

### 1.6.6 Searching

The contig editor’s searching ability and its links to the consensus calculation algorithm are crucial in determining the efficiency with which contigs can be checked and corrected. The consensus is calculated "on the fly" and changes in response to edits. For editing, the most important search functions are those which reveal problems in the consensus whilst ignoring all bases that are adequately well determined. The default search type is therefore by consensus quality. By default this is done in the forward direction and for a quality value of 30, although this is configurable by changing the collowing lines in the gaprc file.

```
set_def CONTIG_EDITOR.SEARCH.DEFAULT_TYPE consquality
set_def CONTIG_EDITOR.SEARCH.DEFAULT_DIRECTION forward
set_def CONTIG_EDITOR.SEARCH.CONSQUALITY_DEF 30
```

Selecting "Next Search" brings up a window which can remain present during normal editor operation. The window allows the user to select the direction of search, the type of search, and a value to search on. The value is entered into a value text box, then pressing the "search" button performs the search. If successful, the cursor is positioned accordingly. An audible tone indicates failure. Pressing the "Cancel" button removes the search window. The search window is automatically removed when the contig editor is exited.

The "Cutoffs" button can be used to select whether or not searching should find matches within the cutoff data.

The Control-s key binding in the editor is equivalent to searching forward for the next match. The Escape Control-s key sequence performs a reverse search. Both key bindings will bring up the search window if it is not currently displayed.

As is described below, there are thirteen different search modes.
1.6.6.1 Search by Position

The presence of padding characters in the consensus can greatly alter the length of the sequence, and the positions of the bases along it. Positions can therefore be defined in two ways: those which include pads and those which do not. This option (termed a search!) moves the cursor to a specified position. The numeric position is specified in the value text box. Eg a value of "1234" causes the cursor to be placed at base number 1234 in the contig. Positioning within a reading is achieved by prefixing the number with the "@" character, eg "@123" positions the cursor at base 123 of the sequence in which the cursor lies. Relative positions can be specified by prefixing the number with a plus or minus character. Eg "+1234" will advance the cursor 1234 bases. If possible, the cursor is positioned within the same sequence. The direction buttons have no effect on this operation.

1.6.6.2 Search by Problem

This positions the cursor at the next place in the consensus sequence which is "*", "-" or "N". The search can be performed either forwards or backwards from the current cursor position. Obviously the characters appearing in the consensus depend on the selected consensus calculation algorithm and the thresholds set.

1.6.6.3 Search by Annotation Comments

This positions the cursor at the start of the next tag which has a comment containing the string specified in the value box. Only currently active tag types are searched. The search performed is a regular expression search, and certain characters have special meaning. Be careful when your string contains ",", ".", ".", ".", ".", ".", ".", ".", ".", ".", ".", ".," or ".,". The search can be performed either forwards or backwards from the current cursor position. Searching with an empty value will find all tags.

1.6.6.4 Search by Tag Type

This positions the cursor at the start of the next tag of the specified type. If the tag type is not active, the tag will be found and underlined but will remain invisible. To change the type, select from the menu that pops up when the mouse is clicked on the button labeled "Type:". The search can be performed either forwards or backwards of the current cursor position. To find all tags, use "Search by Annotation Comments", with an empty text box.

1.6.6.5 Search by Sequence

This positions the cursor at the start of the next segment of sequence that matches the value specified in the text box. The search is case insensitive, ignores pads, and can allow a specified number of mismatches. It is performed on the readings, not on the consensus sequence, and can be either forwards or backwards from the current cursor position.

1.6.6.6 Search by Quality

This positions the cursor at the next place in the consensus sequence where the consensus for each of the two strands disagree. Where there is only data for one strand the search will stop at every base. The search can be performed either forwards or backwards from the current cursor position.

1.6.6.7 Search by Consensus Quality

This positions the cursor on the consensus at the next position where the quality of the consensus is below a given threshold. The quality of the consensus is calculated by the consensus algorithm.
For this search the quality threshold should be entered into the value box and should be within the range of 0 to 100 inclusive.

### 1.6.6.8 Search by file

This steps the cursor through a set of positions specified in a file. The format for the positions in the file is one per line with each line consisting of a reading name, a position within that reading, and an optional comment. If a position is relative to the start of the contig rather than the start of any particular reading, then simply use the first reading in the contig. Positions that are beyond the ends for the reading are still valid, although the editing cursor is moved onto the consensus sequence.

The comment can consist of any string. Multiline comments are possible, but they must be written using `\n` in the comment string rather than an actual newline character (which would signify the start of the next record). The comment for the current position is displayed at the bottom of the editor search window in a text panel which is visible only when in the "search by file" mode.

Any record containing a reading name that is not in the current contig is silently ignored. This allows for a search file to have positions for all contigs. However at present there is no mechanism for stepping through an entire search file bringing up editors for each contig as required. This will be implemented in the future.

An example file follows.

```
xb63c7.s2 102
xb63c7.s2 30 A multi-\nline comment.
xb32a2.s1 56 Oligo, of length 12
xa17b1.r1 5714 Repeat from 5714 to 5780
```

### 1.6.6.9 Search by Reading Name

This positions the cursor at the left end of the reading specified in the value text box. If the value is prefixed with a hash sign it is assumed to be a reading number. Otherwise it is assumed to be a reading name. Eg 

```
#123
```

positions the cursor at the left end of reading number 123. 

```
a16a12.s1
```

positions at the start of reading a16a12.s1. If the value was 

```
a16
```

the cursor is positioned at the first reading which starts with "a16".

### 1.6.6.10 Search by Edit

This positions the cursor at the next place in the contig where an edit has been made. Edits include base insertions, deletions, replacements and confidence value changes. The search can be performed either forwards or backwards from the current cursor position.

### 1.6.6.11 Search by Evidence for Edit (1)

The Evidence for Edit (1) option checks edited bases to find bases in the consensus for which there is no evidence in the original readings. The definition of evidence is that at least one reading had this original base call. Currently this search operates only in the forward direction.

### 1.6.6.12 Search by Evidence for Edit (2)
The Evidence for Edit (2) option checks edited bases to find bases in the consensus for which there is no evidence in the original readings. The definition of evidence is that at least one reading from each strand had this original base call. Currently this searches only in the forward direction.

1.6.6.13 Search by Discrepancies

This finds positions where two or more bases are above a particular quality level, but in disagreement. The quality threshold is given in the value box and should be within the range of 0 to 100 inclusive.

1.6.7 The Commands Menu

The Commands menu is available by either pressing the Commands button at the top of the contig editor window, or by pressing the Control key and the left mouse button, or by pressing right mouse button with the mouse cursor anywhere within the sequence display section of the contig editor. A menu will be revealed containing the following options (which are described in greater detail below).

1.6.7.1 Search

This Contig Editor Commands menu function Performs a search. See Section 1.6.6 [Searching], page 68.

1.6.7.2 Create Tag

This Contig Editor Commands menu function Creates an annotation. See Section 1.6.5 [Annotations], page 66.

1.6.7.3 Edit Tag

This Contig Editor Commands menu function Edits an annotation. See Section 1.6.5 [Annotations], page 66.

1.6.7.4 Delete Tag

This Contig Editor Commands menu function Removes an annotation. See Section 1.6.5 [Annotations], page 66.

1.6.7.5 Save Contig

This Contig Editor Commands menu function writes any edited data to disk. The undo history is cleared and it is no longer possible to quit and abandon these saved changes. The Control-x followed by Control-s will also save the contig editor in the same manner as the Save command.

1.6.7.6 Dump Contig to File

This Contig Editor Commands menu function outputs the current contig, as currently shown (e.g. with status lines) to a file. The user can select the region to dump, the length of each line, and the file name to use. The sequence names can be up to 40 characters, but often projects do not use the full length. To avoid wasted space in the output the number of columns to use for sequence names can be adjusted.
1.6.7.7 Save Consensus Trace

This Contig Editor Commands menu function produces a trace file for the consensus sequence by averaging the traces of the readings. The command brings up a dialogue containing controls to specify the filename, the consensus start and end positions, the strand, and whether to use matching reads.

As the trace of a reading is dependent on the direction it was read, the consensus trace can be computed from all the reads in either the forward or reverse directions, but not both at once. When the "Use only matching reads" toggle is set to "Yes" only the readings of the correct strand that have the same base call as the consensus sequence are used. The option is useful for producing wild-type trace files for a mutation analysis project.

1.6.7.8 List Confidence

This Contig Editor Commands menu function operates in a very similar manner to the main Gap4 List Confidence command (see Section 1.11.6 [List Confidence], page 145), except that it only operates on the current contig, and it uses the current editor consensus confidences rather than the ones saved to disk. It displays a dialogue requesting a range within the contig and a question asking if only summary of the results is required.

Pressing OK or Apply will add to the editor information line a count of the expected number of errors and the error rate. If the "Only update information line" question was answered "No" then the full frequency table will also be output. It will appear in the main text output window in the same format as the "List Confidence" command in the main Gap4 View menu. The Apply button can be used to calculate the number of errors without removing the dialogue.

It is often the very ends of contigs (which are generally low coverage and bad quality) that have most of the errors, and so it is sometimes useful to set a range which includes all of the contig except for around 1000 bases from each end.

1.6.7.9 Report Mutations

This Contig Editor Commands menu function is used to produce a list of all the bases annotated with mutation tags (or those bases which differ from the consensus/reference sequence). If the tags or differences are within segments of sequence which are also annotated with EMBL feature table CDS records, the report will include data describing its effect. The report, which can be sorted by sequence or position, includes the reading names, mutation positions relative to the reference sequence, the actual change, its effect, and the evidence. An example is shown below.

```plaintext
001321_11aF 33885T>Y (silent F) (strand - only)
001321_11aF 34407G>K (expressed E>[ED]) (strand - only)
001321_11cF 35512T>Y (silent L) (double stranded)
001321_11cF 35813C>Y (expressed P>[PL]) (double stranded)
001321_11dF 36314A>R (expressed E>[EG]) (double stranded)
001321_11eF 36749A>R (expressed K>[KR]) (double stranded)
001321_11eF 37313T>K (noncoding) (strand - only)
000256_11eF 36749A>G (expressed K>R) (double stranded)
```
Here the first record is for reading 001321_11aF, position 33885, T changed to T and C (i.e. is heterozygous) to produce no amino acid change, with evidence coming only from the complementary strand. The last record is for reading 000256_11eF, position 36749, A changed to G, producing an amino acid change K to R, with evidence from both strands of the sequence. The penultimate record denotes a heterozygote in a noncoding region.

1.6.7.10 Select Primer

This Contig Editor Commands menu function allows the user to employ the primer selection algorithm OSP to find primers for sequencing experiments. See Section 1.6.10 [Searching for Primers], page 80.

1.6.7.11 Align

This Contig Editor Commands menu function performs a sequence alignment between the currently selected segment of a reading and the consensus sequence. It provides a simple way of extending the visible part of a reading to use its hidden data, which is often useful to double strand a short section of consensus without the need to perform further experiments. On a sequence, highlight the cutoff data to align along with a small section of the good quality non-cutoff data. Then select the align command and adjust the cutoff point as desired. Pads are inserted in the consensus and readings as necessary, although pads will not be inserted in the cutoff data of other sequences.

1.6.7.12 Shuffle Pads

One weakness of the alignment strategy used in the current shotgun assembly algorithm is that padding characters are not always aligned with one another. This Contig Editor Commands menu function attempts to align padding characters using a very simply strategy. It does not solve all pad alignment problems but is a useful first step during cleaning-up operations. After shuffling, all columns consisting entirely of pads are removed.

1.6.7.13 Remove Reading

This Contig Editor Commands menu function marks a reading for subsequent removal. See Section 1.6.9 [Removing readings from the contig], page 79

1.6.7.14 Break Contig

This Contig Editor Commands menu function breaks the contig so that the reading underneath the editing cursor is the left end of a new contig. In order to perform this operation all edits are saved automatically first. Once saved these edits cannot be undone. This operation is identical to the Break Contig command in the main menu. See Section 1.9.1.1 [Break Contig], page 124.

1.6.8 The Settings Menu

The purpose of this menu is to configure the operation of the contig editor, including the consensus calculation, the active tags and the status lines. Settings can be saved using the "Save settings" button. Settings for the following options can be changed.

- Status Line
- Trace Display
- Consensus algorithm
- Highlight Disagreements
The contig editor can display several additional text lines underneath the consensus sequence. This "status" data is of textual form and can provide additional information about the data displayed above. Currently, there are two forms of status line available. These are "Strands" and "Translate Frame". Both status line types update automatically as edits are made that change the consensus.

The status line menu is accessed by cascading off the settings menu. It contains the following.

- Show Strands
- Translate using feature tables
- Translate frame 1+
- Translate frame 2+
- Translate frame 3+
- Translate frame 1-
- Translate frame 2-
- Translate frame 3-
- Translate ± frames
- Translate - frames
- Translate all frames
- Remove all

"Show Strands" creates a single line consisting of the +, -, = and ! characters. These indicate: positive strand only, negative strand only, both strands (in agreement) and both strands (in disagreement) respectively.

The frame translation status lines provide translations in each of the six available reading frames. Alternatively, using the "Translate using feature tables", only segments described in CDS records will be translated. The CDS records are those contained in the reference sequence. Translations can be displayed in either the single character or the three character amino acid codes.

Pressing the right mouse button on the 'name' segment of the status line (on the left hand side) pops up a menu. The commands available may depend on the type of the status line chosen, however currently it will always only contain the "Remove" command. This, as expected, removes the status line from the display. To remove all status lines use the "Remove all" command from the "Status Line" cascading menu.

Note that the data in the status line cannot be cut and pasted, modified or searched; it is not possible to move the cursor into these lines.
1.6.8.2 Trace Display

The editor trace display has two configurations specific to its own usage.

- Auto-display Traces
- Auto-diff Traces

When switched on, auto-display traces will direct certain searches to automatically display relevant traces to aid in solving problems. This works in conjunction with most appropriate searches. The traces chosen to solve the "problem" will, by default, be the best trace from each strand which agrees with the consensus (which is calculated at a low consensus cutoff) and the best trace from each strand which disagrees with the consensus. This selection of traces may be adjusted by modifying the CONTIG_EDITOR.AUTO_DISPLAY_TRACES_CONF configuration variable. The default setting of this is "+ - +d -d". Each of the space separated elements in this string corresponds to a trace file to choose. If one cannot be found, then it is ignored. The order listed here is the order in which they will be displayed in the trace window. The complete list of available trace specifiers is:

+   Best +ve strand trace agreeing with consensus
+p  Best +ve strand dye-primer trace agreeing with consensus
+t  Best +ve strand dye-terminator trace agreeing with consensus
-   Best -ve strand trace agreeing with consensus
-p  Best -ve strand dye-primer trace agreeing with consensus
-t  Best -ve strand dye-terminator trace agreeing with consensus
d  Best trace disagreeing with consensus
+d  Best +ve strand trace disagreeing with consensus
-d  Best -ve strand trace disagreeing with consensus
+2  Second best +ve strand trace agreeing with consensus
+2p Second best +ve strand dye-primer trace agreeing with consensus
+2t Second best +ve strand dye-terminator trace agreeing with consensus
-2  Second best -ve strand trace agreeing with consensus
-2p Second best -ve strand dye-primer trace agreeing with consensus
-2t Second best -ve strand dye-terminator trace agreeing with consensus
2d  Second best trace disagreeing with consensus
+2d Second best +ve strand trace disagreeing with consensus
-2d Second best -ve strand trace disagreeing with consensus

Once this is activated, whenever the user double clicks on a base in the editor sequence display, not only is the reading's trace displayed, but also its designated reference trace plus the difference between them. If its complementary reading is available, its trace and reference trace and their differences are also displayed.
As is shown in the figure below, it is also possible to set the trace difference display to use positive and negative references.

For further information about mutation detection, see Section 2.1 [Search for Mutations], page 185.

1.6.8.3 Consensus Algorithm

This allows selection of the consensus algorithm to use within the Contig Editor. Like the consensus and quality cutoff parameters, it is local to the specific editor being used. The main Consensus algorithm option should be used to globally change the algorithm being used. See Section 1.20.2 [Consensus Algorithm], page 177.
1.6.8.4 Highlight Disagreements

This toggles between the normal sequence display (showing the current base assignments) and one in which those assignments that differ from the consensus are highlighted. It makes scanning for problems by eye much easier.

Several modes of highlighting are available: "By dots" will only display the bases that differ from the consensus, displaying all other bases as full stops. The base colours are as normal (ie reflecting tags and quality). "By foreground colour" and "By background colour" displays all base characters, but colours those that differ from the consensus. This allows easier visual scanning of the context that a difference occurs in, but it may be wise to disable the displaying of tags. Finally the "Case sensitive" toggle controls whether upper and lower case bases of the same base type should be considered differences.

1.6.8.5 Compare Strands

This toggles the consensus calculation routine between treating both strands together or independently. In the independent case any difference between the two strands is shown in the consensus as a ‘-’. Hence these clashes are found as problems by the "Search by problem" option.

1.6.8.6 Toggle auto-save

Selecting auto-save toggles the auto save feature. Initially this is turned off each time the contig editor is invoked. Once toggled the adjacent checkbox will be set to indicate the feature is enabled and the contig will be saved. From that point onwards the contig editor will write its data to disk every 50 edits. Each time an auto save is performed it is announced in the output window. Saving more frequently can still be performed manually by using "Save Contig".

Unlike "saves" made using the manual "Save Contig" command, the "Undo" button will allow the user to undo edits regardless of when the last auto save occurred.

1.6.8.7 3 Character Amino Acids

By default, the codon translation within the status line displays single character amino acid codes. Selecting "3 Character Amino Acids" will toggle the status line to display three character amino acid codes.

1.6.8.8 Group Readings by Templates

When set, this forces readings from the same template to be displayed vertically adjacent to one another. The real "X coordinate" of the reading is not changed, so this has no effect for readings from the same template that are never visible together on the screen at the same time. The purpose of the function is to provide a method for grouping batches of readings together, for example when looking for mutations. They do not need to be from the same template, but can be grouped using the template name.

Note that when this mode is in use the operation of the up and down arrow keys incorrectly affects the editing cursor. Using the mouse to position the editing cursor still works correctly.

1.6.8.9 Show Reading and Consensus Quality

When the quality cutoff value is 0 or higher and either of the "show reading quality" or "show consensus quality" toggles is set, the background for bases is shaded in a grey level dependent on their quality. There are ten levels of shading with the darkest representing poor data and the lightest representing good data. So with the quality cutoff set to 50, all bases with a quality of
less than fifty are shown with a red foreground and a dark grey background, bases with quality just above 50 will have the darkest grey background, and bases with a quality of 100 will have the lightest background. When tags are present the background colour is that of the tag rather than the quality.

The colours used are adjustable by modifying your ".gaprc" file. The defaults are shown below.

```
set_def CONTIG_EDITOR.QUAL0_COLOUR   "#494949"
set_def CONTIG_EDITOR.QUAL1_COLOUR   "#696969"
set_def CONTIG_EDITOR.QUAL2_COLOUR   "#898989"
set_def CONTIG_EDITOR.QUAL3_COLOUR   "#a9a9a9"
set_def CONTIG_EDITOR.QUAL4_COLOUR   "#b9b9b9"
set_def CONTIG_EDITOR.QUAL5_COLOUR   "#c9c9c9"
set_def CONTIG_EDITOR.QUAL6_COLOUR   "#d9d9d9"
set_def CONTIG_EDITOR.QUAL7_COLOUR   "#e0e0e0"
set_def CONTIG_EDITOR.QUAL8_COLOUR   "#e8e8e8"
set_def CONTIG_EDITOR.QUAL9_COLOUR   "#f0f0f0"
set_def CONTIG_EDITOR.QUAL_IGNORE   "#ff5050"
```

1.6.8.10 Show edits

When set, any change between the bases displayed and the original sequence held in the trace files is shown by changing the background colour of the changed base. The detection of these edits depends on the quality values and the "original position" data. Hence the traces do not need to be present in order to detect edits. The colour of the bases reflects the type of change found. The colours are adjustable by editing the ".gaprc" file. The following table lists the colour, gaprc variable name and the meaning.

- **red** CONTIG_EDITOR.EDIT_DEL_COLOUR — Deletion
- **pink** CONTIG_EDITOR.EDIT_BASE_COLOUR — Base change or insertion
- **green** CONTIG_EDITOR.EDIT_PAD_COLOUR — Padding character
- **purple** CONTIG_EDITOR.EDIT_CONF_COLOUR — Confidence value

1.6.8.11 Show Unpadded Positions

The ruler at the top of the contig editor displays every tenth base number in the consensus sequence. Without "show unpadded positions" enabled any character in the consensus is counted, including padding characters. If "show unpadded positions" is enabled the ruler will only count non pad ("*" ) characters. Please note that this may considerably slow down the editor on large databases as the full consensus needs to be calculated in order to plot the ruler. If you just need to obtain the occasional unpadded position it is better to press the Enter key or to use the "unpadded position" search.

1.6.8.12 Set Active Tags

"Set Active Tags" allows configuration of which tag types should be displayed within the editor. Note that searches for tag annotations will only examine active tags, but searching for a specific tag type will find tags even when tags of this type are not visible. In this situation the tag will still be invisible, but as usual the tag location will be underlined. This option is particularly useful for exploring cases where a section of sequence has many overlapping tags. An alternative to using this dialogue is using the Control-Q key, which toggles the display of active tags.
1.6.8.13 Set Output List

"Set output list" pops up a dialogue asking for a list name to be used when outputting reading names (see Section 1.14 [Lists], page 156). Once an output list has been specified, pressing the middle button, or Alt left mouse button, on a reading name will add the name to the end of list. Note that selecting the same name more than once will add the name to the list more than once. The list is never cleared by the editor. This allows multiple editors to append to the same list. If required, use the list menu to clear the list.

1.6.8.14 Set Default Confidences

Replacing bases or inserting new bases in the editor can assign new confidence values to those bases. The default setting is to set these confidence values to 100 which has the effect of forcing the consensus to be that base. The "Set Default Confidences" dialogue allows these default values to be changed. The allowable range of confidence values for a base is from 0 to 100 inclusive. The dialogue also allows selection of confidence -1. This tells the editor to not change the confidence value. When replacing a base this keeps the same confidence value of the base that is being replaced. When inserting a base this uses the average of the confidence value of the two surrounding bases.

1.6.8.15 Set or unset saving of undo

Storing the undo information takes up a great deal of computer memory and slows down the alignment algorithm. Particularly when using the Join Editor for very large overlaps (e.g. after copying batches of readings from one database to another), it can be useful to turn off the saving of undo information. For this reason the settings menu contains an option to turn off (or on) the saving of undo information.

1.6.9 Removing Readings

It is often desirable to completely remove a reading from a contig. When not using the editor this is typically performed using the Disassemble Readings function. See Section 1.9.1.2 [Disassemble Readings], page 125. When using the editor, the "Remove Reading" option on the editor commands menu performs a similar task.

The command marks the reading underneath the editing cursor to be removed once the editor is quitted. Until then, the reading number in the names section of the display is shown with a dark grey background. The reading will also not be used in the calculation of the consensus. Thus, if all readings at a particular section of consensus are marked for removal the consensus sequence will be shown as dashes. Selecting the "Removing Reading" command again with the editing cursor on a reading already marked for removal will cancel the removal request. The keyboard command of Control-H may also be used as a shortcut to the "Removing Reading" command.

Once the editor has been quitted you will be asked whether you wish to disassemble the marked readings. Answering "No" will simply quit the editor as normal without removing any readings. Answering "Yes" will bring up the usual "Disassemble Readings" dialogue. The options here allow removal of all readings from this contig, or non-crucial only. A crucial reading is one that will cause this contig to be broken into two or more segments. A choice is also given as to whether the readings should be completely removed from this database, or for each reading to be placed in its own contig. Pressing "OK" now will remove the readings from the contig, breaking the contig if necessary, and will quit the editor. Pressing "Cancel" will close the "Disassemble Readings" dialogue without making any changes and will not quit the editor.

At any time, quitting the editor and not disassembling the readings will leave a List (see Section 1.14 [Lists], page 156) named "disassemble" containing the readings marked for removal. These may then be disassembled at a later stage if necessary. However the list will only be available until
the next editor is quit (at which stage that editor will create its own, possibly blank, disassemble list), so make a copy if necessary.

1.6.10 Primer Selection

The oligo selection engine is the one used in the program OSP. It is described in Hillier, L., and Green, P. (1991). "OSP: an oligonucleotide selection program," PCR Methods and Applications, 1:124-128. Oligo selection is a complex operation. The normal mode of use is outlined below:

1. Open the oligo selection window, by selecting "Select Primer" from the contig editor commands menu.
2. Position the cursor to where you want the oligo to be chosen. While the oligo selection window is visible, you will still have complete control over positioning and editing within the contig editor.
3. Indicate the strand for which you require an oligo. This is done by toggling the direction arrows ("—>" or "<——").
4. Press the "Find Oligos" button to find all suitable oligos (see the "Parameters" subsection below for further information on controlling this procedure). Information for the closest suitable oligo to the cursor position is given in the output text window. In the contig editor the position of the oligo is marked by a temporary tag on the consensus. The window is recentered if the oligo is off the screen.
5. If this oligo is not suitable (it may have been used before, and failed) the next closest oligo can be viewed by pressing "Next".
6. Suitable templates are automatically identified for the currently displayed oligo (see the "Template selection" subsection below). By default, the template is that closest to the oligo site. If the choice is not suitable (it may be known to be a poor quality template, say) another can be chosen from the "Choose from" pull-down menu. Templates that do not appear on the menu can be specified by selecting simply typing their name in the "Template name" entry box. However, the template must be on the correct strand and be upstream of the oligo.
7. A tag can be created for the current oligo by pressing the button "Accept". The annotation for this tag holds the name of the template and the oligo primer sequence. There are fields to allow the user to specify their own primer name ("serial#") and comments ("flags") for this tag. An example of oligo tag annotation:

```
serial#=
template=a16a9.s1
sequence=CGTTATGACCTATATTTTGTATG
flags=
```
8. The oligo selection window is closed when "Accept" or "Quit" is selected.

1.6.10.1 Parameters

The parameters controlling the selection of oligos can be changed by pressing the "Edit parameters" button. This invokes a dialogue box which allows the specification of further parameters.

By default, the oligos are selected from a window that extends 40 bases either side of the cursor. The size and location of this window relative to the cursor position can be changed in the "Edit parameters" window.

Primer constraints can be specified by melting temperature, length and G+C content.

In gap4 oligos are ranked according to their overall score, where the best oligos have lower scores.
1.6.10.2 Template selection

For simplicity, each reading is considered to represent a template. In practice, many readings can be made off the same template. Suitable templates that are identified are those that satisfy all of the following conditions:

1. are in the appropriate sense,
2. have 5' ends that start upstream of the oligo,
3. are sufficiently close to the oligo to be useful.

This last criterion relates to the insert size for the templates used for sequencing and the average reading length. A template is considered useful if a full reading can be made from it, taking into account both of these factors. The default insert size is 1000 bases (although the size range should be included in the experiment file for each reading, and hence the default would not be required), and the default average reading length is 400 bases. These values can be changed in the "Edit parameters" window.

1.6.11 Traces

The original trace data from which the readings were derived can be displayed by double clicking (two quick clicks) with the left or middle mouse button on the area of interest. Control t has the same effect. The trace will be displayed centered around the base clicked upon and the name of the reading in the contig editor will be highlighted. Double clicking on the consensus displays all the readings covering that position. Double clicking on a reading which already has its trace displayed will cause the corresponding trace to be surrounded by a red border.

Moving the mouse pointer over a base causes the display of an information line at the bottom of the window. This gives the base type, its position in the sequence, and its confidence value.

There are two forms of trace display which are selected using the "Compact" button at the top of the Trace display. The compact form differs by not showing the Info, Diff, Comp. and Cancel buttons at the left of each trace.

Note that gap4 does not store the trace files in the project database: it stores only their names and reads them when required. However it does not know which directory they are stored in, unless this is specified using the "Trace File Location" option (see Section 1.20.8 [Trace File Location], page 179).
The picture shows an example of three displayed traces. The reading number, together with the direction of the reading (+ or -) and the chemistry by which it was determined, is given at the top left of each sub window. The chemistry information is found from comments in the experiment file. 'uf' and 'ur' indicate universal forward and universal reverse, 'cf' and 'cr' indicate custom forward and custom reverse, and 'p' and 't' indicate primer and terminator. There are four buttons ('Info', 'Diff', 'Comp.' and 'Cancel') below this information, and X and Y scale bars to the right.

The "Info" button will display a window like the one shown at the bottom right of the picture. This contains the comments from the relevant SCF file.

The "Diff" buttons are used to produce a new trace showing the differences between two existing traces. To use this, press "Diff" in any window. The mouse cursor then changes to a cross symbol. Pressing the left mouse button anywhere on another trace that has a "Diff" button will create the difference trace. Any other button cancels the operation. The algorithm used for computing the difference trace is adjustable by parameters in the settings menu (see Section 1.6.8.2 [Trace Display Settings], page 75). The trace differencing was originally designed for visual inspection of suspected mutations Bonfield, J.K., Rada, C. and Staden, R. Automated detection of point mutations using fluorescent sequence trace subtraction. Nucleic Acids Res. 26, 3404-3409 (1998).

The "Comp." button complements the displayed trace. If the sequence in the editor has been complemented then the trace will automatically be shown in the complementary sense. This button may be used to toggle the complementarity.

The "Cancel" button will remove the trace.

The X and Y scale bars zoom the trace in the appropriate direction. The default Y scale is to fit the highest peak on the screen without clipping. When the "Show confidence" check-button is selected, the confidence value for each base call will be displayed as a histogram, overlayed on the trace displays. The base confidence values are not computed by gap4, but rather are read from the SCF file which is assumed to have been generated by one of the programs that compute confidence values (such as phred, ATQA or eba). When ABI files are in use, confidence values may not be shown.

The trace is displayed on the right with a scrollbar directly below it and with the reading name in the top left corner. The vertical line seen in these three traces shows the location of the editing cursor in the contig editor window. The lock button on the trace displays ties the editing cursor movement to the scrolling of the trace windows and vice versa.

The trace display supports the display of up to four columns of traces, and can display any number of rows. The number of columns and rows can be configured and saved using the buttons.
at the top of the window. A scrollbar is provided if there are more traces to display than can be viewed with the current settings.

To modify the number of traces that are shown at any one time, and the heights of these, add (and edit) the following lines to your `$HOME/.gaprc` file.

```
set_def TRACE_DISPLAY.ROWS 5
set_def TRACE_DISPLAY.COLUMNS 2
set_def TRACE_DISPLAY.TRACE_HEIGHT 150
```

New traces are always added to the bottom right of the window.

Resizing the width of the trace window, moving the trace window and adjusting the X magnification are all remembered and used when bringing up new trace displays.

The "Close" button at the top right of the Trace Display removes the Trace Display.

An example of the "Compact" form of the trace display is shown below.

![Trace display](image)

**1.6.12 Reference Sequence and Traces**

Reference sequences can be used to provide standard base numbering for contigs. If they have feature table tags which contain CDS records the Contig Editor can use them to translate only the known coding segments, and in the correct reading frame. The primary use for reference sequences is in mutation detection.

Reference Traces provide standards, both positive and negative for mutation detection by trace comparison.

**1.6.12.1 Reference sequences**

In order to put readings and their mutations in context we use a reference sequence and feature table. This enables mutations to be reported using positions defined by the reference sequence, and also allows the effect of the mutations to be noted. To facilitate this gap4 is able to store entries from the EMBL sequence library complete with their feature tables. These feature tables are converted to gap4 database annotations (tags), which means that they can be selectively displayed in the template display and editor, and used to translate only the exons (in the correct reading
1.6.12.2 Reference traces

The preferred way of assigning reference traces to readings is by use of "naming conventions"; that is to have a simple set of rules which control the names given to the trace files. It can be seen in the figures showing the editor that forward and reverse readings from the same patient have names with a common root but which end either F or R. This both ties the two together (so the software knows which is the corresponding complementary trace when the user double clicks on a reading) and also enables the association of readings and their reference traces. Once a convention has been adopted the rules can be defined for pregap4 by loading them via the "Load Naming Scheme" option in its File menu (see Section 3.8 [Pregap4 Naming Schemes], page 236). For any batch of readings the reference traces are defined within pregap4's "Reference Traces" module.

Within the Contig Editor reference traces can be set by right clicking on their names in the editor. When this is done a menu will popup. This allows the user to select whether the trace is to be used as a positive or negative control.

1.6.13 The Editor Information Line

The very bottom line of the editor display is text line used by the editor to display pieces of useful information. Currently this gives information on individual bases, readings, the contig, and tags, as the mouse is moved over the appropriate object. For bases (in both readings and the consensus) this information is only displayed when a mouse button is pressed. The left mouse button displays with format `BASE_BRIEF_FORMAT1` and format `BASE_BRIEF_FORMAT2` is displayed when pressing 'Enter'. By default the only difference between the two is that 'Enter' will display the "unpadded position" of a base in the consensus - i.e. its position in the consensus after pads have been removed. The contents and format of the information displayed is completely configurable by adding the relevant definitions to your `.gaprc` file. The defaults are as follows.

```bash
set_def READ_BRIEF_FORMAT \{Reading:%n(#%Rn) Length:%l(%L) Vector:%Tv Clone:%Cn \Chemistry:%a Primer:%P %c}
set_def CONTIG_BRIEF_FORMAT \{Contig:%n(#%Rn) Length:%l %c}
set_def TAG_BRIEF_FORMAT \{Tag type:%t Direction:%d Comment:%.100c}
set_def BASE_BRIEF_FORMAT1 \{Base confidence:%c (Probability %p) Position %P}
set_def BASE_BRIEF_FORMAT2 \{Base confidence:%c (Probability %p) Position %P \Unpadded position %U}
```
Tag information is shown when the mouse is moved over an annotation. Read information is shown when the mouse is moved over the reading name in the names section of the display. Contig information is displayed when the mouse is moved over the "Consensus" line in the names display. If you wish to leave the contig editor window without changing the information line contents as the mouse moves over other information press and hold the Shift key whilst moving the mouse. This disables the automatic highlighting. The same mechanism also works for other windows (such as the template display).

The general style of the formats is the string to display with particular strings substituting % characters. For instance in the reading format %n is substituted by the reading name. The general format of a % expansion is:

- A percent sign.
- An optional minus sign to request left alignment of the information. When displaying information in a specific field with where that data does not fill the entire space allowed the information will, by default, be right justified. Adding a minus character here requests left justification.
- An optional minimum field width. This is a decimal number indicating how much space to leave for this information.
- An optional precision for numbers or maximum field width for strings. This is given as a fullstop followed by a decimal number.
- An optional 'R' to specify Raw mode. This changes the meaning of many (but not all) of the expansion requests to give a numerical representation of the data. For example %n is a reading name and %Rn is a reading number.
- The expansion type itself. This is either one or two letters. See below for full details of their meanings.

To programmers this syntax may seem very similar to printf. This is intentional, but do not assume it is the same. Specifically the print syntax of %#, %+ and %0 will not work.

### 1.6.13.1 Reading Information

Example output is `Reading:xc04a1.s1(#74) Length:295(474) Vector:m13mp18 Clone:test Chemistry:primer Primer:forward universal`.

<table>
<thead>
<tr>
<th>%</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%n</td>
<td>Reading name. Raw mode: number</td>
</tr>
<tr>
<td>%#</td>
<td>Reading number</td>
</tr>
<tr>
<td>%t</td>
<td>Trace name</td>
</tr>
<tr>
<td>%p</td>
<td>Position</td>
</tr>
<tr>
<td>%l</td>
<td>Clipped length</td>
</tr>
<tr>
<td>%L</td>
<td>Total length</td>
</tr>
<tr>
<td>%s</td>
<td>Start of clip</td>
</tr>
<tr>
<td>%e</td>
<td>End of clip</td>
</tr>
<tr>
<td>%S</td>
<td>Sense (whether complemented) - &quot;+&quot; or &quot;;&quot;. Raw mode: 0/1</td>
</tr>
<tr>
<td>%a</td>
<td>Chemistry - &quot;primer&quot; or &quot;terminator&quot;. Raw mode: 0/1</td>
</tr>
<tr>
<td>%d</td>
<td>Strand - &quot;+&quot; or &quot;;&quot;. Raw mode: 0/1</td>
</tr>
<tr>
<td>%P</td>
<td>Primer - &quot;unknown&quot;, &quot;forward universal&quot;, &quot;reverse universal&quot;, &quot;forward custom&quot; or &quot;reverse custom&quot;. Raw mode: 0/1/2/3/4</td>
</tr>
</tbody>
</table>
%Tn    Template name. Raw mode: template number
%T#    Template number
%Tv    Template vector. Raw mode: template vector number
%Ti    Template insert size
%Cn    Clone name. Raw mode: clone number
%C#    Clone number
%Cv    Clone vector. Raw mode: clone vector number
%c    User defined text, taken from the the first note of type INFO.

1.6.13.2 Contig Information

Example output is Contig:xc04a1.s1(#74) Length:1316.

%-%    Single % sign
%n    Left most reading name. Raw mode: reading number
%s    (As %n)
%e    Right most reading name. Raw mode: reading number
%#    Contig number
%l    Contig length
%E    Expected number of errors (can be slow on large contigs)
%c    User defined text, taken from the the first note of type INFO.

1.6.13.3 Tag Information

Example output is Tag type:OLIG Direction:- Comment:"template=xc04a1 sequence=
CGATTGCAGAATAAGACG".

%-%    Single % sign
%p    Tag position
%d    Tag direction - "+", "-" or "=". Raw mode: 0/1/2
%t    Tag type (always 4 characters)
%l    Tag length
%n    Tag number (0 if unknown)
%c    Tag comment

1.6.13.4 Base Information

Example output is Base confidence:13 (Probability 0.954020) Position 3805 Unpadded position 3678.

%-%    Single % sign
%c    Confidence value (phred style)
%p    Confidence value (as probability)
%P    Padded consensus base position
%U    Unpadded consensus base position
1.6.14 The Join Editor

Contigs are joined interactively using the Join Editor. This is simply a pair of contig editor displays stacked above one another with a "differences" line in between. Note that it is essential to align the contigs over the full length of their overlap. It is much more difficult to achieve this after a join has been made, and until the alignment is correct, the consensus sequence will be nonsense.

The few differences between the Join Editor and the Contig Editor can be seen in the figure below. Otherwise all the commands and operations are the same as those for the Contig Editor. One difference is the Lock button. When set (as it is in the illustration) scrolling either contig, by using the scrollbar or the four movement buttons, will also scroll the other contig.

The Align button aligns the overlapping consensus sequences and adds pads. The alignment routine assumes that the two contigs are already in approximately the right relative position (as they are immediately after the Join Editor has been invoked from Find Internal Joins, or Find Repeats). If they are not they must be positioned manually before using the Align button.

It should be noted that each of the pair of editors comprising the Contig Editor maintains its own undo history, and using Align is likely to add to both undo histories. Hence, to undo the results of the Align command the Undo button in both editors must be used.

Note also that storing the undo information takes up a great deal of computer memory and slows down the alignment process. For this reason the settings menu contains an option to turn off (or on) the saving of undo information. When aligning very long overlaps it is advisable to turn off the undo saving.

When "Join/Quit" is pressed a dialogue box is displayed containing the percentage mismatch of the overlap, and asking if the join should be made. For joins above a certain level of mismatch (20 percent by default) a second confirmation is required.

1.6.15 Using Several Editors at Once

Several editors can be used simultaneously, even on the same contig. In the latter case, it is useful to understand the difference between the data and the view of the data.

Each operating Contig Editor is a view of the data for a particular contig. With two editors viewing the same contig, making changes in either will effect the data that both are viewing, hence the change will be visible in both editors. Similarly, using Undo in either will undo the changes to both.

When quitting and saving changes, other editors for the same contig will act as if a "Save Contig" request has been made by using the "Commands" menu (ie changes are written to disk.
and the undo information will be reset). Answering "no" to the "Save changes" query, simply shuts down the editor without saving. If there is no other editor for this contig then the changes will be lost, otherwise the changes will be retained until the last editor for the contig is exited.

Interaction between Contig Editors and Join Editors is more complicated and generally isn’t advised. However such interactions work consistently with the notion of views of contigs. For example, suppose there are two Contig Editors open on two separate contigs, and in addition to these a Join Editor displaying both contigs. Making the join in the Join Editor will update the two stand-alone Contig Editors so that they are each viewing the correct positions in the new contig, even though they’re both now viewing the same contig.

1.6.16 Quitting the Editor

The "Quit" button quits the editor. If changes have been made since the last save (either a "Save Contig" or an auto- save) you will be asked whether you wish to save these changes. Answering "Cancel" abandons the quit process and provides control of the editor again, otherwise the appropriate action will be taken and the editor quitted.

Within a join editor, the "Quit" button is changed to "Join/Quit". Pressing it will prompt for making the join. You will be told the percentage mismatch of the overlapping consensus sequences. The join can either be accepted, rejected, or cancelled (in which case the editor is not quitted and the join is not made).

1.6.17 Editing Techniques

The editor documentation describes the available controls, but not how these should be used most efficiently. Some editing is performed in a local style or is personal preference, but a great deal of the common editing tasks are best dealt with in specific ways. This section aims to give example methods of resolving the common problems. Typically, problems will be found using one of the editor searches (such as "consensus quality" or "problem"). Used in conjunction with "Auto-display traces" (see Section 1.6.8.2 [Trace Display Settings], page 75) this will automatically bring up a set of traces that are likely to be of assistance in resolving the problem. Prior to working on a contig it can be helpful to use "Shuffle Pads" to try to align padding characters See Section 1.6.7.12 [Shuffle Pads], page 73.

1.6.17.1 Consensus and Quality Cutoffs

The most rapid editing technique (see Section 1.2.5 [The use of numerical estimates of base calling accuracy], page 26) is only available if base call confidence values have been assigned to the reading data using a scale proportional to -log(error_rate). Using the "confidence" consensus method will make use of confidence values to give the most probable consensus sequence and a probability of each base being correct. Using the editor "consensus quality" search then provides an extremely quick way of identifying the lowest quality consensus bases. The List Confidence command will give information on the expected number of errors that can be fixed by examining all consensus bases with a quality less than a particular amount. This gives a good indication to the choice of threshold to use in the consensus quality search. Additionally you will also be told the expected error rates. With this system it is possible to stop editing once a particular average quality has been achieved.

Care should be taken in considering your desired error rate. An average error rate of 1 in 10,000 may be easily achievable. However there could still be consensus bases with very low confidence. Hence it is perhaps best to choose both an average error rate and a minimum consensus confidence for your finishing criteria. The consensus confidence values are scaled such that a confidence of 20 is a 1 in 100 error rate, 30 is 1 in 1000, 40 is 1 in 10000 and so on.
The rest of this section describes methods to use when the aforementioned confidence values are not available.

The Consensus and Quality cutoff values used whilst editing are personal preference. Rather than state suggested values, we discuss the merits of using example values.

The meaning of the consensus and quality cutoff values changes slightly depending on the consensus algorithm in use. For more information on the algorithms and these values see Section 1.11.5 [The Consensus Calculation], page 140.

With the "Base type frequencies" and "Quality weighted base type" methods, a consensus cutoff value of 100 means that every sequence disagreement will yield a dash in the consensus. Hence the "Next Search" button when in "problem" search mode can be used to verify every potential problem. This is a lot of work, but if you wish to make sure that all disagreements are checked this is the easiest way.

With a quality cutoff of -1, lowering the consensus cutoff value to (eg) 90 means that a base in the consensus will only be a dash when over 10% of the bases disagree with the majority at that point. So a base covered by 11 sequences, 10 of which state A and one of which states C would not be considered a problem and would not be found by the problem search. Note that this is regardless of the strand information. So if the As are on the positive strand and the single C is on the negative strand then this is still not considered a problem. However, see below.

Still working with the "Base type frequencies" and "Quality weighted base type" consensus methods, changing the quality cutoff to be 0 or more means that the consensus base is derived from the relative quality of bases instead of simple frequency counts. A quality cutoff of 0 and a consensus cutoff of 90 means that the base will be a dash only when the sum of the quality values for the most common base type (defined by the highest quality sum) is less than 90% of the total. In comparison with a quality cutoff of -1, this means that the above example of 10 A bases and 1 C base would be considered a problem if the C base had a sufficiently high quality.

If you have confidence values for each base available you may consider it unnecessary to check disagreements caused by poor quality data disagreeing with good quality data, although disagreements between good data and good data should always be checked. However it should be obvious from this that with a quality cutoff of 0 and a consensus cutoff of 100% every sequence conflict is still considered a potential problem. A specific change in the consensus cutoff (eg from 100% to 90%) will typically find less problems when the quality cutoff is 0 than when it is -1. This is entirely due to differences between good quality data and poor quality data being excluded.

Finally, the "Compare Strands" editor setting calculates two independent consensus sequences; one for each strand. The consensus shown is then the base calculated in each of the two consensus sequences if they agree, or dash if they do not. The "confidence" consensus algorithm already takes into account strand and chemistry when calculating the consensus base type and confidence, but will only lower the confidence value for strand disagreements, rather than setting the consensus base to be a dash. For all consensus methods enabling "Compare Strands" will force you to check all consensus bases where the evidence from each strand is conflicting.

1.6.17.2 Editing by Base Change or Confidence

Once a location has been found where an edit needs to be made there are two possible methods of resolving the problem. Assuming that the edit is a base replacement, the first way is to simply replace the differing base with the corrected base. This adds the new base at 100% quality. A second solution is to set the confidence of the differing base to 0. Assuming that we have the quality cutoff set to zero or more, this will remove the differing base from the consensus calculation, thus enabling the consensus to be 100% identical.

Both of these methods may be used when replacing bases in the consensus and are selectable using the "Edit Modes" menu. Fixing a problem by adjusting its confidence leaves the original,
conflicting, base visible on the screen. However if the changed reading is the only one on a strand
then adjusting the confidence means that the point only has good data on one strand.

1.6.17.3 Base Overcalls

A common problem is that of base overcalls that will result in perhaps one reading having and
extra base, and all the others being padded by the alignment routines:

<table>
<thead>
<tr>
<th>Read</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read1</td>
<td>ACC*AG</td>
</tr>
<tr>
<td>Read2</td>
<td>ACC*AG</td>
</tr>
<tr>
<td>Read3</td>
<td>ACC*AG</td>
</tr>
<tr>
<td>Read4</td>
<td>ACCCAG</td>
</tr>
<tr>
<td>Read5</td>
<td>ACC*AG</td>
</tr>
<tr>
<td>Consensus</td>
<td>ACC*AG</td>
</tr>
</tbody>
</table>

In this first case we see that Read4 has an extra C, probably due to an overcall. Check that the
trace for Read4 shows an overcall. It is a good idea to check good quality traces for both strands
as well as the trace with the apparent problem. Also note that enabling "Show reading quality"
(Settings menu) will show the reading quality as grey scales.

We now need to remove the column. It would appear that this could be done by removing the
* from each of Readings 1, 2, 3 and 5, and removing the C from Reading 4. However this will only
make edits to those five readings. As we're trying to remove an entire column from the contig, we
need to shift to the left by a single base the position of any readings to the right. Naturally this is
not the ideal method.

By placing the editing cursor in the consensus (on the second A) we can press Delete to remove
the entire column. This automatically makes sure that everything is consistent. If we are editing
at 100% consensus cutoff then this consensus base will be a '-' instead of a '*'. For this to work we
need to make sure that we have "Allow del dash in cons" enabled in the Edit Modes menu. See
Section 1.6.3.2 [Editing Modes], page 62.

1.6.17.4 Base Undercalls

<table>
<thead>
<tr>
<th>Read</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read1</td>
<td>ACCCAG</td>
</tr>
<tr>
<td>Read2</td>
<td>ACCCAG</td>
</tr>
<tr>
<td>Read3</td>
<td>ACCCAG</td>
</tr>
<tr>
<td>Read4</td>
<td>ACC*AG</td>
</tr>
<tr>
<td>Read5</td>
<td>ACCCAG</td>
</tr>
<tr>
<td>Consensus</td>
<td>ACCCAG</td>
</tr>
</tbody>
</table>

In the above case we see that Read4 has a C missing. Once again we must check the traces to be
sure that we wish to edit the reading. If so, then we can either make an edit specifically to Read4
itself (in "replace" mode) or type C in the consensus at this column. The latter will change either
the base type of the * in Read4 to c, or will change its confidence value to 0. This depends upon
the value of the "Edit by base type"/"Edit by confidence" setting in the Edit Modes menu.

When replacing base types, it is preferable to use lowercase letters. This makes the modified
base stand out. However even when using uppercase letters it is always possible to search for edits
at a later stage, although they won’t be as obvious to the human eye. Finally, note that the "Allow
replace in cons" mode must be set to to enable this solution.

1.6.17.5 Multiple Base Disagreements

<table>
<thead>
<tr>
<th>Read</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read1</td>
<td>ACCGAG</td>
</tr>
<tr>
<td>Read2</td>
<td>ACCGAG</td>
</tr>
</tbody>
</table>
Read3  AC*GAG
Read4  ACCGAG
Read5  AC*GAG
Consensus AC-GAG

Now we have a more complex case. Two disagreements out of five readings. Care should be taken to check these traces. Also note the strand of each reading. If the database is highly repetitive, and Read1, Read2, and Read4 are all from one strand, with Read3 and Read5 from the opposite strand then there is a chance that a misassembly has occurred or that the problem is a strand dependent sequencing artifact.

Typically this is clear when using the "Highlight disagreements" mode. See Section 1.6.8.4 [Highlight Disagreements], page 77. By selecting this mode and by also highlighting the reading names (see Section 1.6.2 [The sequence names display], page 61) scanning along the contig will quickly show whether there are other disagreements in common with these two readings versus the other three (which would support the evidence of misassembly).

If any misassembled readings have been spotted then mark them for disassembly (see Section 1.6.9 [Remove Readings], page 79) and they’ll no longer cause conflicts in the consensus. If the problem is a simple case of needing to edit, then making the edit in the consensus will require only one key stroke instead of the two needed to edit the individual readings.

1.6.17.6 Poor Quality

Read1  ACC*AGT*CGTA
Read2  ACC*AGT*CGTA
Read3  ACC*AGT*CGTA
Read4  ACCCAGTCCG
Read5  ACC*AGT*CGTA
Consensus ACC*AGT*CGTA

This is identical to the first case, except we have two edits within Read4 in close proximity. This is usually due to a poor quality reading, which can be checked by examining the trace and confidence values. Whilst we could continue to make edits in the normal fashion it may be wiser to take another approach.

One technique is to adjust the cutoff data for Read4. By marking the data as hidden, this portion of the reading will no longer be used for producing the consensus. However we can only extend the cutoff data at one end or the other; it is not possible to have "hidden" data part way through a reading except by modifying its confidence. Note though that adjusting the cutoff data may mean that we have no data for one strand, which should be solved by extra experiments.

If the reading is poor quality along its entire length, then disassembly is also a viable option. Using Highlight Disagreements (see Section 1.6.8.4 [Highlight Disagreements], page 77.) or Check Assembly (see Section 1.9 [Check Assembly], page 121) is a good way of finding such readings. Note that disassembling readings may have other implications. It could cause a hole in the contig (in which case it will be broken in two) or it could cause a single stranded segment. If this is the case, the user needs to weigh up the work involved with making many edits along the length of this reading against performing another experiment to obtain better quality data.

1.6.17.7 Checking for Errors

It is important to check the final sequence for any errors introduced by incorrect editing. We strongly advise this when making use of the more dangerous options in "Edit Modes" as it is possible to accidentally make changes. The editor provides several methods for checking the edits performed on the data.
The search menu contains two search types (see Section 1.6.6.11 [Search for evidence for edits(1)], page 70) and (see Section 1.6.6.12 [Search for evidence for edits(2)], page 70). "Evidence for edits (1)" searches for the next edited place where none of the original readings agree with the consensus. This helps to spot cases where entire columns have been inserted or deleted. "Evidence for edits (2)" performs the same checks as "Evidence for edits (1)", but on each strand independently. So it will find all edited places where there is not evidence from both strands.

Further checks may be performed outside the editor. Using the Find Read Pairs command all templates containing both forward and reverse readings will be checked to make sure that the relative orientation and distance of the sequences is correct. See Section 1.8.2 [Find Read Pairs], page 110.

Finally, the Check Assembly command can either check the hidden data for each reading to check that it does not diverge from the consensus sequence, or the visible data can be examined to locate segments with a high proportion of disagreements with the consensus. See Section 1.9 [Check Assembly], page 121. Such cases arise from unnoticed section of vector sequence, chimeric reads, or due to a reading being in the wrong copy of a repeated element.

1.6.18 Summary

1.6.18.1 Keyboard summary for editing window

("Left", "Right", "Up", "Down" refer to the appropriate arrow keys.)

- **Escape/Control v** Scroll right one screenful
- **Meta/Alt v** Scroll left one screenful
- **Left or Control b** Move editing cursor left one base
- **Right or Control f** Move editing cursor right one base
- **Up or Control p** Move editing cursor up one base
- **Down or Control n** Move editing cursor down one base
- **Control a** Move editing cursor to start of used
- **Control e** Move editing cursor to end of used
- **Meta/Alt/Escape a** Move editing cursor to start of cutoff
- **Meta/Alt/Escape e** Move editing cursor to end of cutoff
- **Meta/Alt/Escape comma** Move editing cursor to start of contig
- **Meta/Alt/Escape fullstop** Move editing cursor to end of contig
- **Meta/Control/Alt Left** Extend left cutoff data
- **Meta/Control/Alt Right** Extend right cutoff data
- **Control 1** Move pad left
- **Control r** Move pad right
- **<** Zap left cutoff data
- **>** Zap right cutoff data
- **[** Set confidence to 0
- **]** Set confidence to 100
- **Shift Up** Increase confidence of base by 1
- **Shift Down** Decrease confidence of base by 1
- **Control Up** Increase confidence of base by 10
- **Control Down** Decrease confidence of base by 10
- **Delete** Delete base or Shift reading left
- **Backspace** Delete base or Shift reading left
Control Delete Delete base from left and move left
Control d Delete base from right; do not move
Space Shift reading right
Undo key or Control underscore Perform an undo
Control i Toggle insert mode
Control h Toggle a sequence for removal
Control t Display trace
Control s Search forward
Escape Control s Search backwards
Control x Control s Save editor
Control q Toggle tag display
Control c or Control Insert Copy underlined region to paste buffer
Insert Insert a padding character (*)
any ACGT1234DVHKLMNRY5678*- Insert or change base (both cases allowed)

1.6.18.2 Mouse summary for editing window

Left button Position editing cursor to mouse cursor
Update editor information line
Left button (drag) Mark start and end of selection
Shift left button Adjust end of selection
Enter key Update editor information line (unpadded pos.)
Return key As Enter key, but also moves editing cursor
Left button (double click) Display trace
Middle button (double click) Display trace
Control left button Display commands menu
Right button Display commands menu

1.6.18.3 Mouse summary for names window

Left button Toggle user highlight (not in status line)
Middle button/Alt left button Add name to output list (if set)
Right button Display popup menu

1.6.18.4 Mouse summary for scrollbar

Middle button Set scrollbar position
Alt left button Set scrollbar position
Left button Scroll left or right one screenful

In addition to the scrollbar manipulation, the "<<", "<", ">", ">>" buttons also scroll the editor left or right by half a screenful or one base.
1.7 Assembling and Adding Readings to a Database

Assembly is performed by selecting one of the functions from the Assembly menu. The options available are:

- Normal shotgun assembly
- Assemble independently
- Assembly into single stranded regions
- Stack readings
- Put all readings in separate contigs
- Directed assembly
- Enter pre-assembled data
- Screen only

The data for a project is stored in an assembly database (See Section 1.16 Gap Database Files, page 162.) All modes of assembly except CAP2, CAP3 and FAKII can either assemble all the readings for a project in a single operation or can add batches of new data as they are produced. CAP2, CAP3 and FAKII can only be used to assemble all the data for a project as a single operation.

For all modes the names of the readings to assemble are read from a list or file of file names, and the names of readings that fail to be entered are written to a list or a file of file names. If only a single read is to be assembled the "single" button may be pressed and the filename entered instead of the file of filenames.

Now that a sufficient number of readings to get close to contiguity can be obtained quite quickly, and that more repetitive genomes are being sequenced it is sensible to use a "global" algorithm for assembly, such as Cap2, Cap3, FakII or Phrap. These algorithms compare each reading against all of the others to work out their most likely left to right order and so have a better chance of correctly assembling repetitive elements than an algorithm that only compares readings to the ones already assembled.

There is no limit to the length of the individual readings which can be assembled. Hence reference sequences for use in mutation studies or for use as guide sequences can be assembled.

Note that Normal shotgun assembly (see Section 1.7.1 Normal Shotgun Assembly, page 94), Assemble independently (see Section 1.7.1 Assemble Independently, page 94), Assembly into single stranded regions (see Section 1.7.1.2 Assembly Single, page 97), Screen only (see Section 1.7.3 Screen Only, page 101), Put all readings in separate contigs (see Section 1.7.1.4 Assembly new, page 99), may require the parameters maxseq and maxdb to be set beforehand (see Section 1.20.3 Set Maxseq, page 177). The maxseq parameter defines the maximum length of consensus that can be created, and the maxdb parameter the maximum number of readings and contigs that the database can hold (i.e. number of readings + number of contigs).

1.7.1 Normal Shotgun Assembly

In the absence of any of the external assembly engines, which are in general superior, particularly for repetitive data, this is the mode that most users will employ for all assembly. It takes one reading at a time and compares it with all the data already assembled in the database. If a reading matches it is aligned. If the alignment is good enough the reading is entered into the database. If a reading aligns well with two contigs it is entered into one of them, then the two contigs are compared. If they align well they are joined. If the reading does not match it starts a new contig. If a reading matches but does not align well it can either be entered as a new contig or rejected.

A submode allows tagged regions of contigs to be masked and hence restricts the areas into which data is entered. Users select the types of tags to be used as masks. As outlined above readings are
compared in two stages: first the program looks for exact matches of some minimum length, and then for each possible overlap it performs an alignment. If the masking mode is selected the masked regions are not used during the search for exact matches, but they are used during alignment. The effect of this is that new readings that would lie entirely inside masked regions will not produce exact matches and so will not be entered. However readings that have sufficient data outside of masked areas can produce hits and will be correctly aligned even if they overlap the masked data. For this mode the names of readings that do not produce matches are written to the error file with code 5. Note that new readings that carry tags of the types being used for masking will be masked only after they have been entered.

As explained above the user can select to "Apply masking", and if so, the "Select tags" button will be activated and if it is clicked will bring up a dialogue to allow tag types to be selected. See Section 1.20.9 [Tag Selector], page 181.

The "display mode" dialogue allows the type of output produced to be set. "Hide all alignments" means that only the briefest amount of output will be produced. "Show passed alignments" means that only alignments that fall inside the entry criteria will be displayed. "Show all alignments" means that all alignments, including those that fail the entry criteria, are displayed. "Show only failed alignments" displays alignments only for the readings that fail the entry criteria. Adding text to the text output window will increase the processing time.

When comparing each reading the program looks first for a "Minimum initial match", and for each such matching region found it will produce an alignment. If the "Maximum pads per read" and the "Maximum percent mismatch" are not exceeded the reading will be entered. The maximum pads can be inserted in both the reading and the consensus. If users agree we would prefer to swap the maximum pads criteria for a minimum overlap. i.e. only overlaps of some minimum length would be accepted.
Assembly usually works on sets of reading names and they can be read from either a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames.

The routine writes the names of all the readings that are not entered to a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. Occasionally it might be convenient to forbid joins between contigs to be made if a new reading overlaps them both, but the default is to "Permit joins".

If a reading is found to match but does not align within the alignment criteria it can be entered as a new contig or rejected. These two choices are described as "Enter all readings" or "Reject failures". Pressing the "OK" button will start the assembly process.

Note that this option may require the parameter maxseq to be set beforehand (see Section 1.20.3 [Set Maxseq], page 177). This parameter defines the maximum length of consensus that can be created.

Typical output would be:

(Output removed to save space)

Processing 51 in batch
Reading name xb61h12.s1
Reading length 104
Total matches found 2
Contig 9 position 590 matches strand -1 at position 1
Contig 36 position 92 matches strand -1 at position 1
Trying to align with contig 9
Percent mismatch 2.1, pads in contig 0, pads in gel 1
Percentage mismatch 2.1

590 600 610 620 630 640
Consensus TTGAAAAATTTTTTTTTGAAAAATAAAAAGGTGAAAAAGTAAAAAGCAAG

Reading TTGAAAAATTTTTTTTTGAAAAATAAAAAGGTGAAAAAGTAAAAAGCAAG

650 660 670 680
Consensus TAGCATGTAAAATCAAACAAAAATAACTATATT

Reading TAGCATGTAAAATCAAACAAAAATAACTATATT

61 71 81 91
Trying to align with contig 36
Percent mismatch 0.0, pads in contig 0, pads in gel 0
Percentage mismatch 0.0

92 102
Consensus TTGAAAAATTTTTTTTT

Reading TTGAAAAATTTTTTTTT

Overlap between contigs 36 and 9
Length of overlap between the contigs 111
Chapter 1: Sequence assembly and finishing using Gap4

Entering the new reading into contig 9
This gel reading has been given the number 47
Complementing contig 36
Complementing contig 9
Trying to align the two contigs
Percent mismatch 4.4, pads in contig 0, pads in gel 3
Percentage mismatch 5.3

<table>
<thead>
<tr>
<th></th>
<th>86</th>
<th>96</th>
<th>106</th>
<th>116</th>
<th>126</th>
<th>136</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus</td>
<td>AAAAGTTTTAAAAATTTTCAATTTGTTTGGTGGTTCCTTTGACTATTAGAAAAACACCCCCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consensus</td>
<td>AAAAGTTTTAAAAATTTTCAATTTGTTTGGTGGTTCCTTTGACTATTAGAAAA,CACA,CCCCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus</td>
<td>TTGCTCCTGTTGTGCAATTTTTGTTTTAAGTTTTCAATC<em>TTT</em>TATTTTAATA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consensus</td>
<td>TTGCTCCTGTTGTGCAATTTTTGTTTTAAGTTTTC-ATC,TTTTTATTTT-ATA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>61</th>
<th>71</th>
<th>81</th>
<th>91</th>
<th>101</th>
<th>111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consensus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Editing contig 36
Completing the join between contigs 47 and 36

(Output removed to save space)

Batch finished
100 sequences processed
96 sequences entered into database
11 joins made
9 joins failed

1.7.1.1 Assemble Independently

This mode works in exactly the same way as normal shotgun assembly (see Section 1.7.1 [Normal Shotgun Assembly], page 94) with all its options and settings, except that the new batch of data is assembled independently of all the data already in the database. This means that the only overlaps found will be between the readings in the current batch. One role for this mode would be to assemble a batch of data that was known from the way it was produced (say a set of nested clones covering some problem region such as a repeat) to overlap. Use of Assemble Independently will ensure that the batch of readings will only be overlapped with one another, and will not be aligned with other similar regions of the consensus. Once assembled in this way they can be joined to other contigs using Find Internal Joins. See Section 1.8.3 [Find Internal Joins], page 114.

1.7.1.2 Assemble Into Single Stranded Regions

This mode works like normal assembly (see Section 1.7.1 [Normal Shotgun Assembly], page 94) with masking, except that the masking is done for regions that already have sufficient data on both strands of the sequence. This means that new readings will only be assembled into regions that are single stranded or which border, and overlap, such segments. Note that this means that readings that do not match are not entered, therefore those that would actually lie between contigs are rejected.
The "display mode" dialogue allows the type of output produced to be set. "Hide all alignments" means that only the briefest amount of output will be produced. "Show passed alignments" means that only alignments that fall inside the entry criteria will be displayed. "Show all alignments" means that all alignments, including those that fail the entry criteria, are displayed. "Show only failed alignments" displays alignments only for the readings that fail the entry criteria.

When comparing each reading the program looks first for a "Minimum initial match", and for each such matching region found it will produce an alignment. If the "Maximum pads per read" and the "Maximum percent mismatch" are not exceeded the reading will be entered. The maximum pads can be inserted in both the reading and the consensus. If users agree we would prefer to swap the maximum pads criteria for a minimum overlap, i.e. only overlaps of some minimum length would be accepted.

Assembly usually works on sets of reading names and they can be read from either a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames.

The routine writes the names of all the readings that are not entered to a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. Occasionally it might be convenient to forbid joins between contigs to be made if a new reading overlaps them both, but the default is to "Permit joins".

Pressing the "OK" button will start the assembly process.

Note that this option may require the parameter maxseq to be set beforehand (see Section 1.20.3 [Set Maxseq], page 177). This parameter defines the maximum length of consensus that can be created.

### 1.7.1.3 Stack Readings

This assembly mode assumes that all the readings are already aligned and simply stacks them on top of one another in a new contig.

<table>
<thead>
<tr>
<th>Display Mode</th>
<th>Minimum Initial Match</th>
<th>Maximum Pads Per Read</th>
<th>Maximum Percent Mismatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide all alignments</td>
<td>20</td>
<td>25</td>
<td>5.0</td>
</tr>
<tr>
<td>Show passed alignments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Show all alignments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Show only failed alignments</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Input Reading Names from selection file list

List or file name Browse

Store failures to file list

List or file name Browse

Permit joins Yes No

OK Cancel Help

---

The Staden Package Manual
Assembly usually works on sets of reading names and they can be read from either a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames.

The routine writes the names of all the readings that are not entered to a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list.

1.7.1.4 Put All Readings In Separate Contigs

This algorithm simply loads the readings into the database without comparing them, each starting a new contig. This can be of use to those employing the database for storage rather than assembly.

Assembly usually works on sets of reading names and they can be read from either a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames.

The routine writes the names of all the readings that are not entered to a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list.

1.7.2 Directed Assembly

This assembly method assumes that a preprocessing program, such as an external assembly engine, has been used to map the relative positions of the readings to within a reasonable level of accuracy or tolerance. The assembly is "directed" by use of special "Assembly Position" or AP records included in each reading's experiment file. It is expected that these AP records will be added to the experiment files by the preprocessing program, or by a program which parses the output from such a program, and so the details given below are not of interest to the average user.

The experiment file for each reading must contain a special "Assembly Position" or AP line that defines the position at which to assemble the reading. The position is not defined absolutely, but relative to any other reading (the "anchor reading") that has already been assembled. The definition includes the name of the anchor reading, the sense of the new reading, its offset relative to the anchor reading and the tolerance. i.e.:

\[ \text{AP } \text{anchor_reading sense offset tolerance} \]
The sense is defined using + or - symbols.

The offset can be of any size and can be positive or negative. Offset positions are defined from 0, i.e., the first base in a contig or a reading is base number 0.

For normal use tolerance is a non-negative value, and the first base of the new reading must be aligned at plus or minus "tolerance" bases of "offset". If tolerance is zero, after alignment the position must be exactly "offset" relative to the anchor reading. If tolerance is negative then alignment is not performed and the reading is simply entered at position "offset" relative to the anchor reading.

To start a new contig the reading must include an AP line containing the anchor_reading *new* and the sense.

Example AP line:

```
AP fred.021 + 1002 40
```

Example AP line to start a new contig:

```
AP *new* +
```

The algorithm is as follows. Get the next reading name, read the AP line, find the anchor reading in the database, get the consensus for the region defined by anchor_reading + offset +/- tolerance. Perform an alignment with the new reading, check the position and the percentage mismatch. If OK enter the reading.

Obviously the way the positions of readings are specified is very flexible but one example of use would be to employ a file of file names containing a left-to-right ordered list of reading names, with each reading using the one to its left as its anchor reading. In this way whole contigs can be entered.

Although not specifically designed for the purpose this mode of assembly can be used for "assembly onto template".

If required, the alignments can be shown in the Output window by selecting "Display alignments". Only readings for which the "Maximum percent mismatch" after alignment is not exceeded will be entered into the database, unless the "enter all readings" box is checked. In that case a reading that does not match well enough will be placed in a new contig. Specifying a "Maximum percent mismatch" of zero has a special meaning; it implies that there should be no mismatches and so no alignments need to be performed, and hence the consensus does not need to be computed either. For data that has already been padded and aligned using an external tool (such as an external assembly program) setting Maximum percent mismatch to zero can have a significant improvement in the speed of Directed Assembly.
Assembly usually works on sets of reading names and they can be read from either a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames.

The routine writes the names of all the readings that are not entered to a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list.

It is important to note that the algorithm assumes that readings are entered in the correct order, i.e. a reading can only be entered into the defined AP position after the reading relative to which its position is defined. The order of the readings is defined by the order in the list or file of file names, and hence should be ordered by the external assembly engine. But if the browser is used to select a batch of sequences, they are unlikely to be in the correct order by chance, so care must be taken in its use. If reading X specifies an anchor reading that has not been entered the algorithm will start a new contig starting with X.

### 1.7.3 Screen Only

This function is used to compare a batch of readings against the data in an assembly database without entering them. It performs "normal shotgun assembly" and records the percentage mismatch for each matching reading in a file. If required, this file could then be sorted on percentage mismatch and used as a file of file names for "normal shotgun assembly"; in which case the best matches would be entered first. The readings in the batch are only compared to the current contents of the assembly database, and are not compared against the other readings in the batch.

As explained in normal assembly (see Section 1.7.1 [Normal Shotgun Assembly], page 94) the user can select to "Apply masking", and if so, the "Select tags" button will be activated and if it is clicked will bring up a dialogue to allow tag types to be selected. See Section 1.20.9 [Tag Selector], page 181.
The "display mode" dialogue allows the type of output produced to be set. "Hide all alignments" means that only the briefest amount of output will be produced. "Show passed alignments" means that only alignments that fall inside the entry criteria will be displayed. "Show all alignments" means that all alignments, including those that fail the entry criteria, are displayed. "Show only failed alignments" displays alignments only for the readings that fail the entry criteria.

When comparing each reading the program looks first for a "Minimum initial match", and for each such matching region found it will produce an alignment. If the "Maximum pads per read" and the "Maximum percent mismatch" are not exceeded the reading will be entered. The maximum pads can be inserted in both the reading and the consensus. If users agree we would prefer to swap the maximum pads criteria for a minimum overlap. i.e. only overlaps of some minimum length would be accepted.

Screening usually works on sets of reading names and they can be read from either a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames.

The routine writes the names of all the readings and their alignment scores expressed as percentage mismatches to a "file" or a "list" and an appropriate browser is available to enable users to choose the name of the file or list.

Previous versions of the package also had the ability to search for matches in the "hidden" poor quality data at the ends of contigs. This feature is no longer available.

Note that this option may require the parameter maxseq to be set beforehand (see Section 1.20.3 [Set Maxseq], page 177). This parameter defines the maximum length of consensus that can be created.

1.7.4 General Comments and Tips on Assembly

The program has several methods for assembly and it may not be obvious which is most appropriate for a given problem. The following notes may help. They also contain information on methods for checking the correctness of an assembly.

If you have access to an external program that can generate the order and approximate positions of readings then Directed Assembly can be used. The same is true if the experimental method used generates an ordered set of readings (see Section 1.7.2 [Directed Assembly], page 99).

If you have access to a external global assembly program that can produce an assembly and write out correct experiment files then Directed Assembly can still be used by specifying a "tolerance" of -1 (in the experiment file AP lines).

For routine shotgun assembly of whole data-sets or incremental data-sets Normal Shotgun Assembly can be used. Through the idea of "Masked assembly" this option also can also restrict the assembly to particular regions of the consensus (see Section 1.7.1 [Normal Shotgun Assembly], page 94).

Note that Normal shotgun assembly (see Section 1.7.1 [Normal Shotgun Assembly], page 94), Assemble independently (see Section 1.7.1.2 [Assembly Single], page 97), Screen only (see Section 1.7.3 [Screen Only], page 101), Put all readings in separate contigs (see Section 1.7.1.4 [Assembly new], page 99), may require the parameter maxseq to be set beforehand (see Section 1.20.3 [Set Maxseq], page 177). This parameter defines the maximum length of consensus that can be created. If you find that the assembly process is only entering the first few hundred of a batch of readings, try increasing maxseq.

If you have a batch of readings that are known to overlap one another, but which, due to repeats, may also match other places in the consensus, then it can be helpful to use Assemble Independently.
Chapter 1: Sequence assembly and finishing using Gap4

This will ensure that the batch of readings are compared only to one another, and hence will not be assembled into the wrong places (see Section 1.7.1.1 [Assemble independently], page 97).

Almost all readings are assembled automatically in their first pass through the assembly routine. Those that are not can be dealt with in two ways. Either they can be put through assembly again with less stringent parameters, or entered using the "Put all readings in new contigs" routine and then joined to the contig they overlap using Find Internal Joins See Section 1.8.3 [Find Internal Joins], page 114.. If it is found that readings are not being assembled in their first pass through the assembler, then it is likely that the contigs require some editing to improve the consensus. Also it may be that poor quality data is being used, possibly by users over-interpreting films or traces. In the long term it can be more efficient to stop reading early and save time on editing. For those using fluorescent sequencing machines the unused data can be incorporated after assembly using the Contig Editor and Double Strand.

An independent and important check on assembly is obtained by sequencing both ends of templates. Providing the correct information is given in the experiment files gap can check the positions and orientations of readings from the same template (see Section 1.8.2 [Find read pairs], page 110). Any inconsistencies are shown both textually and graphically. In addition this information can be used to find possible joins between contigs.

1.7.5 Assembly Failure Codes

0  The reading file was not found or is of invalid format
1  The reading file was too short (less than the minimum match length)
2  The reading appeared to match somewhere but failed to align sufficiently well (too many padding characters or too high a percentage mismatch)
3  A reading of the same name was already present in the database
4  This error number is no longer used
5  During a masked assembly, no sequence match with this reading was found.
1.8 Ordering and Joining Contigs

After the initial rounds of assembly it is likely that the data for a sequencing project will still not be contiguous. In order to minimise the number of experiments required to finish the project it is useful to be able to get as much from the existing data as possible. The functions described in this section can help to get the current set of contigs into a consistent left to right order, can discover joins between contigs which were missed or overlooked by the assembly engines, and can help in the analysis of repeats which may cause problems for assembly. It is one of the strengths of gap4 that the results from several of these independent types of analysis can be combined in a single display (see Section 1.4 [Contig Comparator], page 34), and where they are seen to reinforce one another, users can feel more confident in their decisions.

A typical Contig Comparator display is shown in the figure above. It is showing results from other functions, as well as the ones described in this section.
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The first function (see Section 1.8.1 [Order Contigs], page 107) automatically orders contigs based on read-pair data. The orderings found can be examined in the Template Display (see Section 1.5.1 [Template Display], page 37).

The next function (see Section 1.8.2 [Find read pairs], page 110) also examines read-pair data, but instead of automatically ordering the contigs, plots out their relationships in the Contig Comparator, from where the user can invoke the Template Display to check them, and use the Contig Selector to reorder them.

Sometimes assembly engines will miss or regard some weak joins as too uncertain to be made. The Find Internal Joins function (see Section 1.8.3 [Find Internal Joins], page 114), compares contigs, including their hidden data, to find matches between the ends of contigs. Again results are presented in the Contig Comparator, and users can invoke the Contig Joining Editor (see Section 1.6.14 [The Join Editor], page 87) to examine and make joins.

Whereas Find Internal Joins makes sure that alignments between contigs continue right to their ends, another search, Find Repeats (see Section 1.8.4 [Find Repeats], page 118) finds any identical segments of sequence, wherever they lie in the consensus. This has several uses. It gives another way of finding potential joins, and it provides a way of annotating (tagging) repeats so that their positions are obvious to users, and can be taken into account by other search procedures. Again results are presented in the Contig Comparator, and users can invoke the Contig Joining Editor (see Section 1.6.14 [The Join Editor], page 87) to examine and make joins.
1.8.1 Order contigs

This routine uses read-pair information to try to work out the left to right order of sets of contigs. It is invoked from the gap4 Edit menu. At present it attempts to order all the contigs in the database, and when finished it produces a listbox window which containing one or more sets (one set per line) of contigs listed by the names of their leftmost readings. By clicking on their names in the listbox the user can request that these "super contigs" should be shown in the standard Template display window (see Section 1.5.1 [Template Display], page 37).

Using the tools available within this window the user can manually move or complement any contigs which appear to have been misplaced. The combination of automatic ordering and the facility to view the results by eye and manually correct any errors make this a powerful tool. The new contig order can be saved to the database by selecting the "Update contig order" command from the "Edit" menu of the Template display. Note, however, that unlike the editing operations in the Contig editor, which are only committed to the disk copy of the database at the user’s request, all the complementing operations in gap4 are always performed both in memory and on the disk. This means that any complementing done as part of the contig ordering process will be immediately committed to disk.

An example of the "Super contig" listbox is shown here.
The example seen in the figures shows a Template display before and after the application of the algorithm.

Before ordering
After ordering

Notice how the operation has reduced the large number of dark yellow (inconsistent) templates by ordering and complementing the contigs so that they are now consistent and show in bright yellow. The few remaining dark yellow templates represent problems, possibly with misassembly or with misnaming of readings. The reliability of these dark yellow templates is also questionable when noting that one or the other of the readings are typically within the middle of large contigs, and hence are not likely to be spanning contigs. The gaps between the contigs, shown in the ruler at the bottom of the template display, are real estimates of size of the missing data, based on the expected lengths of the templates.

The algorithm is based on ideas used to build cosmid contigs using hybridisation data Zhang, P, Schon, EA, Fischer, SG, Cayanis, E, Weiss, J, Kistler, S and Bourne, P, (1994) "An algorithm based on graph theory for the assembly of contigs in physical mapping of DNA", CABIOS 10, 309-317. A difficulty for algorithms of this type is dealing with errors in the data, i.e. pairs of readings that have been incorrectly assigned to the same template (often by simple typing errors made prior to the creation of the experiment files). Our algorithm uses several simple heuristics to deal with such problems but one known problem is that it does not correctly deal with cases where templates span non-adjacent contigs, or where such contigs interleave.
1.8.2 Find Read Pairs

This function is used to check the positions and orientations of readings taken from the same templates. It is invoked from the gap4 View menu.

For each template the relative position of its readings and the contigs they are in are examined. This analysis can give information about the relative order, separation and orientations of contigs and also show possible problems in the data. The search can be over the whole database or a subset of contigs named in a list (see Section 1.14 [Lists], page 156) or file of file names. The results are written to the Output Window and plotted in the Contig Comparator (See Section 1.4 [Contig Comparator], page 34.). Read pair information is also used to colour code the results displayed in the Template Display (see Section 1.5.1 [Template Display], page 37).

Note that during assembly the template names and lengths are copied from the experiment files into the gap database. See Section 10.3 [Experiment Files], page 401. The accuracy of the lengths will depend upon some size selection being performed during the cloning procedures.

Users choose to process "all contigs" or a subset selected from a file of file names ("file") or a list ("list"). If either of the subset options is selected the "browse" button will be activated and can be clicked on to call up a file or list browser dialogue.

1.8.2.1 Find Read Pairs Graphical Output

The contig comparator is used to plot all templates with readings that span contigs. That is, the lines drawn on the contig comparator are a visual representation of the relationship (orientation and overlap) between contigs. When a template spans more than two contigs, all the combinations of pairs of contigs are plotted. However such cases are uncommon.
The figure above shows a typical Contig Comparator plot which includes several types of result in addition to those from Read Pair analysis.

The lines for the read-pairs are, by default, shown in blue. The length of the line is the average length of the two readings within the pair. The slope of the line represents the relative orientation of the two readings. If they are both the same orientation (including both complemented) the line is drawn from top left to bottom right, otherwise the line is drawn from top right to bottom left.

Clicking with the right mouse button on a read pair line brings up a menu containing, amongst other things, "Invoke template display" (see Section 1.5.1 [Template Display], page 37). This creates a template display of the two contigs. The spanning template will be coloured bright yellow if the readings on the template are consistent with one another, or dark yellow if they are not. The ordering of the contigs may need to be altered, or one contig may need complementing, before the readings on the template become consistent. Using the "Invoke join editor" command (see Section 1.6.14 [The Join Editor], page 87) from the same menu will bring up the Join Editor with the two contigs shown end to end.
1.8.2.2 Find Read Pairs Text Output

Two types of results are written to the Output Window: those containing apparently consistent data about the relative orientations and positions of contigs, and those that show inconsistencies in the data. The inconsistencies will be due to misassembly or to misnaming of readings and templates.

In the Output Window the program writes a line of information for each template and a line of information for each reading from that template. In order to restrict this information to fit on a standard 80 column display a few abbreviations are used. An example for two consistent and one problematic template is shown below. Templates with possible problems are separated from those without. The templates shown are sorted by problem; consistent templates at the top followed by increasingly inconsistent templates at the bottom.

<table>
<thead>
<tr>
<th>Template</th>
<th>zf18c8(117), length 1400-2000(expected 1700)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading</td>
<td>zf18a2.s1( +1F), pos 5620 +91, contig 46</td>
</tr>
<tr>
<td>Reading</td>
<td>zf18c8.s1( -117F), pos 1084 +288, contig 127</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Template</th>
<th>zf98f4(659), length 1400-2000(computed 7263)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading</td>
<td>zf98f4.s1( -659F), pos 27 +238, contig 548</td>
</tr>
<tr>
<td>Reading</td>
<td>zf98f4.r1( +800R), pos 5392 +211, contig 46</td>
</tr>
</tbody>
</table>

*** Possibly problematic templates listed below ***

<table>
<thead>
<tr>
<th>Template</th>
<th>zf24g6(262), length 1400-2000(observed 1365)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D Reading</td>
<td>zf24g6.r1( +808R), pos 463 +206, contig 46</td>
</tr>
<tr>
<td>D Reading</td>
<td>zf24g6.s1( -262F), pos 1559 +268, contig 46</td>
</tr>
</tbody>
</table>

1.8.2.3 The Template Lines

To describe the format of the template line we provide a detailed explanation of the lines above for the last Template block.

"Template zf24g6(262)"
This is template with name "zf24g6" and number 262.

length 1400-2000
These are the minimum and maximum lengths specified for this template.

observed(1365)
This section has the general format of "comment(distance)", where "comment" is one of the following.

- **observed** The template has both forward and reverse readings within this contig. From this information the actual size of the template can be seen. In the example this is "1365".
- **expected** The template length is estimated as the average of the specified minimum and maximum size. This will be seen when the template does not span contigs and does not have both forward and reverse primers visible.
- **computed** The template has forward and reverse readings in different contigs. The length is computed by butting the two contigs together, end to end, and finding the resultant separation of the template ends. It is not possible to tell whether the two contigs overlap, and if so by how much. Hence the "computed" lengths should not be considered as absolute.
1.8.2.4 The Reading Lines

"?DPS" The first four characters may be either space or one of "?", "D", "P" or "S". The meaning of each of these is as follows.

? No primer information is available for these readings.

D The distance between forward and reverse primers (ie the template length) is not as expected.

P The primer information for readings on this template is inconsistent. An example of this is where two forward readings exist, both using the universal primer, and the readings are not in close proximity to each other.

S The template strand information is inconsistent. This problem can be seen when the forward and reverse readings are from the same strand, or two forward readings are pointing in opposite directions.

Absence of all of these characters means that the template is consistent.

"Reading zf24g6.r1"

The reading name

"( +808R)"
The reading number. The "+" or "-" character preceding the number represents whether the reading has been complemented ("+" for original, "-" for complemented). The letter following the number indicates the primer information found for this reading. It may be one of:

? Unknown

F Forward, universal primer

f Forward, custom primer (eg a walk)

R Reverse, universal primer

r Reverse, custom primer

"pos 463 +206"
The position and the length of the reading within the contig. In this case the reading starts at position 463 and extends for 206 bases. For a complemented reading the position marks the 3' end of the reading. For both cases the position can be considered as the 'left end' of the reading as displayed within the contig.

"contig 46"
The reading number of the left most reading within this contig.

In the above example the template has two readings. It can be seen that the template starts at contig position 463 and finishes at position 1827. The observed length is 1365, which is just below the expected minimum length of 1400. Hence the template is flagged as having an invalid distance. There are no other inconsistencies for this template and so it is likely that the only "problem" is that the experimental size selection process was not as precise as was thought.
1.8.3 Find Internal Joins

The purpose of this function (which is invoked from the gap4 View menu) is to use sequences already in the database to find possible joins between contigs. Generally these will be joins that were missed or judged to be unsafe during assembly and this function allows users to examine the overlaps and decide if they should be made. During assembly joins may have been missed because of poor data, or not been made because the sequence was repetitive. Also it may be possible to find potential joins by extending the consensus sequences with the data from the 3’ ends of readings which was considered to be too unreliable to align during assembly i.e. we can search in the "hidden data".

If it has not already occurred, use of this function will automatically transform the Contig Selector into the Contig Comparator. Each match found is plotted as a diagonal line in the Contig Comparator, and is written as an alignment in the Output Window. The length of the diagonal line is proportional to the length of the aligned region. If the match is for two contigs in the same orientation the diagonal will be parallel to the main diagonal, if they are not in the same orientation the line will be perpendicular to the main diagonal. The matches displayed in the Contig Comparator can be used to invoke the Join Editor (see Section 1.6.14 [The Join Editor], page 87) or Contig Editor. See Section 1.6 [Editing in gap4], page 58. Alternatively, the "Next" button at the top left of the Contig Comparator can be used to select each result in turn, starting with the best, and ending with the worst. When this is in use, users can find the match in the Contig Comparator which corresponds to the next result by placing the cursor over the Next button. The plotted match and the contigs involved will turn white.
A typical display from the Contig Comparator is shown in the figure above.

To define the match all numbering is relative to base number one in the contig: matches to the left (i.e. in the hidden data) have negative positions, matches off the right end of the contig (i.e. in the hidden data) have positions greater than that of the contig length. The convention for reporting the positions of overlaps is as follows: if neither contig needs to be complemented the positions are as shown. If the program says "contig x in the - sense" then the positions shown assume contig x has been complemented. For example, in the results given below the positions for the first overlap are as reported, but those for the second assume that the contig in the minus sense (i.e. 443) has been complemented.

Possible join between contig 445 in the + sense and contig 405
Percentage mismatch after alignment = 4.9

<table>
<thead>
<tr>
<th>412</th>
<th>422</th>
<th>432</th>
<th>442</th>
<th>452</th>
<th>462</th>
</tr>
</thead>
<tbody>
<tr>
<td>405</td>
<td>TTTCCGACT</td>
<td>GGAAGCGGG</td>
<td>CAGTGAGGC</td>
<td>AACGCAATTA</td>
<td>ATGTGAG,TT</td>
</tr>
<tr>
<td>::::</td>
<td>:::::::</td>
<td>:::::</td>
<td>:::::</td>
<td>:::::</td>
<td>:::::</td>
</tr>
<tr>
<td>445</td>
<td>*TTCCGACT G,AAAGCGGG TAGTG,CGC</td>
<td>AACGCAATTA</td>
<td>ATGTGAG*TT</td>
<td>AGCTCACTCA</td>
<td></td>
</tr>
<tr>
<td>-127</td>
<td>-117</td>
<td>-107</td>
<td>-97</td>
<td>-87</td>
<td>-77</td>
</tr>
<tr>
<td>472</td>
<td>482</td>
<td>492</td>
<td>502</td>
<td>512</td>
<td></td>
</tr>
</tbody>
</table>
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### 1.8.3.1 Find Internal Joins Dialogue

The contigs to use in the search can be defined as "all contigs", a list of contigs in a file "file", or a list of contigs in a list "list". If "file" or "list" is selected the browse button is activated and gives access to file or list browsers. Two types of search can be selected: one, "Probe all against all" compares all the contigs defined against one another; the other "Probe with single contig",
compares one contig against all the contigs in the list. If this option is selected the Contig identifier panel in the dialogue box is ungreyed. Both sense of the sequences are compared.

If users elect not to "Use standard consensus" they can either "Mark active tags" or "Mask active tags", in which cases the "Select tags" button will be activated. Clicking on this button will bring up a check box dialogue to enable the user to select the tags types they wish to activate. Masking the active tags means that all segments covered by tags that are "active" will not be used by the matching algorithms. A typical use of this mode is to avoid finding matches in segments covered by tags of type ALUS (ie segments thought to be Alu sequence) or REPT (ie segment that are known to be repeated elsewhere in the data (see Section 1.2.7.1 [Tag types], page 29). "Marking" is of less use: matches will be found in marked segments during searching, but in the alignment shown in the Output Window, marked segments will be shown in lower case.

The default setting for the consensus is to "Use hidden data" which means that where possible the contigs are extended using the poor quality data from the readings near their ends. To ensure that this additional data is not so poor that matches will be missed, the program uses algorithms which can be configured from the "Edit hidden data parameters" dialogue. Two algorithms are available. Both slide a window along the reading until a set criteria is met. By default an algorithm which sums confidence values within the window is used. It stops when a window with < "Minimum average confidence" is found. The other algorithm counts the number of uncalled bases in the window and stops when the total reaches "Max number of uncalled bases in window". The selected algorithm is applied to all the readings near the ends of contigs and the data that extends the contig the furthest is added to its consensus sequence.

If your total consensus sequence length (including a 20 character header for each contig that is used internally by the program) plus any hidden data at the ends of contigs is greater than the current value of a parameter called maxseq, Find Internal Joins may produce an error message advising you to increase maxseq. Maxseq can be set on the command line (see Section 1.21 [Command line arguments], page 182) or by using the options menu (see Section 1.20.3 [Set Maxseq], page 177).

The search algorithms first finds matching words of length "Word length", and only considers overlaps of length at least "Minimum overlap". Only alignments better than "Maximum percent mismatches" will be reported.

There are two search algorithms: "Sensitive" or "Quick". The quick algorithm should be applied first, and then the sensitive one employed to find any less obvious overlaps.

The sensitive algorithm sums the lengths of the matching words of length "Word length" on each diagonal. It then finds the centre of gravity of the most significant diagonals. Significant diagonals are those whose probability of occurrence is < "Diagonal threshold". It then uses a dynamic programming algorithm to align around the centre of gravity, using a band size of "Alignment band size (percent)". For example: if the overlap was 1000 bases long and the percentage set at 5, the aligner would only consider alignments within 50 bases either side of the centre of gravity. Obviously the larger the percentage and the overlap, the slower the alignment.

The quick algorithm can find overlaps and align 100,000 base sequences in a few seconds by considering, in its initial phase only matching segments of length "Minimum initial match length". However it does a dynamic programming alignment of all the chunks between the matching segments, and so produces an optimal alignment. Again a banded dynamic algorithm can be selected, but as this only applies to the chunks between matching segments, which for good alignments will be very short, it should make little difference to the speed.

After the search the results will be sorted so that the best matches are at the top of a list. This list can be stepped through, one result at a time using the Contig Joining Editor, by clicking on the "Next" button at the top left of the Contig Comparator.
1.8.4 Find Repeats

The purpose of this function (which is invoked from the gap4 View menu) is to find exact repeats in contig consensus sequences. An exact repeat is defined as a run of consecutive identical ACGT characters; no mismatches or gaps are permitted.

If it has not already occurred, selection of this function will automatically transform the Contig Selector into the Contig Comparator. See Section 1.4 [Contig Comparator], page 34. Each match found is plotted as a diagonal line in the Contig Comparator. The length of the diagonal line is proportional to the length of the match.

If the match is for two contigs in the same orientation the diagonal will be parallel to the main diagonal, if they are not the line will be perpendicular to the main diagonal. The matches displayed in the Contig Comparator can be used to invoke the Join Editor (see Section 1.6.14 [The Join Editor], page 87) or Contig Editors (see Section 1.6 [Editing in gap4], page 58), and an Information button will display data about the match in the Output window. e.g.

Repeat match
From contig xb54a3.s1(#26) at 78
With contig xb62h3.s1(#3) at 1
Length 37

This means that position 78 in the contig with xb54a3.s1 (reading number 26) at its left end matches 37 bases at position 1 in the contig with xb62h3.s1 (number 3) at its left end.

Users can elect to search a "single" contig, or compare "all contigs", or a subset of contigs defined in a list or a file. If "file" or "list" is selected the browse button is activated and gives access to file or list browsers. If they choose to analyse a single contig the dialogue concerned with selecting the contig and the region to search becomes activated. The "Minimum Repeat" defines the smallest match that the algorithm will report. The algorithm will search only for repeats in the forward direction "Find direct repeats", or only those in the reverse direction "Find inverted repeats", or both "Find both".

If "Mask active tags" is selected the "Select tags" button is activated. Clicking on this button will bring up a check box dialogue to enable the user to select the tags types they wish to activate.
Masking the active tags means that all segments covered by tags that are "active" will not be used in the matching algorithm. A typical use of this mode is to avoid finding matches in segments covered by tags of type ALUS (ie segments thought to be Alu sequence) or that already covered by REPT tags. See Section 1.2.7.1 [Tag types], page 29.

After the search is complete clicking on "Yes" in the "Save tags to file" panel will activate the "File name" box and all repeats on the list will be written to a file. This file can be used with "Enter tags" (see Section 1.12.2 [Enter Tags], page 147) to create REPT tags for all the repeats found. Note that "Enter tags" will remove all the results plotted in the contig comparator.

Note that the current version of Find Repeats has a limit to the number of repeats it can store. The limit depends on the current maximum consensus length, so if you want to increase the limit, reset the maximum consensus length. This can be done using the "Set maxseq" item in the "Options" menu.
Chapter 1: Sequence assembly and finishing using Gap4

1.9 Checking Assemblies and Removing Readings

After assembly, and prior to editing, it can be useful to examine the quality of the alignments between individual readings and the sections of the consensus which they overlap. This may reveal doubtful joins between sections of contigs, poorly aligned readings, or readings that have been misplaced. By using this analysis in combination with other gap4 functions such as Find internal joins (see Section 1.8.3 [Find Internal Joins], page 114) and Find repeats (see Section 1.8.4 [Find Repeats], page 118), it is also possible to discover if readings have been positioned in the wrong copies of repeat elements. The functions for checking the alignment of readings in contigs are described below. See Section 1.9 [Checking Assemblies], page 121.

If readings are found to be misplaced or need removing for other reasons, gap4 has functions for breaking contigs (see Section 1.9.1.1 [Breaking Contigs], page 124), and removing readings (see Section 1.9.1.2 [Disassembling Readings], page 125). These functions can be accessed through the main gap4 Edit menu or from within the Contig Editor.

If readings are removed from contigs to start new contigs of one reading, these contigs can then be processed by Find internal joins (see Section 1.8.3 [Find Internal Joins], page 114) and the Join editor (see Section 1.6.14 [The Join Editor], page 87), which should reveal all the other positions at which the reading matches.
1.9.0.1 Checking Assemblies

The Check Assembly routine (which is invoked from the gap4 View menu) is used to check contigs for potentially misassembled readings by comparing them against the segment of the consensus which they overlap. It has two modes of use: the first simply counts the percentage mismatch between each reading and the consensus it overlaps, and the second performs an alignment between the hidden data for a reading and the consensus it overlaps. If the percentage is above a user-defined maximum, a result is produced. That is, one mode compares the "visible" part of the readings, and the other aligns and compares the hidden data. Results are displayed in the Output Window and plotted on the main diagonal in the Contig Comparator. See Section 1.4 [Contig Comparator], page 34.

From the Contig Comparator the user can invoke the Contig Editor to examine the alignment of any problem reading. See Section 1.6 [Editing in gap4], page 58. If the reading appears to be correctly positioned the user can either edit it, or in the case of poor alignment of the hidden data, place a tag, so that it does not produce a result if the search is done again. Note however such data will then also be ignored by the automatic double stranding routine. See Section 1.10.1 [Double Stranding], page 126. A typical textual output from the analysis of hidden data is shown below.

```
Reading 802(fred.s1) has percentage mismatch of 25.86

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>375</td>
<td>385</td>
<td>395</td>
<td>405</td>
<td>415</td>
<td>425</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Reading</td>
<td>*CCTGT</td>
<td>TTTAAATTG-TGG-C-C</td>
<td>CCG*-TTAACC</td>
<td>CGG</td>
<td>GT*CAAC**CTGG</td>
</tr>
<tr>
<td>Consensus</td>
<td>ACATGT</td>
<td>T*TAAATTGATGAA</td>
<td>CACC</td>
<td>CG*AA</td>
<td>AAA</td>
</tr>
<tr>
<td></td>
<td>2929</td>
<td>2939</td>
<td>2949</td>
<td>2959</td>
<td>2969</td>
</tr>
</tbody>
</table>
```

Users select either to search only one contig ("single"), all contigs ("all contigs"), or a subset of contigs contained in a "file" or a "list". If "file" or "list" is selected the "browse" button will be activated and clicking on it will invoke a file or list browser. If a single contig is selected the "Contig identifier" dialogue will be activated and users should enter a contig name.

Selecting between analysing the visible or hidden data is done by clicking on "yes" or "no" in the "Use cutoff data" dialogue. All alignments that are worse than "Maximum percentage of mismatches" will produce a result in the Output Window and the Contig Comparator. If "Use cutoff data" is selected then dialogue to enable the user to restrict the quality and length of the hidden data that the program aligns is activated. First, to avoid finding very short mismatching regions (where percentage mismatch figures could be very high) users can set a "Minimum length
of alignment" figure. Secondly to ensure that the hidden data is not so bad that alignments will necessarily be poor, the program uses the following algorithm. It slides a window of size "Window size for good data scan" along the hidden data for each reading and stops if it finds a window that contains more than "Max dashes in scan window" non-ACGT characters.

To check the used data for each reading ("Use cutoff data" is set to "No") the program compares all segments of size 'window' against the consensus sequence that they lie above (obviously no alignment is required). If the percentage mismatch within any segment is above the specified amount, then the entire 'alignment' of the reading and consensus is displayed. Note that in the output the program will first give the percentage mismatch over the window length, and then the percentage over the whole reading. To check the overall percentage mismatch of readings, simply set the "Window size for used data" to be longer than the reading lengths. To check for divergence of segments within readings set the window size accordingly.

The "Information" window produced by selecting "Information" from the Contig Comparator "Results" menu produces a summary of the results sorted in order os percentage mismatch.

By clicking with the right mouse button on results plotted in the Contig Comparator a pop-up menu is revealed which can be used to invoke the Contig Editor (see Section 1.6 [Editing in gap4], page 58). The editor will start up with the cursor positioned on the problem reading. If the reading is found to be misplaced it can be marked for removal from within the Editor (see Section 1.6.7.13 [Remove Reading], page 73). However, prior to this it may be beneficial to use some of the other analyses such as Find internal joins (see Section 1.8.3 [Find Internal Joins], page 114) and Find repeats (see Section 1.8.4 [Find Repeats], page 118), which may help to find its correct location. Both of these functions produce results plotted in the Contig Comparator (see Section 1.4 [Contig Comparator], page 34) and any alternative locations will give matches on the same vertical or horizontal projection as the problem reading.
1.9.1 Removing Readings and Breaking Contigs

Occasionally contigs require more drastic changes than simple basecall edits. Sometimes it is necessary to remove readings that have been put in the wrong place, or to break contigs that should not have been joined. Gap4 contains functions to help with these problems, and two types of interface.

If a contig needs to be broken cleanly into two new contigs, with all the readings, other than the two at the incorrect join, still linked together, then Break Contig (see Section 1.9.1.1 [Breaking Contigs], page 124), or (see Section 1.6.7.14 [Break Contig], page 73) should be used. The former interface is available via the main gap4 Edit menu, and the latter as an option in the Contig Editor.

If one or more readings need removing from from contig(s), even if their removal will break the contiguity of a contig, then (see Section 1.9.1.2 [Disassemble Readings], page 125), or (see Section 1.6.7.13 [Remove Reading], page 73) should be used. The former interface is available via the main gap4 Edit menu, and the latter as an option in the Contig Editor. Readings can be removed from the database completely, or moved to start individual new contigs, one for each reading.
1.9.1.1 Breaking Contigs

The Break Contig function (which is available from the gap4 Edit menu) enables contigs to be broken by removing the link between two adjacent readings. The user defines the name or number of the reading that, after the break, will be at the left end of the new contig. That is, the break is made between the named reading and the reading to its left.

It is also possible to interactive select places to break the contig when using the Contig Editor. See Section 1.6.7.14 [Break Contig], page 73.
1.9.1.2 Disassembling Readings

This function is used to remove readings from a database or move readings to new contigs. There are two interfaces which allow sets of readings to be disassembled. One is to identify the readings interactively when using the Contig Editor (see Section 1.6.9 [Remove Readings], page 79), and the other, described below, is available as a separate option from the main gap4 Edit menu.

If readings are removed from the database all reference to them is deleted. If a reading is moved to a new contig, the new contig will contain only that single reading. Such contigs can then be processed by Find internal joins (see Section 1.8.3 [Find Internal Joins], page 114) and the Join editor (see Section 1.6.14 [The Join Editor], page 87), which should reveal all the other positions at which the reading matches.

Disassembly can also distinguish between "crucial" and "non-crucial" readings: a crucial reading is defined as one that, if removed, would leave a single stranded region in the contig or cause a break in the contig. Conversely non-crucial readings are those that are entirely covered by other readings from both strands of the sequence. So if only non-crucial readings were removed the amount of single stranded data would not change. Note that when readings are removed from the database the program cleans up by moving data to fill up any holes made in its list of readings, and this will cause some high numbered readings to be given different numbers.

The set of readings to be processed can be read from a "file" or a "list" and clicking on the "browse" button will invoke an appropriate browser. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames. The "Removal mode" is either "All readings" or "Non-crucial only" which are explained above. The "task" is to "Remove readings from database" or simply "Move readings to new contigs" which means each will start a new contig. For large batches of readings this processing can be quite slow.
1.10 Finishing Experiments

Gap4 contains several functions for helping to select experiments to finish an assembly project. These functions (which are all available from the gap4 Experiments menu) are able to automatically analyse the contigs to find the regions which need attention, and to suggest appropriate experiments.

Prior to performing any experiments it can be worthwhile to try to make the most of the existing data by moving the boundary between the hidden and visible data of readings to cover single stranded readings. (see Section 1.10.1 [Double Strand], page 126)

The following "Experiment Suggestion" functions analyse the contigs to find problems, and then suggest the best templates to use for further experiments.

Primers and templates for primer walking experiments can be suggested. (see Section 1.10.2 [Suggest Primers], page 128). Sometimes resequencing on a long gel machine will help to fill a single stranded region or join a pair of contigs. (see Section 1.10.3 [Suggest Long Readings], page 130). Compressions and stops can be solved by resequencing using a different chemistry. (see Section 1.10.4 [Compressions and Stops], page 132). In order to select oligos to use as probes for clones near the ends of contigs a further function is available. (see Section 1.10.5 [Suggest Probes], page 134).

1.10.1 Double Stranding

The purpose of this function (which is available from the gap4 Edits menu) is to use hidden data to fill regions of contigs that have data on only one strand (see Section 1.2.6 [Use of the "hidden" poor quality data], page 28). First the routine finds a region that has data for only one strand. Then it examines the nearby readings on the other strand to see if they have hidden data that covers the single stranded region. If so it finds the best alignment between this hidden data and the consensus over the region. If this alignment is good enough the data is converted from hidden to visible. This process is continued over all the selected contigs. The function can be run on a subsection of a single contig, on all contigs, or on a subset of contigs that are named in a file of a list.

Significant portions of the sequence can be covered by this operation, hence saving a great deal of experimental work, and it can be used as a standard part of cleaning up a sequencing project. However it must be noted that an increased number of edits may be required after its application. The amount of cutoff data used depends on the number of mismatches and the percentage mismatch in the alignment. That is, it depends on the quality of the alignment, not the quality of the data: if it aligns it is assumed to be correct!

The program reports its progress in the Output window as shown in the following example.

```
Wed 03:52:46 PM: double strand
-----------------------------------------------------------------
Double stranding contig xf48g3.s1 between 1 and 6189
Double stranded zf23b2.s1  by 121 bases at offset 3752
Double stranded zf18g11.s1  by 194 bases at offset 5652
Positive strand:
  Double stranded 315 bases with 2 inserts into consensus
  Filled 0 holes
Complementing contig 358
Double stranded zg29a11.s1  by 42 bases at offset 5265 - Filled
Double stranded zf38c7.s1  by 131 bases at offset 5015 - Filled
Negative strand:
  Double stranded 174 bases with 1 insert into consensus
  Filled 2 holes
```
The contigs to process can be a particular "single" contig, "all contigs", or a subset of contigs whose names are stored in a "file" or a "list". If a file or list is selected the browse button will be activated, and if it is clicked, an appropriate browser will be invoked. If the user selects "single" then the dialogue for choosing the contig and the section to process becomes active.

Only alignments with not more than "Maximum number of mismatches" and "Maximum percentage of mismatches" will be accepted.
1.10.2 Suggest Primers

The purpose of this function (which is available from the gap4 Experiments menu) is to suggest custom primer experiments to extend and "double strand" contigs. First the routine finds regions of contigs with data on only one strand. Then it selects templates and primers, which if used in sequencing experiments, would produce data to cover these single stranded regions. This information is written to a file or a list and also appears in the Output window. For each primer suggested a tag is automatically created containing the template name and the sequence. See also Section 1.10.3 [Suggest Long], page 130, and Section 1.10.1 [Double Strand], page 126.

The following example shows how the results appear in the Output window.

Wed 04:53:08 PM: Suggest Primers

Selecting oligos for contig xf23a3.s1 between 1 and 12379
At 3873 - template zf23b2, primer GAACTGGATAATACGAC, number 1
At 5847 - template zf18g11, primer CCTCCAATAGCGTGAAG, number 2
At 7924 - template zf22d11, primer GTAAAGTGAATTCAAGGAAG, number 3
At 9033 - template zf97c10, primer ATGATAGAAATCTCGTGG, number 4
At 9972 - template zf98b5, primer CGGAAAGTTGAAGAAG, number 5
At 10506 - template zg09a9, primer ACACATCATTTTCGAGG, number 6
At 10958 - template zf24c1, primer CAGTTTACGAGAAAGTCC, number 7
At 11529 - template zg29a12, primer ACCTTCCAAAGATGTC, number 8
At 11897 - template zf97d7, primer AACCGATTTCGTAATG, number 9
Complementing contig 358
At 11400 - template zf38b1, primer CGAAGACCCAAAGAAG, number 11
At 9902 - template zf98a4, primer CTTTTCTCTTCTCACTTTCC, number 12
At 7104 - template zf22h10, primer GTGTCAGAAATTCGGC, number 13
At 6564 - template zf21e6, primer CGGATCAAATATGGATGG, number 14
At 1499 - template zf98a11, primer CGTGATTTTTACACTATTTCC, number 15
At 774 - template zf19c4, primer TCCAATTTTCGTAATG, number 16
Complementing contig 46

The following shows the contents of the corresponding file. The fields are template name, reading name, primer name, primer sequence, position and direction.

zf23b2 zf23b2.s1 B0334.1 GAAACTGGATAATACGAC 3818 +
zf18g11 zf18g11.s1 B0334.2 CCTCCAATAGCGTGAAG 5789 +
zf22d11 zf22d11.s1 B0334.3 GTAAAGTGAATTCAAGGAAG 7883 +
zf97c10 zf97c10.s1 B0334.4 ATGATAGAAATCTCGTGG 8984 +
zf98b5 zf98b5.s1 B0334.5 CGGAAAGTTGAAGAAG 9932 +
zg09a9 zg09a9.s1 B0334.6 ACACATCATTTTCGAGG 10460 +
zf24c1 zf24c1.s1 B0334.7 CAGTTTACGAGAAAGTCC 10902 +
zg29a12 zg29a12.r1 B0334.8 ACCTTCCAAAGATGTC 11487 +
zf97d7 zf97d7.s1 B0334.9 AACCGATTTCGTAATG 11855 +
zf23a3 zf23a3.s1 B0334.10 CAAAGCAATTTCGCCAG 12339 +
zf38b1 zf38b1.s1 B0334.11 CGAAGACCCAAAGAAG 930 +
zf98a4 zf98a4.s1 B0334.12 CTTTTCTCTTCTCACTTTCC 2427 +
zf22h10 zf22h10.s1 B0334.13 GTGTCAGAAATTCGGC 5220 +
zf21e6 zf21e6.s1 B0334.14 CGGATCAAATATGGATGG 5771 +
zf98a11 zf98a11.s1 B0334.15 CGTGATTTTTACACTATTCC 10833 +
zf19c4 zf19c4.s1 B0334.16 TCCAATTTTCGTAATG 11565 +
The contigs to process can be a particular "single" contig, "all contigs", or a subset of contigs whose names are stored in a "file" or a "list". If a file or list is selected the browse button will be activated and, if it is clicked, an appropriate browser will be invoked. If the user selects "single", then the dialogue for choosing the contig and the section to process becomes active.

The primer sequences, their template names and their reading names can be written to a file or a list and an appropriate browser can be used to aid its selection.

For each single stranded region located, the program will search for a primer on its 5' side in the region "search start position", to "search end position". That is, it will try to locate a primer starting at "search start position" and then will look increasingly further away until it reaches "search end position".

If required, by employing the "number of primers per match" entry box, the user can request that the program tries to suggest more than one primer per problem. The "primer start number" is an attempt to generate a unique name for each primer suggested. If the number was set to, say 11, and the database was named B0334, then the first primer would be named B0334.11, the next B0334.12, etc in the output file.

The "Edit parameters" button invokes a dialogue box which allows the specification of further parameters. Primer constraints can be specified by melting temperature, length and G+C content.
1.10.3 Suggest Long Readings

This routine (which is available from the gap4 Experiments menu) suggests which templates could be resequenced on a long gel machine to fill in single stranded regions or extend contigs. The "Estimated long reading length" tells the routine the expected length of reading that will be produced by the sequencing machine. The routine finds all single stranded regions, and where possible suggests solutions. Solutions will not be suggested using readings from templates that have inconsistent read-pair information.

The example output below shows a list of problem segments followed by suggested templates.

Prob 1..1: Extend contig start for joining.
   Long  c91d3.s1  367. T_pos=366, T_size=1000..1500 (1250), cov 189
   Long  c99e12.s1  340. T_pos=191, T_size=1000..1500 (1250), cov 216

Prob 1..456: No +ve strand data.
   No solution.

Prob 1597..1736: No +ve strand data.
   Long  c53c6.s1  1074. T_pos=341, T_size=1000..1500 (1250), cov 32
   Long  e04c11.s1  1076. T_pos=376, T_size=1000..1500 (1250), cov 34
   Long  e05h9.s1  1081. T_pos=377, T_size=1000..1500 (1250), cov 39
   Long  e05a1.s1  1198. T_pos=329, T_size=1000..1500 (1250), cov 156*
   Long  c53b11.s1  1382. T_pos=216, T_size=1000..1500 (1250), cov 340*

Prob 2530..2532: No +ve strand data.
   Long  e03a8.s1  2283. T_pos=199, T_size=1000..1500 (1250), cov 308*
   Long  e05b10.s1  2331. T_pos=200, T_size=1000..1500 (1250), cov 356*

Prob 3974..4067: No -ve strand data.
   No solution.

Prob 4067..4067: Extend contig end for joining.
   D Long  e06a3.s1  3588. T_pos=366, T_size=1000..1500 (1582), cov 76
   Long  c53b1.s1  3709. T_pos=360, T_size=1000..1500 (1250), cov 197

Some brief notes on the above output; looking at the suggested rerun of reading e05a1.s1.

Prob 1597..1736: No +ve strand data.
   A single stranded region has been identified in this contig at bases 1597 to 1736 inclusive.

"?D Long" The optional two letters before the word "Long" are used to flag possibly inconsistent templates (templates that are definitely inconsistent are ignored). "?" means that no primer information is available for the template that the reading is from. "D" means that the template size is not within the expected minimum and maximum. In this case the observed size is displayed (see below).

"Long e05a1.s1 1198."
   A possible solution; rerun reading e05a1.s1 as a long gel. The first used base at the 5’ end of this reading is at position 1198 in the contig. Typically this roughly corresponds to the primer position for this reading in the contig.

T_pos=329
   The last used base at the 3’ end of the reading is estimated to be the 329th base of the template. Together with the template lengths this gives us an estimate of how much template there is available for a long gel or for walking.
The estimated size for this template is 1250 bases. Gap4 is supplied a minimum and maximum size when a reading is assembled. In this case the minimum is 1000 bases, and the maximum 1500. When forward and reverse reads assembled into the same contig estimate the real length reasonably accurately. Otherwise (as can be seen here), the estimated length is simply the average of the supplied minimum and maximum lengths.

We would expect a long gel to cover our "hole" by 156 bases. This estimate is based purely on the position of the start of the reading in relation to the start of the hole, and the estimated length of a long gel. The asterisk here marks that this coverage is more than enough to completely solve the problem by plugging the positive strand hole.

For the problem "3974..4067" there is "No solution" listed. This is due to the fact that there are no suitable readings within the estimated long gel reading length of this problem.
1.10.4 Compressions and Stops

This option (which is available from the gap4 Experiments menu) searches through a region of a contig looking for stop (STOP) or compression (COMP) tags. These tags could have been added using the Contig Editor or by a suitable external program which can analyse traces to detect these types of problems. For each such tag found the routine produces a list of readings that could be resequenced to try to solve the problem. Obviously the types of experiments available will change as the technology improves but at present the program produces output that suggests "Taq terminator" experiments. We welcome suggestions for other experiment types or news of any programs that can automatically assign the tags. The results, in the form of suggestions, are written to the Output window.

Note that the Taq reading length is used as a guideline for deciding which readings are suitable candidates for solving a problem. All readings in the correct orientation and with their 5' ends within this length are assumed to solve the problem. The actual distance is listed in the output; an example of this is shown below.

- Prob 1544..1545: COMP tag on strand 0 (forward)
  Taq for xd26d8.s1 1365 179

- Prob 1554..1554: STOP tag on strand 0 (forward)
  Taq for xd26d8.s1 1365 189

- Prob 5276..5288: COMP tag on strand 1 (reverse)
  Taq for xc34g1.s1 5299 23
  Taq for xc34g1.slt 5298 22
  Taq for xc34d6.s1 5316 40
  Taq for xc45e1.s1 5463 187

- Prob 24042..24046: COMP tag on strand 1 (reverse)
  Taq for xc50a12.s1 24167 125
  Taq for xc33d1.s1 24188 146
  Taq for xc36h4.s1 24208 166
  Taq for xc51c8.s1 24232 190

The format of the above output is:

```
Prob <start>..<end>: <type> tag on strand <st>
  Taq for <read> @ <pos> <distance>
```

Where:
<start>..<end> marks the inclusive range for the tag in the contig.
<type> is the type of the current tag.
<st> is the strand of the reading that the tag is placed upon.
<read> is the gel reading name.
<pos> is the position of the 5’ end of <read> in the contig.
<distance> is the distance of the 5’ end from the tag.
1.10.5 Suggest Probes

The suggest probes function (which is available from the gap4 Experiments menu) looks for oligos at the end of each contig suitable for use with an *oligo probing strategy* invented by Jonathan Flint. Flint, J., Sims, M., Clark, K., Staden, R., and Thomas, K. An oligo-screening strategy to fill gaps found during shotgun sequencing projects. DNA Sequence 8, 241-245. The probing strategy is used part way through a sequencing project to find clones which should help to extend contigs. The gap4 function described here is used to select oligos from readings that are near the ends of the current contigs. These oligos are synthesised and then used to probe a pool of sequencing clones. Those which it selects are then sequenced in the hope that they will lengthen the contigs.

The dialogue contains the usual methods of selecting the set of contigs to operate on. For each end of the selected contigs, oligos are chosen using the OSP Hillier, L., and Green, P. (1991). "OSP: an oligonucleotide selection program," PCR Methods and Applications, 1:124-128. selection criteria which is dependent on the maximum and minimum size of oligos specified. The "search from" and "search to" parameters control the area of consensus sequence in which to search for oligos. For example, if they are set to 10 and 100 respectively the a section of consensus sequence used is 90 bases long and starts 10 bases from the end of the contig.

Once an oligo is found it is screened against all the existing consensus sequence. An oligo is rejected if it matches with a score greater than or equal to the "maximum percentage match". If a file of vector filenames has been specified then the oligos are also screened against the vector sequences.

Typical output for a single contig follows. The output shows all oligos that have passed the screening process. The information listed includes the distance of this oligo from the end of the contig (Dist ??), the score returned from the OSP selection (primer=??), the melting temperature (Tm=??), the best percentage match found (match=??%) and the oligo sequence.

Contig zf37b5.s1(495): Start
Rejected 8 oligos due to non uniqueness
Contig zf37b5.s1(495): End
No oligos found
Contig zf48g3.s1(315): Start
Pos 71, Dist 70, primer=16, Tm=52, match=75%, GCGTTTTACAATACTTCTC
Pos 80, Dist 79, primer=16, Tm=50, match=72%, AATACTTCTCAGGCAAC
Pos 69, Dist 68, primer=16, Tm=52, match=75%, GTGCGTTTTACAATACTTC
Pos 48, Dist 47, primer=20, Tm=50, match=72%, AAAATACCATTGCAGGCTC
### Probes found

<table>
<thead>
<tr>
<th>Contig zf37b5.s1(495): Start</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejected 6 oligos due to non uniqueness</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contig zf37b5.s1(495): End</th>
</tr>
</thead>
<tbody>
<tr>
<td>No oligos found</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contig zf48g3.s1(315): Start</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejected 1 oligo due to non uniqueness</td>
</tr>
</tbody>
</table>

The selected oligos can then be written to a file by filling in the "output filename" and will have OLIG tags created for them when the "Create tags" checkbutton is selected. This output window vanishes once OK is pressed, but the text in the main Output Window is left intact.
1.11 Calculating Consensus Sequences

In this section we describe the types of consensus which gap4 can produce, the formats they can be written in, and the algorithms that can be used. The algorithms are not only used to produce consensus sequence files, but in many other places throughout gap4 where an analysis of the current quality of the data is required. One important place is inside the Contig Editor (see Section 1.6 [Editing in gap4], page 58) where they are used to produce an "on-the-fly" consensus, responding to every edit made by the user.

The currently active consensus algorithm is selected from the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).

There are four main types of consensus sequence file that can be produced by the program: Normal, Extended, Unfinished, and Quality. They are all invoked from the File menu.

"Normal" is the type of consensus file that would be expected: a consensus from the non-hidden parts of a contig. "Extended" is the same as "Normal" but the consensus is extended by inclusion of the hidden, non-vector sequence, from the ends of the contig.

"Unfinished" is the same as "Normal" except that any position where the consensus does not have good data for both strands is written using A,C,G,T characters, and the rest (which has good data for both strands) is written using a different set of symbols. This sequence can be used for screening against new readings: only the regions needing more readings will produce matches. By screening readings in this way, prior to assembly, users can avoid entering readings which will not help finish the project, and which may require further editing work to be performed.

"Quality" produces a sequence of characters of the same length as the consensus, but they instead encode the reliability of the consensus at each point.

Consensus sequence files can also encode the positions of the currently active tag types by changing the case of the tagged characters (marking) or writing them in a different character set (masking) (see Section 1.2.7.2 [Active tags and masking], page 29).

The consensus algorithms are usually configured to produce only the characters A,C,G,T and ".", but it is possible to set them to produce the complete set of IUB codes. This mode is useful for some types of work and allows the range of observed base types at any position to be coded in the consensus. How the IUB codes are chosen is described in the introduction to the consensus algorithms (see Section 1.11.5 [The Consensus Algorithms], page 140).

Depending on the type of consensus produced, the consensus sequence files can be written in three different formats: Experiment files (see Section 10.3 [Experiment File], page 401), FASTA (Pearson, W.R. Using the FASTA program to search protein and DNA sequence databases. Methods in Molecular Biology. 25, 365-389 (1994)) or staden formats. If experiment file format is selected a further menu appears that allows users to select for the inclusion of tag data in the output file. For FASTA format the sequence headers include the contig identifier as the sequence name and the project database name, version number and the number of the leftmost reading in the contig as comments. e.g. ">xyzzy.s1 B0334.0.274" is database B0334, copy 0, and the left most reading for the contig is number 274, which has a name of xyzzy.s1. For staden format the headers include the project database name and the number of the leftmost reading in the contig. e.g. "<B0334.00274—→" is database B0334 and the left most reading for the contig is number 274. Staden format is maintained only for historical reasons - i.e. there may still be a few unfortunate people using it. Obviously Experiment file format can contain much more information, and can serve as the basis of a submission to the sequence library.
1.11.1 Normal Consensus Output

This is the usual consensus type that will be calculated (and is available from the gap4 File menu). The currently active consensus algorithm is selected from the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).

Contigs can be selected from a file of file names or a list. In addition, tagged regions can be masked or marked (see Section 1.2.7.2 [Active tags and masking], page 29), and output can be in Experiment file, fasta or staden formats. If experiment file format is selected a further menu appears that allows users to select for the inclusion of tag data in the output file.

The contigs for which to calculate a consensus can be a particular "single" contig, "all contigs", or a subset of contigs whose names are stored in a "file" or a "list". If a file or list is selected the browse button will be activated, and if it is clicked, an appropriate browser will be invoked. If the user selects "single" then the dialogue for choosing the contig, and the section to process, becomes active.

If the user selects either "mask active tags" or "mark active tags" the "Select tags" button is activated, and if it is clicked, a dialogue panel appears to enable the user to select which tag types should be used in these processes. If "mask" is selected all segments covered by the tag types chosen will not be written as ACGT but as defi symbols. If "mark" is selected the tagged segments will be written in lowercase characters. Masking is useful for producing a sequence to screen against other sequences: only the unmasked segments will produce hits.

The "strip pads" option will remove pads ("*"s) from the consensus sequence. In the case of experiment files this will also automatically adjust the position and length of the annotations to ensure that they still mark the correct segment of sequence.

The routine can write its consensus sequence (plus extra data for experiment files) in "experiment file", "fasta" and "staden" formats. The output file can be chosen with the aid of a file browser. If experiment file format is selected the user can choose whether or not to have "all annotations", "annotations except in hidden", or "no annotations" written out with the sequence. If the user elects to include annotations the "select tags" button will become active, and if it is clicked, a dialogue for selecting the types to include will appear.
1.11.2 Extended Consensus Output

This consensus type (which is available from the gap4 File menu) is useful for those who are too impatient to complete their sequence and want to compare it, in its fullest extent, to other data. The sequence produced therefore includes hidden data from the ends of the contigs.

The currently active consensus algorithm is selected from the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).

Contigs can be selected from a file of file names or a list. In addition tagged regions can be masked or marked (see Section 1.2.7.2 [Active tags and masking], page 29), and output can be in fasta or staden formats.

The contigs for which to calculate a consensus can be a particular "single" contig, "all contigs", or a subset of contigs whose names are stored in a "file" or a "list". If a file or list is selected the browse button will be activated, and if it is clicked, an appropriate browser will be invoked. If the user selects "single" then the dialogue for choosing the contig and the section to process becomes active.

Where possible the contigs are extended using the poor quality data from the readings near their ends. To ensure that this additional data is not too poor the program uses the following algorithm. It slides a window of size "Window size for good data scan" along the hidden data for each reading and stops if it finds a window that contains more than "Max dashes in scan window" non-ACGT characters. The data that extends the contig the furthest is added to its consensus sequence.

If the user selects either "mask active tags" or "mark active tags" the "Select tags" button is activated, and if it is clicked, a dialogue panel appears to enable the user to select which tag types should be used in these processes. If "mask" is selected all segments covered by the tag types chosen will not be written as ACGT but as defi symbols. If "mark" is selected the tagged segments will be written in lowercase characters. Masking is useful for producing a sequence to screen against other sequences: only the unmasked segments will produce hits.

The "strip pads" option will remove pads ("*"s) from the consensus sequence.
The routine can write its consensus sequence in "fasta" and "staden" formats. The output file can be chosen with the aid of a file browser.

### 1.11.3 Unfinished Consensus Output

This option is available from the gap4 File menu. An "Unfinished" consensus is one in which any position where the consensus does not have good data for both strands is written using A,C,G,T characters, and the rest (which has good data for both strands) is written using a different set of symbols (d,e,f,i). This sequence can be used for screening against new readings: only the regions needing more readings will produce matches. By screening readings in this way, prior to assembly, users can avoid entering readings which will not help finish the project, and which may require further editing to be performed. This type of consensus when written in staden format, consists of A,C,G,T for single stranded regions and d,e,f,i for finished sequence (d=a,e=c,f=g,i=t).

The currently active consensus algorithm is selected from the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).

Contigs can be selected from a file of file names or a list, and output can be in fasta or staden formats.

![Save consensus: unfinished dialog](image)

The contigs for which to calculate a consensus can be a particular "single" contig, "all contigs", or a subset of contigs whose names are stored in a "file" or a "list". If a file or list is selected the browse button will be activated, and if it is clicked, an appropriate browser will be invoked. If the user selects "single" then the dialogue for choosing the contig and the section to process becomes active.

The "strip pads" option will remove pads ("*s) from the consensus sequence.

The routine can write its consensus sequence in "fasta" and "staden" formats. The output file can be chosen with the aid of a file browser.

### 1.11.4 Quality Consensus Output

The Quality Consensus Output option described here (which is available from the gap4 File menu) applies either of the two simple consensus calculations (see Section 1.11.5.1 [Consensus Calculation Using Base Frequencies], page 142) and (see Section 1.11.5.2 [Consensus Calculation Using Weighted Base Frequencies], page 142) to the data for each strand of the DNA separately. The currently active consensus algorithm is selected from the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).
Chapter 1: Sequence assembly and finishing using Gap4

It produces, not a consensus sequence, but an encoding of the "quality" of the data which defines whether it has been determined on both strands, and whether the strands agree. The categories of data and the codes produced are shown in the table. For example 'c' means bad data on one strand is aligned with good data on the other.

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Good Good (in agreement)</td>
</tr>
<tr>
<td>b</td>
<td>Good Bad</td>
</tr>
<tr>
<td>c</td>
<td>Bad Good</td>
</tr>
<tr>
<td>d</td>
<td>Good None</td>
</tr>
<tr>
<td>e</td>
<td>None Good</td>
</tr>
<tr>
<td>f</td>
<td>Bad Bad</td>
</tr>
<tr>
<td>g</td>
<td>Bad None</td>
</tr>
<tr>
<td>h</td>
<td>None Bad</td>
</tr>
<tr>
<td>i</td>
<td>Good Good (disagree)</td>
</tr>
<tr>
<td>j</td>
<td>None None</td>
</tr>
</tbody>
</table>

The contigs for which to calculate a consensus can be a particular "single" contig, "all contigs", or a subset of contigs whose names are stored in a "file" or a "list". If a file or list is selected the browse button will be activated, and if it is clicked, an appropriate browser will be invoked. If the user selects "single" then the dialogue for choosing the contig and the section to process becomes active.

The routine can only write its consensus sequence in "staden" format. The output file can be chosen with the aid of a file browser.

1.11.5 The Consensus Algorithms

The consensus calculation is a very important component of gap4. It is used to produce an "on-the-fly" consensus, responding to every individual change in the Contig Editor (see Section 1.6 [Editing in gap4], page 58) and is used to produce the final sequence for submission to the sequence libraries. Some years ago Bonfield, J.K. and Staden, R. The application of numerical estimates of base calling accuracy to DNA sequencing projects. Nucleic Acids Res. 23, 1406-1410 (1995) we put forward the idea of using base call accuracy estimates in sequencing projects, and this has been partially realised with the values from the Phred program (Ewing, B. and Green, P. Base-Calling of Automated Sequencer Traces Using Phred. II. Error Probabilities. Genome Research. Vol 8 no 3. 186-194 (1998)). These values are widely used and have defined a decibel type scale for base
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call confidence values and gap4 is currently set to use confidence values defined on this scale. An overview of our use of confidence values is contained in the introductory sections of the manual (see Section 1.2.5 [The use of numerical estimates of base calling accuracy], page 26).

As is described elsewhere (see Section 1.11.6 [List Consensus Confidence], page 145) being able to calculate the confidence for each base in the consensus sequence makes it possible to estimate the number of errors it contains, and hence the number of errors that will be removed if particular bases are checked and, if necessary, edited.

Gap4 caters for base calls with and without confidence values and hence provides a choice of algorithms. There are currently three consensus algorithms that may be used. The choice of the best algorithm will depend on the data that you have available and the purpose for which you are using gap4.

The currently active consensus algorithm is selected from the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).

The only way to produce a consensus sequence for which the reliability of each base is known, is to use reading data with base call confidence values. Their use, in combination with the Confidence Value algorithm (see Section 1.11.5.3 [Consensus Calculation Using Confidence Values], page 143), is strongly recommended.

For base calls without confidence values use the Base Frequencies algorithm (see Section 1.11.5.1 [Consensus Calculation Using Base Frequencies], page 142). This is also a fast algorithm so it may be appropriate for very high depth assemblies such as for mutation studies.

For data with simple base call accuracy estimates rather than those on the decibel scale, the Weighted Base Frequencies algorithm should be used (see Section 1.11.5.2 [Consensus Calculation Using Weighted Base Frequencies], page 142).

All confidence values lie in the range 0 to 100. When readings are entered into a database, gap4 assigns a confidence of 99 to all bases without confidence values. For all three algorithms, a base with confidence of 100 is used to force the consensus base to that base type and to have a confidence of 100. However, if two or more base types at any position have confidence 100, the consensus will be set to "unknown", i.e. "-", and will have a confidence of 0. Note that dash ("-".) is our preferred symbol for "unknown" as, within a sequence, it is more easily distinguished from A,C,G,T than "N".

The consensus sequence is also assigned a confidence, even when base call confidence values are not used to calculate it. The scale and meaning of the consensus confidence changes between consensus algorithms. However the consensus cutoff parameter always has the same meaning. A consensus base with a confidence 'X' will be called as a dash when 'X' is lower than the consensus cutoff, otherwise it is the determined base type.

Both the consensus cutoff and quality cutoff values can be set by using the "Configure cutoffs" command in the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177). Within the Contig Editor (see Section 1.6 [Editing in gap4], page 58) these values can be adjusted by clicking on the "<" and ">" symbols adjacent to the "C:" (consensus cutoff) and "Q:" (quality cutoff) displays in the top left corner of the editor. These buttons are repeating buttons - the values will adjust for as long as the left mouse button is held down. Changing these values lasts only as long as that invocation of the contig editor.

The consensus algorithms are usually configured to produce only the characters A,C,G,T,* and ",", but it is possible to set them to produce the complete set of IUB codes. This mode is useful for some types of work and allows the range of observed base types at any position to be coded in the consensus. The IUB code at any position is determined in the following way.

We assume that the user wants to know which base types have occurred at any point, but may want some control over the quality and relative frequency of those that are used to calculate the
"consensus". For the simplest consensus algorithm there is no control over the quality of the base calls that are included, but the Consensus Cutoff can be used to control how the relative frequency affects the chosen IUB code. All base types whose computed "confidence" exceeds the Consensus Cutoff will be included in the selection of the IUB code. For example if only base type T reaches the Consensus Cutoff the IUB code will be T; if both T and C reach the cutoff the code will be Y; if A, C and T each reach the cutoff the code will be H; if A, C, G and T all reach the cutoff the code will be "N". For the Confidence Value algorithm the Quality Cutoff can be used to exclude base calls of low quality, so that all those that do not reach the Quality Cutoff are excluded from the IUB code calculation. Otherwise the logic of the code selection is the same as for the two simpler algorithms.

Both the consensus cutoff and quality cutoff values can be set by using the "Configure cutoffs" command in the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177).

The algorithms are explained below.

1.11.5.1 Consensus Calculation Using Base Frequencies

This algorithm can be used for any data, with or without confidence values. Each standard base type is given the same weight. The consensus will be the most frequent base type in a given column provided that the consensus cutoff parameter is low enough. All unrecognised base types, including IUB codes, are treated as dashes. Dashes are given a weight of 1/10th that of recognised base types. Pads are given a weight which is the average of their neighbouring bases.

The confidence of a consensus base for this method is expressed as a percentage. So for example a column of bases of A, A, A and T will give a consensus base of A and a confidence of 75. Therefore a consensus cutoff of 76 or higher will give a consensus base of ".-".

In the event that more than one base type is calculated to have the same confidence, and this exceeds the consensus cutoff, the bases are assigned in descending order of precedence: A, C, G and T.

The quality cutoff parameter (Q in the Contig Editor) has no effect on this algorithm.

1.11.5.2 Consensus Calculation Using Weighted Base Frequencies

This method can be used when simple, unquantified, base call quality values are available. Instead of simply counting base type frequencies it sums the quality values. Hence a column of 4 bases A, A, A and T with confidence values 10, 10, 10 and 50 would give combined totals of 30/80 for A and 50/80 for T (compared to 3/4 for A and 1/4 for T when using frequencies). As with the unweighted frequency method this sets the confidence value of the consensus base to be the the fraction of the chosen base type weights over the total weights (62.5 in the above example).

The quality cutoff parameter controls which bases are used in the calculation. Only bases with quality values greater than or equal to the quality cutoff are used, otherwise they are completely ignored and have no effect on either the base type chosen for the consensus or the consensus confidence value. In the above example setting the quality cutoff to 20 would give a T with confidence 100 (100 * 50/50).

In the event that more than one base type is calculated to have the same weight, and this exceeds the consensus cutoff, the bases are assigned in descending order of precedence: A, C, G and T.

This is Rule IV of Bonfield, J.K. and Staden, R. The application of numerical estimates of base calling accuracy to DNA sequencing projects. Nucleic Acids Research 23, 1406-1410 (1995).
1.11.5.3 Consensus Calculation Using Confidence values

This is the preferred consensus algorithm for reading data with Phred decibel scale confidence values. As will become clear from the following description, it is more complicated than the other algorithms, but produces a much more useful result.

A difficulty in designing an algorithm to calculate the confidence for a consensus derived from several readings, possibly using different chemistries, and hopefully from both strands of the DNA, is knowing the level of independence of the results from different experiments - namely the readings. Given that sequencing traces are sequence dependent, we do not regard readings as wholly independent, but at the same time, repeated readings which confirm base calls may give us more confidence in their accuracy. In addition, if we get a particularly good sequencing run, with consequently high base call confidence values, we are more likely to believe its base call and confidence value assignments. The final point in this preamble is that the Phred confidence values refer only to the probability for the called base, and they tell us nothing about the relative likelihood of each of the other 3 base types appearing at the same position. These difficulties are taken into account by our algorithm, which is described below.

In what follows, a particular position in an alignment of readings is referred to as a "column". The base calls in a column are classified by their chemistry and strand. We currently group them into "top strand dye primer", "top strand dye terminator", "bottom strand dye primer" and "bottom strand dye terminator" classes.

Within each class there may be zero or many base calls. For each class we check for multiple occurrences of the same base type. For each base type we find the highest confidence value, and then increase it by an amount dependent on the number of confirming reads. Then Bayes formula is used to derive the probabilities and hence the confidence values for each base type.

To further describe the method it is easiest to work through an example. Suppose we have 5 readings with the following characteristics covering a particular column.

| Dye primer, top strand, 'A', confidence 20 |
| Dye primer, top strand, 'A', confidence 10 |
| Dye primer, top strand, 'T', confidence 20 |
| Dye terminator, top strand, 'T', confidence 10 |
| Dye primer, bottom strand, 'A', confidence 5 |

Hence there are three possible classes.

Examining the "dye primer top strand" class we see there are three readings (A, A and T). The highest A is 20. We add to this a fixed quantity to indicate one other occurrence of an A in this set. For this example we add 5. Now we have an adjusted confidence of 25 for A and 20 for T. This is equivalent to a .997 probability of A being correct and .99 probability of T being correct. To use Bayes we split the remaining probabilities evenly. A has a probability of .997 and so the remaining .003 is spread amongst the other base types. Similarly for the .01 of the T. The result is shown in the table below.

<table>
<thead>
<tr>
<th>A</th>
<th>C</th>
<th>G</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>.997</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
</tr>
</tbody>
</table>

Bayesian calculations on this table then give us probabilities of approximately .766 for A, .00154 for C, .00154 for G and .231 for T.

The other classes give probabilities of .033 for A, C, G and .9 for T, and .316 for A, and .228 for C, G and T.
To combine the values for each class we produce a table for a further Bayesian calculation. Once again we fill in the probabilities and spread the remainder evenly amongst the other base types.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>C</th>
<th>G</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer Top</td>
<td>.766</td>
<td>.00154</td>
<td>.00154</td>
<td>.231</td>
</tr>
<tr>
<td>Term Top</td>
<td>.0333</td>
<td>.0333</td>
<td>.0333</td>
<td>.9</td>
</tr>
<tr>
<td>Primer Bot</td>
<td>.316</td>
<td>.228</td>
<td>.228</td>
<td>.228</td>
</tr>
</tbody>
</table>

From this Bayes gives the final probabilities of .135 for A, .0002 for C, .0002 for G and .854 for T. This is what would be expected intuitively: the T signal was present in both dye primer and dye terminator experiments with 1/100 and 1/10 error rates whilst the A signal was present on both strands with 1/100 and 1/3 error rates. Hence the consensus base is T with confidence 8.4 \((-10\times\log_{10}(1-.854))\).

If a padding character is present in a column we consider the pad as a separate base type and then evenly divide the remaining probabilities by 4 instead of 3.

1.11.5.4 The Quality Calculation

The Quality Calculation described here (which is available from the gap4 File menu) applies either of the two simple consensus calculations (see Section 1.11.5.1 [Consensus Calculation Using Base Frequencies], page 142) and (see Section 1.11.5.2 [Consensus Calculation Using Weighted Base Frequencies], page 142) to the data for each strand of the DNA separately. It produces, not a consensus sequence, but an encoding of the "quality" of the data which defines whether it has been determined on both strands, and whether the strands agree. This quality is used as the basis for problem searches, such as find next problem, and the Quality Display within the Template Display (see Section 1.5.1.5 [Quality Plot], page 44).

The categories of data and the codes produced are shown in the table. For example 'c' means bad data on one strand is aligned with good data on the other.

+Strand -Strand

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Good Good (in agreement)</td>
</tr>
<tr>
<td>b</td>
<td>Good Bad</td>
</tr>
<tr>
<td>c</td>
<td>Bad Good</td>
</tr>
<tr>
<td>d</td>
<td>Good None</td>
</tr>
<tr>
<td>e</td>
<td>None Good</td>
</tr>
<tr>
<td>f</td>
<td>Bad Bad</td>
</tr>
<tr>
<td>g</td>
<td>Bad None</td>
</tr>
<tr>
<td>h</td>
<td>None Bad</td>
</tr>
<tr>
<td>i</td>
<td>Good Good (disagree)</td>
</tr>
<tr>
<td>j</td>
<td>None None</td>
</tr>
</tbody>
</table>

the "Configure cutoffs" command in the

In the "Consensus algorithm" dialogue in the main gap4 Options menu (see Section 1.20.2 [Consensus Algorithm], page 177), setting the configuration to treat readings flagged using the "Special Chemistry" Experiment File line (CH field) (see Section 10.3 [Experiment File], page 401) affects this calculation. When set, the reading counts for both strands in the Consensus and Quality Calculations, and hence is equivalent to having data on both strands.
1.11.6 List Consensus Confidence

The Confidence Value consensus algorithm (see Section 1.11.5.3 [Consensus Calculation Using Confidence Values], page 143) produces a consensus sequence for which the expected error rate for each base is known. The option described here (which is available from the gap4 View menu) uses this information to calculate the expected number of errors in a particular consensus sequence and to tabulate them.

The decibel type scale introduced in the Phred program uses the formula \(-10 \times \log_{10}(error\_rate)\) to produce confidence values for the base calls. A confidence value of 10 corresponds to an error rate of 1/10; 20 to 1/100; 30 to 1/1000; etc.

So for example, if 50 bases in the consensus had confidence 10, we would expect those 50 bases (with an error rate of 1/10) to contain 5 errors; and if 200 bases had confidence 20, we would expect them to contain 2 errors. If these 50 bases with confidence 10, and 200 bases with confidence 20 were the least accurate parts of the consensus, they are the bases which we should check and edit first. In so doing we would be dealing with the places most likely to be wrong, and would raise the confidence of the whole consensus. The output produced by List Confidence shows the effect of working through all the lowest quality bases first, until the desired level of accuracy is reached. To do this it shows the cumulative number of errors that would be fixed by checking every consensus base with a confidence value less than a particular threshold.

The List Confidence option is available from within the Commands menu of the Contig Editor and the main gap4 View menu. From the main menu the dialogue simply allows selection of one or more contigs. Pressing OK then produces a table similar to the following:

<table>
<thead>
<tr>
<th>Value</th>
<th>Frequencies</th>
<th>Expected errors</th>
<th>Cumulative frequencies</th>
<th>Cumulative errors</th>
<th>Cumulative error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1/971</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.79</td>
<td>1.79</td>
<td>1.79</td>
<td>1/976</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1/976</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1.50</td>
<td>2.00</td>
<td>2.00</td>
<td>1/985</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>11.94</td>
<td>14.24</td>
<td>14.24</td>
<td>1/1061</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.63</td>
<td>3.63</td>
<td>3.63</td>
<td>1/1065</td>
</tr>
<tr>
<td>6</td>
<td>263</td>
<td>66.06</td>
<td>80.94</td>
<td>80.94</td>
<td>1/1867</td>
</tr>
<tr>
<td>7</td>
<td>151</td>
<td>30.13</td>
<td>111.06</td>
<td>111.06</td>
<td>1/2841</td>
</tr>
<tr>
<td>8</td>
<td>164</td>
<td>25.99</td>
<td>614.06</td>
<td>614.06</td>
<td>1/5168</td>
</tr>
<tr>
<td>9</td>
<td>96</td>
<td>12.09</td>
<td>710.14</td>
<td>710.14</td>
<td>1/8344</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>8.00</td>
<td>790.14</td>
<td>790.14</td>
<td>1/14069</td>
</tr>
</tbody>
</table>

The output above states that there are 164068 bases in the consensus sequence with an expected 169 errors (giving an average error rate of one in 971). Next it lists each confidence value along with its frequency of occurrence and the expected number of errors (as explained above, frequency \(x\) error\_rate). For any particular confidence value the cumulative columns state: how many bases in the sequence have the same or lower confidence, how many errors are expected in those bases, and the new error rate if all these bases were checked and all the errors fixed.

Above it states that there are 790 bases with confidence values of 10 or less, and estimates there to be 157 errors in those 790 bases. As we expect there to be about 169 errors in the whole consensus this implies that manually checking those 790 bases would leave only 12 undetected errors. Given that the sequence length is 164068 bases this means an average error rate of 1 in 14069. It is important to note that by using this editing strategy, this error rate would be achieved.
by checking only 0.48% of the total number of consensus bases. This strategy is realised by use of
the consensus quality search in the gap4 Contig Editor (see Section 1.6.6.7 [Search by Consensus
Quality], page 69).
1.12 Miscellaneous functions

1.12.1 Complement a Contig

This function (which is available from the gap4 Edit menu) is used to complement a contig, which means that it will complement and reverse all its readings and reorder them to produce a contig with the opposite orientation. It operates on a single contig selected via a dialogue box.

1.12.2 Enter Tags

This routine (which is available from the gap4 Edit menu) is used to add a set of tags (see Section 1.2.7 [Annotation readings and contigs], page 29) stored in a file, to the database. The file format (see below) is identical to the output produced by the "save tags to file" option of "Find Repeats". See Section 1.8.4 [Find Repeats], page 118. The format is a subset of the experiment file format. See Section 10.3 [Experiment Files], page 401. The two are close enough for Enter tags to use an experiment file as input. The only input required is the name of the file to read and a file browser can be used to aid its selection.

Note that "Enter tags" will remove any results plotted in the Contig Comparator.

The start of a typical file is shown below.

```
CC Repeat number 0, end 1
ID zf48g3.s1
TC REPT b 1031..1072
TC Repeats with contig zf48g3.s1, offset 957
```

```
CC Repeat number 0, end 2
ID zf48g3.s1
TC REPT b 957..998
TC Repeats with contig zf48g3.s1, offset 1031
```

```
CC Repeat number 1, end 1
ID zf48g3.s1
TC REPT b 1102..1130
TC Repeats with contig zf48g3.s1, offset 953
```

```
CC Repeat number 1, end 2
ID zf48g3.s1
TC REPT b 953..981
TC Repeats with contig zf48g3.s1, offset 1102
```
1.12.3 Show Relationships

This function (which is available from the gap4 View menu) is used to show the relationships of the gel readings in the database in three ways.

1. All contig descriptor lines followed by all gel descriptor lines.
2. All contigs one after the other sorted, i.e. for each contig show its contig descriptor line followed by all its gel descriptor lines sorted on position from left to right.
3. Selected contigs: show the contig line and, in left to right order, the gel readings. This can be done for a list or a file of contigs. For a single contig the output can be restricted to a user-defined region.

In the above illustration, a single contig, all contigs, a file or list of contigs can be selected. For a single contig, the contig identifier and range selector becomes enabled. Choosing a file or list enables the "browse" button which will invoke either the file or list browser respectively. When "all" contigs is selected a further choice is available: whether to 'Show readings in positional order'. This question determines whether to output in method 1 (No) or 2 (Yes) listed above.

The function is particularly useful for creating files or lists of reading names. To create a list of reading names run Show Relationships to produce the desired output to the Output Window. Then either use cut and paste from this window to a list editor, or use the right mouse button in the output window to request the "Output to list" option. In this latter case the header "CONTIG LINES" and "GEL LINES" lines should be removed (although most functions will happily ignore, with warnings, a list containing unknown reading names).

In the output window the reading names are underlined, indicating that they are hyperlinks. Double clicking on a name with the left mouse button will bring up the contig editor showing the start of that sequence, or it will move an existing contig editor to display that position. (You may wish to turn off the "Scroll on output" button if you do not wish the text output window to scroll to the bottom as it displays the "Edit contig" title.) Clicking on a reading name with the right mouse button will bring up a popup menu containing Edit contig, Template display, List reading notes and List contig notes.

Below is an example showing a contig from position 1 to 689. The left gel reading is number 6 and has archive name HINW.010, the rightmost gel reading is number 2 and has archive name HINW.004. On each gel descriptor line is shown: the name of the archive version, the gel number, the position of the left end of the gel reading relative to the left end of the contig, the length of the gel reading (if this is negative it means that the gel reading is in the opposite orientation to its archive), the number of the gel reading to the left and the number of the gel reading to the right.

<table>
<thead>
<tr>
<th>CONTIG LINES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTIG LINE LENGTH</td>
</tr>
<tr>
<td>LEFT RIGHT</td>
</tr>
<tr>
<td>NAME</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>HINW.010</td>
</tr>
<tr>
<td>HINW.007</td>
</tr>
<tr>
<td>HINW.009</td>
</tr>
<tr>
<td>HINW.999</td>
</tr>
<tr>
<td>HINW.017</td>
</tr>
<tr>
<td>HINW.031</td>
</tr>
<tr>
<td>HINW.004</td>
</tr>
</tbody>
</table>
1.12.4 Sequence Search

The purpose of this function (which is available from the gap4 View menu) is to find matches between the consensus sequence and short segments of sequence defined by the user. The segments of sequence (or "strings") can be typed into the dialogue provided or can be the sequences covered by consensus tag types (see Section 1.2.7.1 [Tag types], page 29) selected by the user. The latter mode hence provides a way of checking to see if a tagged segment of the sequence occurs elsewhere in the consensus. The function was previously known as "Find Oligos".

Users can elect to search against a "single" contig, "all contigs", or a subset of contigs defined in a list (see Section 1.14 [Lists], page 156) or a file. If "file" or "list" is selected the browse button is activated and gives access to file or list browsers. If they choose to analyse a single contig the dialogue concerned with selecting the contig and the region to search becomes activated.

Both strands of the consensus are scanned using a very simple algorithm: insertions and deletions are not allowed, but mismatches are. The "Minimum percent match" defines the smallest percentage match which will be reported by the algorithm. A value of 75 means that at least 75% of the bases must match the target sequence.

The user can elect to use tags or to specify their own sequences for the search. Selecting "Use tags" will activate the "Select tags" browse button. Clicking on this button will bring up a check box dialogue to enable the user to select the tags types they wish to activate. Alternatively selecting "Enter sequence" will activate a text entry box and the user can enter a string of characters. Only the characters ACGTU are allowed and there is no limit to the length of the string.

If it has not already occurred, selection of this function will automatically transform the Contig Selector into the Contig Comparator. See Section 1.4 [Contig Comparator], page 34. Each match found is plotted as a diagonal line in the Contig Comparator. The length of the diagonal line is proportional to the length of the search string. Self matches from the tag search are not reported.

If the match between the search string and the contig are in the same orientation, the diagonal match line will be parallel to the main diagonal, otherwise the line will be perpendicular to the main diagonal. Matches found between a tag and a contig can be used to invoke the Join Editor (see Section 1.6.14 [The Join Editor], page 87) or Contig Editors (see Section 1.6 [Editing in gap4], page 58). Matches between a specified sequence and a contig will only invoke the Contig Editor. All of the matches found are displayed in the Output Window e.g.
Match found between tag on contig 315 in the + sense and contig 495
Percentage mismatch 16.7

957 967 977 987 997
315 CATAAGGATTTCATATTTTATCCAGTTGGGGAATCTAT
     ::::::::::: :::::::::::::::::: ::::
495 GATTGGGATTTCATATTTTATCCAGTTGGGACCCTAAG
     2   12  22  32  42
1.12.5 Extract Readings

This function (which is available from the gap4 File menu) is used to produce copies of readings stored in the assembly database. The readings, and information about them, are written to disk in experiment file format (see Section 10.3 [Experiment file format], page 401) and will include any edits made and tags created. They are written in their original orientation. No change is made to the copies in the assembly database: this process creates copies and should not be confused with "Disassemble readings". See Section 1.9.1.2 [Disassemble Readings], page 125. The names of the readings to extract can be read from a list or a file of file names. Clicking on the browse button will invoke an appropriate browser dialogue. If just a single reading is to be assembled choose "single" and enter the filename instead of the file or list of filenames. The files are written into the "Destination directory" with their original file names.

If required, the files will include additional information suitable for processing by either "Enter pre-assembled data" or "Directed assembly" (see Section 10.3.1 [Experiment file format explained], page 401). Both contain the ON and AV Experiment File records. Pre-assembled data also contains SE and PC records whilst Directed assembly contains AP records. It is recommended that Directed Assembly format is always used in preference to the Preassemble format.

To merge databases use the "Directed assembly" format to output the contigs required. Then, within the database you wish to merge the data use the Directed Assembly (see Section 1.7.2 [Directed Assembly], page 99) command. By using Directed Assembly with new blank databases it is also possible to create database subsets or to split databases.
1.12.6 Automatic Clipping by Quality and Sequence Similarity

Our consensus calculation algorithms use the data for all the unclipped bases covering each position in a contig. However, some assembly engines may leave the ends of readings unaligned, and these unaligned bases could therefore lead to the production of an incorrect consensus. The two clipping methods described here (which are available from the gap4 Edit menu) are designed to overcome this potential problem.

In addition to improving the reliability of the consensus calculation, clipping in this way tidies up the alignments, so helping the user to concentrate on the better data. It is important to note that in no case is the clipped sequence thrown away. The contig editor can show this hidden data, and the clip points may be manually adjusted to reveal any clipped sequence.

1.12.6.1 Difference Clipping

The difference clipping method (which is available from the gap4 Edit menu) works in stages. First it calculates the most likely consensus sequence. Then it compares each reading with that consensus sequence and identifies areas at the ends of the reading where there are enough differences to indicate the possibility of badly aligned bases. The clip points are adjusted accordingly.

To identify the clip points for each reading the algorithm first finds a good matching segment near the middle of the reading. Then steps, base by base, from this point to the left accumulating a score as it goes by using +1 for a match and -2 for a mismatch. It sets the left clip point at the position of the highest score. The right clip point is set in an equivalent way. These new clip points are used only if they are more severe than the existing ones. The portions of readings which have been clipped are then tagged using a DIFF tag type. To see which segments have been clipped use the contig editor search tool.

After clipping the algorithm then identifies any holes (breaks in the contigs) that may have been created and fills them up again by extending the sequence(s) with the fewest number(s) of expected errors.
1.12.6.2 Quality Clipping

The quality clipping function (which is available from the gap4 Edit menu) clips the ends of readings when the average (over 31 bases) confidence value is lower than a user defined threshold. As with the difference clipping method the clips are only adjusted when the newly calculated clip points are more stringent than the originals.

After clipping Gap4 then identifies any holes (breaks in the contigs) that may have been created and fills them up again by extending the sequence(s) with the fewest number of expected errors.

An example output follows.

Hole from 32652 to 32725: extend #1378 and #1385 with 3.157324 expected errors

We have observed that when using confidence values expressed as -10*log(err_rate), it is sometimes better not to clip using the confidence values, but to use the difference clipping method (see Section 1.12.6.1 [Difference Clipping], page 153).
1.13 Results Manager

Some commands within gap4 produce "results" that are updated automatically as data is edited. The Result Manager provides a way to list these results, and to interact with them.

A result is an abstract term used to define any collection of data. Typically this data can be displayed, manipulated and is usually updated automatically when changes are made that affect it. In gap4 each set of matches from a particular search plotted on the Contig Comparator (see Section 1.4 [Contig Comparator], page 34) is a result, as are entire displays such as the Template Display.

The "results" window, shown above, can be invoked either from the View menu in the main display or from the View menu of the Contig Comparator. Each result is listed in the window on a separate line containing the time that the result was created (which may not be the same as when it was last updated), the name of the function that created the result, and the result number. The number is simply a unique identifier to help distinguish two results produced by the same function.

Each item in the list is consuming memory on your computer. Running functions over and over again without removing the previous results will slow down your machine and it will, eventually, run out of memory. Removing items from the list solves this.

Pressing the right mouse button over an listed item will display a popup menu of operations that can be performed on this result. The operations available will always contain "Remove" which will delete this result and shut down any associated window, but others listed will depend on the result selected. In the illustration above the popup menu for the "Repeat search" can be seen. Here the operations relate to a set of repeat matches currently being displayed in the Contig Comparator (not shown).

The Contig Comparator functions ("Find internal joins", "Find read pairs", "Find repeats", "Check assembly" and "Find Sequences") are all listed in the Results Manager once per usage of the function. It is worth remembering that the only places to completely remove the plots from one of these functions is using the "Remove" command within the Results Manager or to use the "Clear" button within the Contig Comparator to remove all plots.
Chapter 1: Sequence assembly and finishing using Gap4

1.14 Lists

For many operations it is convenient to be able to process sets of data together - for example to calculate a consensus sequence for a subset of the contigs. To facilitate this gap4 uses lists.

Most gap4 commands dealing with batches of files or sets of readings or contigs can use either files of filenames or lists. When selecting list names from within dialogues the "browse" button will display a window containing all the currently existing lists. To select a list simply double click on the list name. Alternatively the name may simply be typed in.

The List menu on the main menubar contains commands to Edit, Create, Delete, Copy, Load, and Save lists. Some of these display a list editor. This is simply a scrollable text window supporting simple editing facilities (see Section 9.1.3 [Text Windows], page 375).

The "Clear" button clears the list. The "Ok" button removes the list editor window. It is not necessary to use "Ok" here before supplying the list name for input to another option.

1.14.1 Special List Names

Some lists are automatically updated or are generated on-the-fly as needed. The lists named "contigs" and "readings" correspond to the currently selected contigs in the contig selector window and the currently selected readings in the template displays. Note that lists (with any names) can also be created from selected items in the contig editor. See Section 1.6.8.13 [Set Output List], page 79. The "allcontigs" and "allreadings" lists are created as needed and always contain an identifier for every contig and every reading identifier.

Because of the way the lists are implemented, as is outlined below, there are some useful "tricks" that can be employed. A list name consisting of a contig identifier surrounded by square brackets ('[' and ']') will cause the creation of a list containing all of the readings within that contig. For example, to use the Extract Readings option (see Section 1.12.5 [Extract Readings], page 152) to extract all the readings from contig 'xb54f8.s1', the list name given in the Extract Readings dialogue would be '[xb54f8.s1]'..

A list name surrounded by curly brackets ('{ and '}') will cause the creation of a list containing all of the readings in the contigs named in the specified list name. So '{contigs}' is equivalent to all the readings in the contigs contained in the 'contigs' list. Hence the 'allreadings' list is identical to '{allcontigs}'.

These tricks can be used anywhere where a list name is required except for editing and deletion of lists. As a final example, to produce a file of filenames for the currently selected contigs, save the list named '{contigs} to file.'

1.14.2 Basic List Commands

The basic operations that can be performed on lists include copying, loading, saving, editing, creation and deletion. Joining and splitting can only be performed using the list editors and using cut and paste between windows.

The Load and Save commands require a list name and a file name. If only the name of the file is given the list is assumed to have the same name. If it is desired to load or save a list from/to a file of a different name then both should be specified. Creating a list that already exists (or loading a file into an already existing list) is allowed, but will produce a warning message.

1.14.3 Contigs To Readings Command

This command produces a list or file of reading names for a single contig or for a set of contigs. The user interface provides a dialogue to select the contigs and to select a list name or filename.
1.14.4 Minimal Coverage Command

This command produces a minimal list of readings that together span the entire length of a contig. The dialogue allows contigs names to be defined using a list or a file of filenames. The output produced, can be sent to a list or a file of filenames. An example use of this function is to determine a minimal set of overlapping readings for resequencing.

1.14.5 Unattached Readings Command

This command finds the contigs that consist of single readings. The output can be written to a list or a file of filenames. One example use of the option is for tidying up projects by removing the trivial and unrequired contigs. In this case the list would be used as input to disassemble readings (see Section 1.9.1.2 [Disassembling Readings], page 125).

1.14.6 Search Sequence Names

This command allows searching for sequences matching a given pattern. The function produces both a list in the text output window and a gap4 "list" of reading names. The highlighted output is clickable, with the left mouse button invoking the contig editor and the right mouse button displaying a popup-menu allowing additional operations (contig editor, template display, reading notes and contig notes).

The text search may be performed as either case-sensitive or case-insensitive. Additionally the pattern search types are available.

- **sub-string**  Matches any reading name where the pattern matches all or part of the name.
- **wild-cards**  Searches for a pattern using normal filename wild-card matching syntax. So * matches any sequence of characters, ? matches any single character, [chars] matches a set of characters defined by chars, and \char matches the literal character char. Character sets may use a minus sign to match a range. For example x*.\[fr\][1-9] matches any name starting with x and ending with fullstop followed by either f or r followed by a single digit between 1 and 9 inclusive. To match a substring using wild-cards prepend or append the search string with *
- **regular expression**  This uses the Tcl regular expression syntax to perform a match. These patterns are naturally sub-strings unless anchored to one or both ends using the ^expression$ syntax. A full description of regular expressions is beyond the scope of this manual.

1.14.7 Search Template Names

This searches for template names matching a given pattern. The gap4 list produce will contain just the template names, but the information listed in the text output window lists the template names and the readings contained within each template. The reading names are hyperlinks and so double left-clicking on them will bring up the contig editor whilst right-clicking brings up a popup menu.

For a description of the types of template search patterns see Section 1.14.6 [Search Sequence Names], page 157.

1.14.8 Search Annotation Contents

This searches the contents of annotations on both the individual reading sequences and the consensus sequences. A gap4 list will be produce containing the annotation number, contig and position. In the text output window a more complete description is available listing the annotation
type and the contents of each annotation. Both the list and text-output window will contain a
highlighted section which is a hyperlink. Double clicking on this with the left mouse button will
bring up the contig editor at that point. Clicking with the right mouse button will display a
popup-menu with further options.

For a description of the types of annotation search patterns see Section 1.14.6 [Search Sequence
Names], page 157.
1.15 Notes

A ‘Note’ is an arbitrary piece of text which can be attached to any reading, any contig, or to a database as a whole. Each note also contains a note type, a creation date and a modification date. Any number of notes can be attached to each reading, contig or database. They can be considered as positionless tags.

1.15.1 Selecting Notes

The primary interface to creating, viewing and editing notes is the Note Selector window. This is accessible from a variety of places, including anywhere a contig or reading name (or line in a graphical plot) is displayed, and also by using the "Edit Notebooks" command in the main gap4 Edit menu.

The Note Selector initially starts up showing the database notes (unless selected from a specific contig or reading plot). The picture above shows three notes attached to the main gap4 database record. These are of type OPEN and RAWD, both of which have a specific meaning to gap4, and type COMM.

The View Menu is used to see a list of notes for readings or contigs. If Reading Notes or Contig Notes is selected, the interface will ask for a reading or contig identifier by adding an extra line to the Note Selector Window, just beneath the menus. Typing one in and pressing return will then list the notes for that reading or contig.

To speed up selection, it is possible to use the right mouse button on the Contig Selector Window and in the contig rulers at the bottom of many plots (such as the Template Display), to select the "List Notes" option. This will start the Note Selector if it is not already running, and will direct it to display notes for the desired contig. Similarly, the right mouse button can be used to popup a menu from a reading in the Template Display or from a reading name in the Contig Editor.

To edit a note, double click anywhere in the Note Selector on the line for the note.

To delete a note, single click on the note line to highlight it and then select "Delete" from the Note Selector Edit menu. To delete several notes at once, first highlight a range by left clicking and dragging the mouse to mark a region of notes, and then use Delete. Alternatively notes may be deleted by double clicking to bring up the note editor and selecting Delete from the Note Editor File menu.

To create a new note use the "New" command from the Edit menu. The note will be added to whatever data type is currently shown. To create a note for a particular contig, select that contig using the Contig Notes option in the View menu, and then use New to create a new note. New notes will have type COMM and the contents can be in any format.
1.15.2 Editing Notes

Double clicking on a note in the Note Selector, or creating a new note, will bring up the Note Editor Window. This is a simple text editor, allowing use of keyboard arrow keys and the mouse to position and edit text. It also has keyboard bindings for many of the simple emacs movement commands.

At the top of the Notes Editor are three buttons. The leftmost is the File menu which contains the "Save", "Delete" and "Exit" options. Next to this is the Type selector. This menu name displays the currently selected note type. To change the note type select the appropriate type from the Type menu. The final button gives access to the online Help.

Listed underneath the menu are the creation and modification dates. The creation date is fixed when a note is created. The modification date is adjusted every time a note is edited. (Simply viewing a note will not update the modification date, but saving changes to it will.)

Underneath these is the note text itself. For convenience, the first line of each note is shown in the note selector window (so it can be helpful to make it identifiable).

1.15.3 Special Note Types

Several types of note have special meanings. These include the OPEN, CLOS and RAWD note types.

OPEN CLOS

Notes of type OPEN and CLOS should contain pure Tcl code. If they exist, they will be executed when the database is opened (OPEN) and closed (CLOS). Take great care in creating and editing a note with these types! The purpose is to allow configuration options to be attached to a database, and hence allow for different gap4 configurations to be used when a UNIX directory contains more than one database. In general use of the '.gaprc' file (see Section 1.20.1 [Options Menu], page 176) is probably safer.

If there is a problem with a database containing a malformed OPEN or CLOS note, it may be opened using gap4 -no_exec_notes. This will prevent gap4 from executing the OPEN and CLOS notes and so allow them to be fixed using the Note Editor.
This note specifies an alternative to the RAWDATA environment variable and should be set to be the full directory name for the location of the trace files for the database. If both the environment variable and the note are exist then the note will take priority. This automatic use of this note can be disabled be using the `-no_rawdata_note` command line option to gap4.

When created on a reading or a contig, this note may be displayed in the contig editor "information line" (see Section 1.6.13 [The Editor Information Line], page 84) when the user moves the mouse over the editor sequence name list.

It is possible to create your own types by editing the `$STADENROOT/tables/NOTEDB` file. The format is fairly self explanatory, and is very similar to the `GTAQDB` file. Each note type should consist of the long name followed by a colon and `id=4_letter_short_name`, optionally followed by `dt="any default text for this note"`. Lines may be split at colons by adding a backslash to the end of the line. See the standard `NOTEDB` file for examples.
1.16 Gap4 Database Files

Gap4 stores the data for each sequencing project (e.g. the data for a single cosmid or BAC) in a gap4 assembly database, so at the start of a sequencing project the user should employ gap4 to create the database for the project (see Section 1.16.2 [Opening a New Database], page 163). New database are created with sufficient index space for around 8000 readings, but this can be extended if required.

Gel reading data in experiment file format (see Section 10.3 [Experiment File Format], page 401) is entered into the database using the methods available from the assembly menu (see Section 1.7 [Entering Readings into the Database (Assembly)], page 94).

To assemble more data for the project or to edit or analyse readings already entered the user should open the same project database (see Section 1.16.3 [Opening an Existing Database], page 163).

Although the database files are designed to be free of corruption it is advisable to make regular backups (see Section 1.16.4 [Making Backups of Databases], page 163).

Database names can have from one to 240 letters and must not include a full stop or spaces. The database itself consists of two files; a file of records and an index file. If the database is called ‘FRED’ then version 0 of the database comprises the pair of files named ‘FRED.0’ and ‘FRED.0.aux’, the latter of these being the index file. The "version" is the character after the full stop in these filenames. Versions are not limited to numbers alone, but must be single characters.

When a database is opened for writing a ‘BUSY’ file is created. For the ‘FRED’ database this will be named ‘FRED.0.BUSY’. When the database is closed the file is deleted. The file is used by gap4 to signify that the database is opened for writing and is part of its mechanism to prevent more than one person editing a database at any time. Before opening a database for writing, gap4 checks to see if the BUSY file for that database exists. If it does the database is opened only for reading, if not it creates the file, so that any additional attempts to open the database for writing will be blocked. A side effect of this mechanism, is that in the event of a program or system crash the BUSY file will be left on the disk, even though the database is not being used. In this case users must remove the BUSY file (after checking that it really isn't in use!) using, on UNIX the `rm` command before opening the database. Eg "rm FRED.0.BUSY". On Windows use the Recycle Bin.

The gap4 database is robustly designed. Killing the program whilst updating the database should never yield an inconsistent state. A "roll-back" mechanism is utilised to undo any partially written updates and revert to the last consistent database. Hence quitting abnormally may result in the loss of some data. Always quit using the Exit command within the File menu.

However it is advised that copies of the database are made regularly to safeguard against any software bugs or disk corruptions.

1.16.1 Directories

By default, Gap4 expects files to be in the current directory. In dialogues which request filenames, full pathnames can be specified, however it is generally tidier to keep files specific to a particular project in the same directory as the project database. Creating new databases and opening new databases will change directory to the directory containing the opened project.

It is possible to change the current directory by selecting "Change directory" from the File menu. Be warned that changing to a directory other than that containing the database and the trace files may mean that gap4 can no longer find the trace files. The solutions to this problem are discussed elsewhere (see Section 1.20.8 [Trace File Location], page 179).
1.16.2 Opening a New Database

To create a new gap4 database select the "New" command from the File menu. This brings up a dialogue prompting the new filename. Type the name of the database to create without specifying the version number. To create version 0 of a database named 'FRED' typing FRED will create the two database files, ‘FRED.0’ and ‘FRED.0.aux’.

If the database already exists you will be asked whether you wish to overwrite it. Any database that was already open will be closed before the new database is created. The new database is then opened, ready for input.

Note that Gap4 database names are case sensitive.

1.16.3 Opening an Existing Database

To open an existing database select the "Open..." command from the File menu. This brings up a file browser where the database name can be selected. The databases will be listed in a NAME.V notation (where V is the version number). Double clicking on the database name will then open this database.

If the program already had a database open it will close it before the new one is opened. If the new database is already in use by gap4 a dialogue will appear warning you that the database has been opened in read only mode. This mode prevents any edits from being made to the database by greying out certain options and disabling the editing capabilities in the contig editor.

A database may also be opened by specifying the database name and version on the unix command line. To open version 0 of the database ‘FRED’ use "gap4 FRED.0".

1.16.4 Making Backups of Databases

The importance of making regular backups of your data cannot be over stated. Using the "Copy database" command from the File menu brings up a dialogue asking for a new database version. Type in a single character for the new version and press "ok" or return. If the new database already exists you will be asked whether you wish to overwrite it. Any subsequent changes you make will still be to the database that you originally opened, not to the database you have just saved to.

The database file may sometimes become fragmented. An option available when saving is to use garbage collection. This creates the new database by only copying over the used portions of data (and hence reduces fragmentation). However it is quite a lot slower than the standard "Copy database" mechanism, so if this causes problems add "set_def COPY_DATABASE.COLLECT 0" to your ‘.gaprc’ file to change the default to no garbage collection. It should be noted that garbage collection also performs a rigorous database consistency check.

Do not always use the same version character for your backups. Instead keep several different backups. Otherwise you may find that both your current database and the backup have problems. It is also wise to run "check database" to verify data integrity. See Section 1.18 [Check Database], page 168.

It is also possible to backup databases from outside gap4 by using standard unix commands to copy both the record and index files. Care should be taken when doing this to ensure that the database is not being modified whilst copying. See your unix or Windows manuals for further details or the copy_db manual page (see Section 11.2 [Copy_db], page 420) for the external garbage collecting database copy program.
1.16.5 Reading and Contig Names and Numbers

For various reasons there are restrictions on the characters used in file names and the length of the file names.

Characters permitted in file names:

ABCDEFGHIJKLMNOPQRSTUVWXYZabcdefghijklmnopqrstuvwxyz0123456789._-

A reading name or experiment file name must not be longer than 40 characters.

These restrictions also apply to SCF files which means, in turn, also to the names given to samples obtained from sequencing instruments. For example do not give sample names such as 27/OCT/96/r.1 when using and ABI machine: the / symbols will be interpreted as directory name separators on UNIX!

As each reading is entered into a project database it is given a unique number. The first is numbered 1, the second 2 and so on. Their reading names are read from the ID line in the experiment files and copied into the database. As new readings are created and existing ones removed the reading numbers change in an unpredictable fashion. Hence when taking notes on a project always record the reading name instead of the reading number.

The maximum number of readings a database can hold is 99,999,999.

Many options ask for a reading or contig identifier. A contig identifier is simply any reading name or number within that contig. A reading identifier is either the reading name or the hash ("#") character followed by the number. For example, if the reading name is fred.gel with number 99 users could type "fred.gel" or "#99" when asked to identify the contig.

Generally when prompting for a contig or reading name a default is supplied. This is the last name you used, or if you've only just opened the database, the name of the longest contig in the database. For more information about selecting contigs within the program see See Section 1.3.1 [Selecting Contigs], page 31..
1.17 Copy Readings

1.17.1 Introduction

During large scale sequencing projects where the genome is cloned into e.g. BACs prior to being subcloned into sequencing vectors it is generally the case that the ends of the DNA from one BAC will overlap that of two other BACs. Unless it is being used for quality control, it is a waste of time to sequence the overlapping regions twice, and so most labs transfer the relevant data between the adjacent gap4 databases. This is the function of copy_reads which copies readings from a "source" database to a "destination" database.

The consensus sequences for user selected contigs in each of the two databases are compared in both orientations. If an overlapping region is found, readings of sufficient quality are automatically assembled into the destination database. In the source database readings which have been added to the destination database will be tagged with a "LENT" tag and the equivalent readings in the destination database will be tagged with a "BORO" (borrowed) tag.

1.17.1.1 Copy Reads Dialogue

The Copy reads function is available from either the File menu of gap4 or from the command line.
The program must be able to write to both databases. It is recommended that you create backups of both databases before commencing using "Copy database". See Section 1.16.4 [Making Backups of Databases], page 163.

From within gap4: The source database must be entered into the "Open source database" entry box at the top of the dialogue box. The adjacent Browse buttons will list only gap4 databases, that is files ending in aux. Either select from the browser by double clicking on the name or type in the database name. The ending of .aux is ignored. The destination database is always the database which is currently open in gap4.

The location of the traces of the source database can either be determined from the rawdata note (see Section 1.20.8 [Trace File Location], page 179) held within the database ("read from database") or can be entered via the "directory" option. The program will add the location of the source traces into the rawdata note of the destination database. If the environment variable RAWDATA is set, this will be taken to be the location of the destination database traces and will also be added to the rawdata note of the destination database. If there are no traces for the source database, no rawdata note will be created.

One or more contigs from the source database can be compared. These are selected either by clicking on "all contigs" or providing a file containing a list of contig names (any reading name from within that contig, typically the first reading name). Only contigs over a user defined length will be used. A minimum reading quality can be set so that only readings with an average quality over the specified amount will be entered into the destination database.

Contigs from the destination database can be chosen by either selecting "all contigs" or providing a file of contig names.

The consensus sequence is determined for each contig in both databases using either the standard consensus algorithm or "Mask active tags". The latter option will activate the "Select tags" button. Clicking on this button will bring up a check box dialogue to enable the user to select the tags types they wish to activate. Masking the active tags means that all segments covered by tags that are "active" will not be used by the matching algorithms. A typical use of this mode is to avoid finding matches in segments covered by tags of type ALUS (ie segments thought to be Alu sequence) or REPT (ie segment that are known to be repeated elsewhere in the data (see Section 1.2.7.1 [Tag types], page 29).

The consensus searching parameters are equivalent to those found in the find internal joins algorithm (see Section 1.8.3 [Find Internal Joins], page 114). The search algorithm first finds matching words of length "Word length", and only considers overlaps of length at least "Minimum overlap". Only alignments better than "Maximum percent mismatch" will be reported. Find internal joins has the option of either a quick or sensitive algorithm. Here, it is only necessary to use the quick algorithm. The quick algorithm can find overlaps and align 100,000 base sequences in a few seconds by considering, in its initial phase only matching segments of length "Minimum initial match length". However it does a dynamic programming alignment of all the chunks between the matching segments, and so produces an optimal alignment. A banded dynamic algorithm can be selected, but as this only applies to the chunks between matching segments, which for good alignments will be very short, it should make little difference to the speed. The alignments between the consensus sequences can be displayed in the text output window by selecting "Display consensus alignments".

If a match between two consensus sequences is found, the readings in that overlap are assembled into the destination database using the "directed assembly" function (see Section 1.7.2 [Directed Assembly], page 99). Only readings for which the "Maximum percent mismatch" is not exceeded, and which have an average reading quality higher than the specified minimum, will be entered into the database. Again, the alignments can be shown in the Output window by selecting "Display sequence alignments".

From the command line:
copy_reads [-win] [-source_trace_dir ("")] [-contigs_from <file> (all contigs)] [-min_contig_len (2000)] [-min_average_qual (30.0)] [-contigs_to <file> (all contigs)] [-mask <none mask> (none)] [-tag_types <list> ("")] [-word_length (8)] [-min_overlap (20)] [-max_pquality (30.0)] [-min_match (20)] [-band (1)] [-display_cons] [-align_max_mism (10.0)] [-display_seq] [source database] [destination database]

The values in brackets () are the default values. The only mandatory values are the source and destination databases. Details on these values are given in the copy_reads man page (see Section 11.3 [Copy reads], page 421).

The -win option will bring up a new program which presently only has one function (copy reads). This is accessed from the "File" menu. This brings up a dialogue the same as that from within gap4 except for an extra entry box to select the destination database.
Chapter 1: Sequence assembly and finishing using Gap4

1.18 Check Database

This function (which is available from the gap4 File menu) is used to perform a check on the logical consistency of the database. No user intervention is required. If the checks are passed the message "Database is logically consistent" is written to the Output Window. If the database is not found to be consistent diagnostic messages will appear in the Output Window and Doctor Database from the Edit menu should be used to correct the problem. See Section 1.19 [Doctor Database], page 171.

Several options, such as assembly, automatically perform a check database prior to executing. If the database is found to be inconsistent the option will not continue. However some checks are considered as "non fatal" and will not block such operations. Currently the only non fatal checks are the positional checks for annotations and for readings that are never used. To fix the database, use the Doctor Database "ignore check database" setting to disable the inconsistency checking. See Section 1.19.2 [Ignoring Check Database], page 174.

The following sections define the checks and the order in which they are performed.

1.18.1 Database Checks

- Number of contigs used is \( \leq \) number allocated
- Disk and memory values for "number of contigs" are consistent
- Number of readings used is \( \leq \) number allocated
- Disk and memory values for "number of readings" are consistent
- Disk and memory values for "actual database size" are consistent
- Actual database size \( \leq \) maximum size
- Data_class is either DNA(0) or protein(1).
- Number of free annotations \( \geq \) 0 and \( \leq \) number allocated
- Contig order is consistent
- Number of free notes \( \geq \) 0 and \( \leq \) number allocated
- First note has prev_type as GT_Database
- Detect note loops

1.18.2 Contig Checks

- Has a left reading number
- Has a right reading number
- The left reading has no left neighbour
- The right reading has no right neighbour
- Chain right to
  - check loops
  - check holes
  - flag a reading as used
- When finished chaining
  - check length is correct
  - check right reading number is correct
- Reference only valid reading numbers
- Chain left to
  - check loops
− flag readings as used, if not done so in right chaining;
• When finished chaining, check left reading number is correct
• Chain along annotation list to
  − flag as used
  − detect annotation loops
  − annotation is within the contig
  − annotation is rightwards of previous
• First note has prev_type as GT_Contigs
• Detect note loops

1.18.3 Reading Checks
• Memory and disk values tally for
  − left neighbour
  − right neighbour
  − relative position
  − length + sense
• Left neighbour is a valid reading number
• Right neighbour is a valid reading number
• Reading is not used zero times
• Reading is not used more than once
• Hand holding: \(\text{lnbr}[\text{rnbr}[\text{reading}]] == \text{reading}\)
• Relative position of reading \(>=\) position of left neighbour
• Length \(!=\) 0
• Used sequence length \(!=\) "right clip position" - "left clip position"
• Has valid strand (0 or 1)
• Has valid primer
• Has valid sense (0 or 1)
• Chain along annotation list to
  − flag as used
  − detect annotation loops;
  − annotation is rightwards of previous
• First note has prev_type as GT_Readings
• Detect note loops

1.18.4 Annotation Checks
• No loops in free annotation list
• Is neither used nor is on the free list
• Annotation is not used more than once
• Is used, yet is still on the free list
• Length \(!=\) 0
• Has valid strand (0 or 1)
1.18.5 Note Checks
- No loops in free note list
- Is neither used nor is on the free list
- Hand holding: (note->next->prev == note)
- Note is not used more than once
- Is used, yet is still on the free list

1.18.6 Template Checks
- Minimum insert length $\leq$ maximum insert length
- Has valid vector
- Has valid clone
- Has valid strand

1.18.7 Vector Checks
- Level $> 0$
- Level $\leq$ MAX.LEVEL \( (\text{MAX.LEVEL currently is } 10; \text{ a } "\text{feasibility}" \text{ check}) \)

1.18.8 Clone Checks
- Has valid vector
1.19 Doctor Database

Doctor Database (which is available from the gap4 Edit menu) is used to make arbitrary changes to the database. It should be extremely unlikely that its use will be required, and if so, is for experts only. Very few checks are performed on the user’s input and there are few limitations on what can be done. Consequently, this option should never be used without first making a backup using "Copy database". See Section 1.16.4 [Making Backups of Databases], page 163. It is very easy to create inconsistencies within the database. Do not feel that values (such as the maximum gel reading length) can be safely changed simply because they are shown in a dialogue.

The main window consists of a menubar containing "File", "Structures" and "Commands" menus. The menus contain:

- File
  - New
  - Quit

- Structures
  - Database
  - Reading
  - Contig
  - Annotation
  - Template
  - Original clone
  - Vector
  - Note

- Commands
  - Check
  - Ignore check database
  - Extend structures
    - Reading
    - Annotation
    - Template
    - Clone
    - Vector
  - Delete contig
  - Shift readings
  - Reset contig order
  - Output annotations to file
  - Delete annotations

The New command in the Commands menu brings up another Doctor Database window complete with its own menubar. This is useful for comparing structures. Whilst Doctor Database
is running all other program dialogues, including the main gap4 menubar, are blocked. Control is reenabled once the last Doctor Database window is removed. Remember to perform a Check Database (Commands menu) before quitting to double check for database consistency.

1.19.1 Structures Menu

The gap4 database consists of records of several predefined types. The types correspond to the commands available within the Structures menu. All of these, except for the "Database" command, insert a dialogue between the menubar and whatever is underneath it. In the picture below we have selected "Annotations" from the menu which has prompted for "Which annotation (1-380)" (the 1-380 is the valid range of inputs available).

In the panel beneath the "Which annotation" question is a panel detailing another annotation structure. In general the structure type and number are shown at the top of the panel (in this case annotation number 100). Beneath this are the structure fields on the left followed by the values for these fields on the right. Sometimes gap4 may store a value as numeric, but display the structure as both a numeric and a string describing this value. For instance here the annotation strand is "1" which is gap4's way of storing "reverse".

Some values have an arrow next to them, such as with the "next" field in the illustration. Clicking on this arrow will display the structure referenced by this value. Here it is another annotation (annotation 357). It is stated that the annotation is part of Contig number 6. Clicking on the arrow next to this will reveal that contig structure.

Selected notes on editing the structures follows.

1.19.1.1 Database Structure

There is only a single Database structure. A description of its more important fields follows.

num_contigs
The number of currently used contigs

num_readings
The number of currently used readings

Ncontigs
The number of currently allocated contigs
**Nreadings**  The number of currently *allocated* readings

**contigs**  readings
**annotations**  templates
clones
**vectors**
**notes**  Record numbers of arrays holding the record numbers of each item

**free_annotations**
A linked list of unused annotations

**free_notes**  A linked list of unused notes

### 1.19.1.2 Reading Structure

Some Reading Structure fields reference the record number in the gap4 database of a string. Where this string is short, such as the reading name, both the record number and the contents of the string can be edited. To edit a single name the string should be changed. To swap two reading names around either edit both strings or swap the two name record numbers.

The **annotations** value references an annotation number. If this is zero then this reading has no annotations.

The **length** is the complete length of sequence, including hidden data. The **sequence_length** is the length of only the used sequence. The location of the hidden data is specified by the **start** and **end** values. Note that $sequence\_length=end-start-1$.

A **left** or **right** value of zero means that this reading has no left or right neighbour.

### 1.19.1.3 Contig Structure

A Contig Structure is defined as a list of readings. The **left** and **right** values specify the first and last reading numbers in the doubly linked list representing the contig.

### 1.19.1.4 Annotation Structure

Annotations are stored as linked lists. Each reading and each contig has a (possibly blank) list. All other unused annotations are held on the free list. The **next** value is used to reference the next annotation number. A value of zero represents the end of the list.

### 1.19.1.5 Template Structure

The Template name field can be edited as both a string and the record number pointing to that string. The Template Structure display has links to a vector number and a clone.

### 1.19.1.6 Original Clone Structure

The original clone name is often the name of the database. The use of original clones is primarily for large scale sequencing. When breaking down a sequence into cosmids and then into sequencing templates, we say that each cosmid is a clone.
1.19.1.7 Note Structure

A Note may be considered as a positionless annotation (without the position, length or strand fields). Notes store both their creation and last-modification dates. Notes may be attached, in a linked-list fashion, to readings, contigs, or the database structure.

1.19.2 Ignoring Check Database

Many functions use the Check Database function to determine whether the database is consistent. Often editing an inconsistent database can yield more and more inconsistencies. However it is sometimes useful to use such an editing function in the process of fixing the database. In such cases, the "Ignore check database" toggle should be set.

An example of the use is for the Break Contig function. If we find that a database is inconsistent due there being a gap in the contig, the obvious solution is to fix this using Break Contig. But Break Contig checks for consistency, and refuses to work if the database is inconsistent.

1.19.3 Extending Structures

Sometimes it is required to allocate new structures. The "Extend structure" item on the command menu reveals a cascading menu containing the different structure types. Once a type has been selected a dialogue appears asking how many extra structures to create.

The new structures created can then be modified using the Structures menu. Expect strange behaviour if these structures are not initialised correctly.

1.19.4 Listing and Removing Annotations

The Commands menu contains two commands for manipulating lists of annotations. Output annotations to file saves a list of annotations to file. The dialogue requests a filename to save the annotations to and an annotation type. Only one type can be specified.

The format of the file is "Annotation_number Type Position Length Strand".

The "Delete annotations" command requests a file of annotations in this format. The function then removes these annotations from readings and contigs and adds them to the free annotation list.

1.19.5 Shift Readings

The Shift Readings option allows the user to change the relative positions of a set of neighbouring readings starting at a selected reading. Hence it can be used to change the alignment of readings within a contig. It prompts for the number of the first reading to shift and then the relative distance to move by. A negative shift will move the readings leftwards.

The reading and all its rightward neighbours are moved by the requested distance. Tags on the readings and the consensus are moved accordingly. The command also automatically updates then length of the contig.

1.19.6 Delete Contig

The Delete Contig function removes a contig and all its readings. Annotations on the removed readings and contig are added to the free annotations list.
1.19.7 Reset Contig Order

The contig order information contains a list of contig numbers. If a contig number does not appear within this list, or if it appears more than once, then the contig order is inconsistent and windows such as the Contig Selector may not work. The Reset Contig Order function resets the contig order to a consistent state, but will lose the existing contig order information.
1.20 Configuring

1.20.1 Introduction

The Options menu allows selection of the Consensus algorithm and the genetic code to use, and adjustment of various parameters used throughout Gap4. It also provides a way of setting more trivial things such as fonts and colours.

Most of these options have "OK Permanent" buttons in addition to the normal "OK" button. The "OK Permanent" button will save the current settings to the '.gaprc' file in the user's home directory. On Windows 95 this may be C:\.

In general users will not need to be aware of this method as the most important configuration options are all available from within the graphical user interface. However there are many additional configurable parameters which may be referred to throughout the manual. These too are stored in the '.gaprc' files.

When gap4 starts up it will first load the complete set of configurations from the '$STADENROOT/tables/gaprc' file. Next it loads '.gaprc' from the user's home directory, and finally '.gaprc' from the user's current project directory. This means that the setting stored in the '.gaprc' file in the user's project directory will have priority over those found in the home directory, which, in turn, have priority of those found in the Staden Package installation directories.

Note that searching for the '.gaprc' files only applies when starting gap4 and not when opening new or different databases. Hence if the user double clicks on a database '.aux' file then gap4 will read the '.gaprc' file found in the same directory as the database. If users start up gap4 from the Start menu and then open the project, the '.gaprc' file in the project directory will not be read.

The format of commands in the '.gaprc' file are:

"#" followed by anything is a comment.

"set_def VARIABLE value" sets the parameter "VARIABLE" to the value "value". Note that value must be enclosed in double quotes if it contains spaces.

"set_defx temp VARIABLE value" sets a parameter in a temporary list named "temp". This has no effect unless it is then used within a set_def command. In this case we use "$temp" as the "value" parameter of a set_def command.

An example follows:

set_def FIJ.MAXMIS.VALUE 30.00

set_def TEMPLATE.PRIMER_REVERSE_COLOUR "green"

set_def CONTIG_EDITOR.DISAGREE_MODE 2
set_def CONTIG_EDITOR.DISAGREE_CASE 0
set_def CONTIG_EDITOR.MAX_HEIGHT 25

Note that some adjustments will effect more than just gap4. For example, the colours of traces are stored in the '.tk_utilsrc' file, and this file is used by both gap4 and trev. For colour blind users it can be useful to change these particular settings. For example the following is a '.tk_utilsrc' file to change the colours for the trace displays.

set_def TRACE.COLOUR_A white
set_def TRACE.COLOUR_C blue
set_def TRACE.COLOUR_G black
set_def TRACE.COLOUR_T "#ff8000"
set_def TRACE.LINE_WIDTH 2

1.20.2 Consensus Algorithm

Gap4 currently contains 3 consensus algorithms (see Section 1.11.5 [The Consensus Algorithms], page 140). This option (which is available from the gap4 Options menu) allows the algorithm to be selected.

Note the consensus algorithm is used in several places throughout gap4: Assembly (see Section 1.7.1 [Normal Shotgun Assembly], page 94), producing a consensus sequence file (see Section 1.11.5 [The Consensus Algorithms], page 140), in the Contig Editor (see Section 1.6 [Editor introduction], page 58), for Experiment Suggestion (see Section 1.10 [Finishing Experiments], page 126), and in the plot of the confidence values (see Section 1.5.2 [Consistency Display], page 46).

1.20.3 Set Maxseq/Maxdb

The "Set maxseq/maxdb" option (which is available from the gap4 Options menu) may be used to adjust the maximum size of the total consensus sequence contained within gap4. This includes concatenations of consensus sequences (with extra space for text headers) and the cutoff data at either end of each contig.

When opening an already assembled project, maxseq is automatically increased accordingly (if required), so "Set maxseq" only needs to be used when adding in more data, such as when using the sequence assembly algorithms.

The maxdb option controls the maximum combined number of readings and contigs allowed. Note that changing this does not take effect on the currently opened database so be sure to set it before opening your database.

Both these values can also be adjusted by using the –maxseq and –maxdb command line arguments. See Section 1.21 [Command Line Arguments], page 182.

1.20.4 Set Fonts

"Set fonts" (which is available from the gap4 Options menu) controls the fonts used for the various components of gap4’s windows. Note that for the correct operation of some displays, careful font selection is necessary. For example it is not wise to choose a proportional font for the Contig Editor, which displays fixed width sequence alignments. For more complete documentation, see Section 9.7 [Font Selection], page 381..

1.20.5 Configuring Menus

When used for the first time gap4 will start up in beginner mode. What this means is that some of the less widely used options will not appear in the menus. The "Configure menus" command in the Options menu may be used to change between "beginner" and "expert" mode. In expert mode all the menu items will be displayed.

To permanently set the menu level users select the appropriate level and press the "OK Permanent" button. This will save the menu level information to the ‘.gaprc’ file in their home directory.

If desired, other menu levels may be created by the package administrator. This is achieved by editing the $STADENROOT/tables/gaprc_menu_full’ file, changing the MENU_LEVELS definition and adding the appropriate labels to the end of each command. Each command specified in the menu file ends in a list of menu levels in which it is active. To make a command active for several levels, enclose the level identifiers in a Tcl list, such as {m e}. If this is missing, the command will be active at all menu levels.
1.20.6 Set Genetic Code

This function allows the user to change the genetic used in all the options. The codes are defined as a set of codon tables stored in the directory tables/gcodes distributed with the package. The current list of codes and their codon table file names is shown at the end of this section.

The user interface consists of the dialogue shown below. The user selects the required code by clicking on it, and then clicking "OK" or "OK permanent". The former choice selects the code for immediate use, and the latter also selects it for future uses of the program.

When the dialogue is left the codon table selected will be displayed, as below, in the Output Window.

```
F ttt S tct Y tat C tgt
F ttc S tcc Y tac C tgc
L tta S tca * taa W tga
L ttg S tcg * tag W tgg
```

The following table shows the list of available genetic codes and the files in which they are stored for use by the package. They were created from genetic code files obtained from the NCBI.
1.20.7 Alignment Scores

The Alignment Scores command (which is available from the gap4 Options menu) may be used to adjust the gap open and gap extension penalties for some of the alignment algorithms used within gap4. At present this will affect all alignments except the Find Internal Joins function and most of the assembly algorithms.

For dealing with sequences where the alignment differences have been caused by real evolutionary events, these parameters will probably need changing from the defaults. The default values are set up with the assumption that any alignment differences are due to base calling errors, and hence the gap extension penalty will be high.

The alignment matrix may also be adjusted, but this is not listed in the dialogue. To do this take a copy of "$STADENROOT/tables/nuc_matrix", edit the copy, and set the alignment.MATRIX_FILE parameter in your ".gaprc" file.

1.20.8 Trace File Location

Gap4 does not store the trace data within the gap4 database. Instead it stores the filename of the trace file. Usually the trace files are kept within the same directory as the gap4 database. If this is not the case gap4 needs to know where they are.

To make sure that gap4 can still display the traces we need to specify any alternative locations where traces may be found. The "Trace File Location" command (which is available from the gap4 Options menu) performs this task. It brings up a dialogue asking for the directory names. If there is just one directory to specify, its name should be typed in. If there are several directories to search through, they must all be typed in, separated by the colon character (":"). To include a directory name that contains a colon, use a double colon.

For example, on windows to specify two directories, use (eg) "F::\tfiles1:G::\tfiles2".

In addition to specifying directories, RAWDATA may also be used to indicate that the trace files are contained within a tar file. The syntax for RAWDATA is then TAR=filename. This can be combined with directories if desired (for example, ./traces/online:TAR=/traces/archive.tar). In order to speed up accessing of traces within the tar file a command line utility named index_tar
may be used. This produces a text index containing the filenames held within the tar and their offsets within it. Programs will then use this index file to provide a fast way of accessing the trace. The syntax for `index_tar` is: `index_tar tar_filename > tar_filename.index`. (For example "index_tar traces.tar > traces.tar.index".)

If the gap4 database has been opened with write-access this directory location will be stored as a database RAWD note (see Section 1.15.3 [Special Note Types], page 160), which is read by gap4 when it opens the database. The demonstration data supplied with the package includes an example database (named DEMO.0) that has a RAWD note to specify that traces are fetched from a tar file within the same directory.

An alternative way of specifying the trace file location is by setting the RAWDATA environment variable. On Unix and Windows NT this is straightforward (although system and shell specific). However on Windows 95 this may prove difficult (and at least require a reboot), so manually setting the environment variable is no longer recommended.
1.20.9 The Tag Selector

Each command using tags (for example to mask tagged sequence segments) can utilise the Tag Selector to determine which tag types are to be used. As each command has its own particular use for tags, the default tags are command specific.

The Tag Selector dialogue (which is available from the relevant gap4 options) consists of a set of checkbuttons plus commands to select all tags or to deselect all tags. The "OK" button quits the display and accepts the selected list as the current list of active tags. The "Cancel" button quits the display without making any changes. The "As default" button marks the current selected tags as the defaults to be used for all future uses of this command. These selections are not saved to disk and will be lost when the program quits. To permanently set the default tag types, users must modify their ‘.gaprc’ file. Brief instructions on how to edit this file follow. They are also contained within the copy of the file distributed with the package: ‘$STADENROOT/tables/gaprc’. Search for "Tag type lists".

1.20.10 The GTAGDB File

To plot tags, gap uses a file describing the available tag types and their colours. It is possible for users to edit their own local copies of this file to create new tag types.

The environment variable GTAGDB is used to specify the location of tag type databases. The GTAGDB variable consists of one or more file pathnames separated by colons. The first file read defines a set of tags and colours. Subsequent files can define additional tags and also override the earlier tag definitions. To achieve this gap4 loads each file from the GTAGDB variable in the order of rightmost first to leftmost last. Thus, as is similar to the unix shell PATH variable, the leftmost pathnames have highest precedence for the resultant tag definitions. The default GTAGDB specified in the staden login and profile scripts is:

GTAGDB: $HOME/GTAGDB: $STADTABLE/GTAGDB

Hence the ‘$STADTABLE/GTAGDB’ file is read and the ‘$HOME/GTAGDB’ and ‘GTAGDB’ (a file in the current directory) files are merged if present. To add a new tag type only to the database local to the current directory, create a ‘GTAGDB’ file in the current directory.

The BNF grammar for the tag database is as follows:
Quoting strings is optional for single words, but necessary when writing a string containing spaces. In plain English, this means that to define the compression tag (COMP) to be displayed in red, with no default annotation string we write:

```
compression: id="COMP": bg=red
```

Any lines starting with hash ("#") are considered as comments. Lines ending in backslash ("\") are joined with the next line. Hence the above definition can be written in a clearer form using:

```
# For marking compressions
compression: \
  id="COMP": \
  bg=red:
```

An example including a default annotation string of "default string" follows:

```
# For general comments
comment: \
  id="COMM": \
  bg=MediumBlue: \
  dt="default string"
```

Allowed names for colours are those recognised by the windowing system.

### 1.21 Command Line Arguments

- **-check**

  Specifies whether to run the "Check Database" option when opening new databases. **-check** forces this to always be done and **-nocheck** forces it to never be done. By default Check Database is always performed when opening databases in read-write mode and never performed when opening in read-only mode.

- **-maxdb**

  Specifies the maximum number of readings plus contigs. This value is not automatically adjusted whilst the program is running, but is not allowed to be set to a value too small for the database to be opened. It controls the size of some areas of memory (approximately $16 \times \text{maxdb}$ bytes) used during execution of gap. The default value is 8000.

- **-maxseq**

  Specifies the maximum number of characters used in the concatenated consensus sequences. This parameter is generally not required as the value is normally computed and adjusted automatically. However a few functions (such as assembly) still need to know a maximum size before hand. The default is 100000 bases.

- **-ro**

  **-read_only**

  Opens the database (if specified on the command line) in read only mode. This does not apply to databases opened using the file browser.
-no_exec_notes
   Does not search for and execute any Notes of type OPEN or CLOS. This may be an important security measure if you are using foreign databases.

-no_rawdata_note
   Does not make use of the RAWD note type for specifying the trace file search path.

--
   Treat this as the last command line option. Only useful if the database name is specified and the name starts with a minus character (not recommended!).
Chapter 2: Searching for point mutations using pregap4 and gap4

2 Searching for point mutations using pregap4 and gap4

The original version of these methods was described in James K Bonfield, Cristina Rada and Rodger Staden, "Automated detection of point mutations using fluorescent sequence trace subtraction", Nucleic Acids Res. 26, 3404-3409, 1998. The more recent work has been done by Mark Jordan and James Bonfield with advice from Graham Taylor, Andrew Wallace, Will Wang and others.

2.1 Introduction to mutation detection

Our methods for detecting mutations are based on the alignment and comparison of the fluorescent traces produced by Sanger DNA sequencing. To use clinical terminology, samples from patients are compared to standard reference traces. Patient and reference traces should be produced using the same primers and sequencing chemistry, ideally from both strands of the DNA. The data shown in the examples below is from exon 11 of the BRCA1 gene.

The basic idea is illustrated in the following two figures which are screen dumps from our program gap4 (see Section 1.2 [Gap4 introduction], page 3). The first shows a sample containing a point mutation and the second contains a heterozygous base position. The displays are bisected vertically: at the top left is the sample trace from one strand of the DNA, below that the reference trace for that strand, and underneath the difference between these traces which is obtained by subtracting one from the other. On the right is corresponding data from the other DNA strand (shown complemented).

![Figure 1. Top and bottom strand differences for a point mutation.](image-url)
Figure 2. Top and bottom strand differences for a heterozygous base.

As can be seen, although no vertical scaling is performed the difference trace is quite flat or is consistently either above or below the mid-line, except at the sites of mutations. Near these are strong peaks, but notice that only for the mutated base are there peaks both above and below the mid-line. The context effects caused by the mutation produce peaks only in one direction.

It is perhaps necessary to point out that analysis of the traces is essential because base callers make mistakes: they can assign the wrong base types and also assign single bases where the DNA is heterozygous. An example of the latter can be observed in Figure 2: on one strand the base caller has assigned a "-" symbol at position 251, at least indicating uncertainty, but on the other strand it has assigned "T". The DNA is clearly heterozygous at this position. This means that simply looking for differences between patient sequences and reference sequences will cause point mutations and heterozygous bases to be missed (of course base calling errors will also create false differences).

These trace displays alone are very useful for visual inspection of data and are all some users want. However we also have programs which automatically analyse the trace differences and tag the bases which have significant peaks as possible sites of mutation.

Trace viewing is initiated from within the gap4 editor (see Section 1.6 [Editing in gap4], page 58). Each record in the editor shows an individual reading with its number and name at the left. Negative numbers denote readings which have been complemented. Several sequences have special status. At the top is a sequence labelled with a letter S at the left edge. This is the reference sequence, here the EMBL entry HSLBRCA1 which covers the entirety of the BRCA1 gene. The numbering at the top of the display corresponds to positions in this reference sequence. The program has also coloured (green) all exons on the reference sequence. The bottom DNA sequence in the editor is labelled "CONSENSUS". For mutation detection work this sequence is forced to be identical to the reference. Below the CONSENSUS sequence is the amino acid sequence for the reference. This is calculated on the fly using the feature table of the reference sequence and so translates only exons and in their correct reading frames. Two other sequences (near the top) are labelled R and F. These are the readings providing the reverse and forward reference traces for this segment of the data.
Figure 3. A set of aligned sequence readings displayed in the gap4 editor.

At the very bottom of the editor is an information line which is used to display data about items touched by the mouse cursor. Here it is showing data about one of the positions tagged as possibly being heterozygous. It includes the observed base types (G and A) and the scores achieved by the automated analysis.

The editor can be set to show only differences between readings and the reference; all matching bases appear as dots. For example, Figure 4. shows the same data as Figure 3, but with the editor set to show differences, and the information line showing details about a possible mutation.

Figure 4. An alternative view of aligned sequence readings in the gap4 editor.
One column contains several bases tagged in red, signifying possible heterozygotes, and some in orange denoting possible point mutations. During visual inspection the program can be made to move the cursor from one tag to the next and to display the aligned traces as shown above in Figures 1 and 2.

It is also possible to have positive controls for displaying the trace differences; i.e. reference traces which contain the mutation. In this case the traces appear as shown in figure 5. Here the forward and reverse positive controls are shown to the right of the normal plots. In Figure 5 the positive control difference plots are quite flat hence, in this case, providing confirmation of the presence of the heterozygous base.

![Figure 5. Top and bottom strand differences and positive control for a heterozygous base.](image)

As mentioned above the package contains programs which can automatically compare the traces and their reference sequences. The output from these programs are the tags shown in the editor. Users can check the traces at these positions using the displays shown in Figures 1, 2 and 5; if necessary removing or adding tags. Alternatively users can rely entirely on visual inspection and create all tags themselves.

Once all the mutations are correctly tagged the program can produce a report which includes the reading names, mutation positions relative to the reference sequence, the actual change, its effect, and the evidence. An example is shown below in Figure 6.
Figure 6. How gap4 reports mutations.

Here the first record is for reading 001321_11aF, position 33885, T changed to T and C (i.e. is heterozygous) to produce no amino acid change, with evidence coming only from the complementary strand. The last record is for reading 000256_11eF, position 36749, A changed to G, producing an amino acid change K to R, with evidence from both strands of the sequence. The penultimate record denotes a heterozygote in a noncoding region.

### 2.1.1 Mutation Detection Programs

The software handles batches of trace data from sequencing instruments. It performs all processing except base calling (although it can employ third party programs such as phred for this step). This includes file format conversions, quality clipping, scanning for mutations and heterozygotes, multiple sequence alignment, easy visual inspection of traces, production of reports, and the accumulation and storage of readings and traces. The software also handles the initialisation/configuration of standard reference files and databases for any project. The two main programs are pregap4 and gap4. Pregap4 (see Section 3.2 [Pregap4 introduction], page 202) prepares data for gap4 by automatically using a variety of smaller programs, including those used to search for mutations: tracediff (see Section 3.6.21 [Trace Difference], page 229) and hetscan (see Section 3.6.22 [Heterozygote Scanner], page 231). Gap4 (see Section 1.2 [Gap4 introduction], page 3) is used to store the aligned readings, to view the sequences and traces, and to produce a report listing the observed mutations.

Any number of sequences can be processed in a single run, and for each individual patient sample the operation is generally performed in two steps. First, via pregap4, the traces are aligned and compared to the reference traces and any possible mutations or heterozygous bases marked. Secondly, the data is transferred into a gap4 database from where users can visually check the differences between the reference and patient traces.

The programs tracediff (see Section 3.6.21 [Trace Difference], page 229) and hetscan (see Section 3.6.22 [Heterozygote Scanner], page 231), can automatically compare patient and reference traces to find point mutations and heterozygous bases. Users can set parameters which control the sensistivity of the algorithms (and hence which determine the ratio of false negative and positive results). Tracediff adds tags of type "mutation" to the patient files, and hetscan of type "heterozygous". The tags contain the numerical scores achieved at the site of the reported base changes, and they can be viewed via the gap4 editor (see Section 1.6 [Editing in gap4], page 58). Tracediff and hetscan are normally run via pregap4 (see Section 3.2 [Pregap4 introduction], page 202).

The description of the programs given below is presented in reverse order of use i.e. gap4 then pregap4, but first we give further details about the use of reference data.

```plaintext
001321_11aF 33885T>Y (silent F) (strand - only)
001321_11aF 34407G>K (expressed E>[ED]) (strand - only)
001321_11cF 35512T>Y (silent L) (double stranded)
001321_11cF 35813C>Y (expressed P>[PL]) (double stranded)
001321_11dF 36314A>R (expressed E>[EG]) (double stranded)
001321_11eF 36749A>R (expressed K>[KR]) (double stranded)
001321_11eF 37313T>K (noncoding) (strand - only)
000256_11eF 36749A>G (expressed K>R) (double stranded)
```
2.1.2 Mutation Detection Reference Data

The mutation detection methods require reference traces and optionally reference sequences. Reference traces are used for automatic mutation detection and for visual inspection of trace differences. Reference sequences are used in gap4 to provide a base numbering standard, and if required to provide feature table entries to control translation and mutation reporting.

2.1.3 Reference Sequences

Reference sequences are used in gap4 (see Section 1.2 [Gap4 introduction], page 3). Here they can be used to define a numbering system independent of gaps introduced to produce alignments. The numbering can start at any point in the reference sequence. If the reference sequence is entered with a feature table the features are converted to tags and can be used to control translation of the sequence in the contig editor. For mutation detection work the reference sequence and feature table enable mutations to be reported using positions defined by the reference sequence, and also allows the effect of the mutations to be noted. Gap4 is able to store entries from the EMBL sequence library complete with their feature tables. These feature tables are converted to gap4 database annotations (tags), which means that they can be selectively displayed in the template display and editor, and used to translate only the exons (in the correct reading frame). Obviously it may be useful to augment the feature tables with the sites of known polymorphisms or deleterious mutations so that they can be displayed in gap4 as landmarks. When it comes to producing a report of the observed mutations the feature table is used to work out if a mutation is expressed and if so what the amino acid change is. Additional tags can be created to specify the positions of the primers or restriction sites used to obtain data covering segments of the sequence. For any project the reference sequence need only be set up once. Either project databases can be started with the reference sequence already configured or the reference can be assembled along with the reading data. The reference sequence can be designated (or reassigned) as follows. In pregap4 (see Section 3.2 [Pregap4 introduction], page 202) it can be named in the module "Reference Traces". In the gap4 editor it can be set by right clicking on its name. Once set it should appear labelled "S" at the left edge of the editor.

2.1.4 Reference Traces

References traces are used by the automatic mutation detection programs tracediff (see Section 3.6.21 [Trace Difference], page 229) and hetscan (see Section 3.6.22 [Heterozygote Scanner], page 231), and by the trace difference display in the gap4 editor (see Section 1.6 [Editing in gap4], page 58). Ideally forward and reverse reference traces should be available and should be obtained using the same primers and sequencing chemistry as the patient data. From the "settings" menu of the editor the trace display can be set to "Auto-Diff traces". Once this is activated, whenever the user double clicks on a base in the editor sequence display, not only is the reading's trace displayed, but also its designated reference trace plus the difference between them. If its complementary reading is available, its trace and reference trace and their differences are also displayed. These trace displays and the editing cursor scroll in synch.
Top and bottom strand differences for a heterozygous base.

The preferred way of assigning reference traces to readings is by use of "naming conventions"; that is to have a simple set of rules which control the names given to the trace files. It can be seen in the figures showing the editor that forward and reverse readings from the same patient have names with a common root but which end either F or R. This both ties the two together (so the software knows which is the corresponding complementary trace when the user double clicks on a reading) and also enables the association of readings and their reference traces. Once a convention has been adopted the rules can be defined for pregap4 by loading them via the "Load Naming Scheme" option in its File menu (see Section 3.8 [Pregap4 Naming Schemes], page 236). For any batch of readings the reference traces are defined within pregap4’s "Reference Traces" module. Note that this mode of operation, by allowing the specification of only one forward and one reverse trace, limits each batch of traces processed to those which correspond to a given pair of reference traces. The size of the batch is unlimited.

The alternative way of specifying the reference traces is to right click on their names in the editor. This also allows positive trace controls to be specified (which is not possible in pregap4).
2.1.5 Using The Template Display With Mutation Data

Figure 7. The template display showing the whole of the BRCA1 gene (exons in green).

The view obtained from the Template display and shown in Figure 7 is not of practical use but serves here to illustrate the overall arrangement of the data for our chosen example the BRCA1 gene. This figure shows the entirety of the EMBL entry HSLBRCA1 with its exons marked in green. Only exon 11 has patient trace data stacked above it.
Figure 8. A zoomed-in version of the data shown in Figure 7.

Here we can see all the readings covering exon 11. Forward readings are light blue, reverse readings orange, primers are marked in yellow, mutations in red and orange. A common mutation appears in the leftmost set of readings and illustrates the value of using the template display for visualising the overall pattern of the tagged mutations.

2.1.6 Configuring The Gap4 Editor For Mutation Data

The current version of the gap4 editor contains very many options that are not needed for mutation data. Given sufficient demand a version tailored for mutation studies could be produced. For now it might make it easier to understand the program if its origin as a genome assembly program is borne in mind. Here we outline the options and settings relevant to mutation studies. The assignment of reference sequence and traces is described above. From the editor they can be set by right clicking on the reading names.

Gap4 enables segments of sequences to be annotated (or tagged). Each tag has a type (eg primer) and each type has an associated colour. Each instance of a tag can include editable text. This text can be viewed and edited by right clicking on the tag and selecting "Edit tag", after which a text box will appear. Gap4 can display annotations/tags as background colour and the user can specify which tag types are shown. For mutation studies the following tag types may usefully be activated, and all others turned off. Using the "Set Active Tags" option in the "Settings" menu first click on "Clear all". Then click on "primer". To add further types you must hold down the "Ctrl" key on the keyboard while clicking. Now scroll down and click on "Mutation", "Heterozygous" and "FEATURE CDS". Add any others required, then click "OK".

The following configurations are performed via the "Settings" menu.
Gap4 has three consensus generation algorithms. When using a reference sequence it is convenient if the consensus shown in the editor is forced to be the same as the reference. This will be the case if either the "Weighted base frequencies" or the "Confidence values" consensus algorithms are being used. This selection is made using the "Consensus algorithm" option.

Translations are shown in what gap4 refers to as the "Status" line. To enable automatic translation of the exons defined in the reference sequence, in the "Status Line" option set "Translate using feature tables".

To enable automatic display of trace differences, in the "Trace Display" option set "Auto-Diff Traces".

To show only the base differences between the consensus/reference, set "Highlight Disagreements". These can be shown by dots or colour.

To show base confidence values set "Show reading quality" and also make sure that the value in the box labelled "Q" at the top left of the editor is set to 0 or greater.

To force forward and reverse reading pairs to be shown in adjacent records in the editor set "Group readings by templates" (NB this assumes that an appropriate naming scheme has been used).

If a reference sequence is assigned, the numbering at the top of the sequence will reflect the base positions in that sequence. Any pads in the reference sequence are ignored. If no reference sequence is assigned, the numbering will ignore pads if the "Show unpadded positions" option is activated.

At the bottom of the "Settings" menu is an option to "Save settings". Use of this will mean that the current configuration will be set automatically next time the editor is used (and hence the steps just described only need to be performed once).

2.1.7 Using The Gap4 Editor With Mutation Data

The current version of the editor has a fixed width and a maximum height. If too many sequences are present at any position a vertical scrollbar on the right edge can be used to move them up and down. The CONSENSUS line will always be visible, but at present, the reference sequence is scrolled along with all the other sequences and so may disappear. Horizontal scrolling is achieved in the usual ways, plus by use of the >, >> and <, << buttons. The reading names can be moved left and right using the scrollbar above them.

Configure the editor as described above.

The traces for readings (and their reverse) can be examined over their full length one at a time by simply double clicking on them then scrolling along. Any mutations observed can be labelled by right clicking on the base in the editor display and invoking the "Create tag" option. This brings up a dialogue box. At the top is a button marked "Type:comment"; clicking on this will bring up another dialogue with a list of all the tag types; choose the appropriate one ("Heterozygous" or "Mutation"). There are obviously many advantages to examining the traces like this using gap4. However, if the automated mutation detection methods are trusted, or used in way that makes them trustworthy for the type of study being undertaken, then there are quicker ways of examining the data.

The "Next Search" button at the top of the editor gives access to many types of search, one of which is "tag type". If this is selected a button appears labelled "Tag type COMM(Comment)". Clicking on this will bring up a dialogue showing all the available tag types. If the user selects, say "Mutation", each time the "Next Search" button is used the program will position the editing cursor on the next mutation tag. Double clicking will automatically bring up the appropriate traces as shown in figures 1, 2 and 5 (see Section 2.1 [Introduction to mutation detection], page 185). The user can view the traces and if necessary alter the tag (eg delete it if it is a false positive).
Once all the data has been checked and all mutations and heterozygous bases have been tagged a report can be generated using the "Report Mutations" option in the editor "Commands" menu. Note that it is also possible to simply report all differences between base calls and the reference, but the usual procedure is for the program to report all bases tagged as "Mutation" or "Heterozygous". Example output is shown above in Figure 6 (see Section 2.1 [Introduction to mutation detection], page 185). The report appears in the gap4 "Output window" which can be saved to disk by right clicking on the text and selecting "Output to disk".

2.1.8 Processing Batches Of Mutation Data Trace Files

It is not clear which is the best way of organising the data for the simplest and most efficient processing using the current programs, but for now we make the following suggestions.

We assume that the region of the DNA being studied has a standard set of forward and reverse primer pairs covering all segments of interest and that a standard reference sequence in EMBL format is available.

We recommend that batches of data from single primer pair combinations are processed separately, using separate temporary gap4 databases. For example, exon 11 of BRCA1 can be covered by five pairs of forward and reverse primers and we suggest that batches of traces obtained from each of these primer pairs should be processed using five gap4 databases.

Each processing run should create a new database and should enter, not only the new sets of patient data for that particular primer pair, but also the corresponding reference sequence and reference traces.

Obviously when several primer pairs are needed to cover a given region of the DNA (eg for BRCA1) the same reference sequence would be used for all the primer pairs.

An alternative to the above is to create a template database for each primer pair which contains the data for the corresponding forward and reverse reference traces plus the fully annotated reference sequence. These template databases are copied to create a temporary database for each new batch of data for the given primer pair.

Whichever of these two strategies is adopted each batch of new data is processed, analysed and assembled into these temporary databases, inspected visually, and a mutation report generated.

The use of separate temporary databases simplifies the assignment of reference traces and the use of the report generation function.
Figure 9. An overview of a database containing data for only one primer pair of BRCA1

For long term storage and to facilitate larger studies, the content of each of these temporary databases is then transferred to archive databases, after which the temporary databases are no longer needed. The archive databases could be restricted to individual primer pairs or could accommodate data covering the whole of the reference sequence.

2.1.9 Processing Batches Of Mutation Data Trace Files Using Pregap4

All the data processing other than visual inspection of traces and report generation is handled by the program pregap4 (see Section 3.2 [Pregap4 introduction], page 202). Pregap4 achieves this by running a set of individual programs selected by the user.
2.1.10 Configuration Of Pregap4 For Mutation Data

General Configuration
This option allows the user to select whether the trace names used for the samples should be the same as their file names or should be the names stored inside the files.

Phred
Phred is a base caller which also assigns confidence values to each base. Generally the data passed to pregap4 has already been base called. However not all base callers assign confidence values and so it can be useful to apply phred or ATQA (which does not base call but does assign confidence values). Alternatively "Estimate Base Accuracies" can be applied which is a simple program for providing numerical values which reflect the signal to noise ratio for each base, and which can be used instead of confidence values.
(Note that if quality clipping is used, its score thresholds depend on whether confidence values of eba values are used).

**Trace Format Conversion**
This option can be used to convert bulky files such as those of ABI to a compact format such as SCF or ZTR without loss of the data required for trace display.

**Initialise Experiment Files**
The input to gap4 and several of the other programs used here is a data format known as Experiment file format. This step, which has no configurable parameters is essential for mutation data processing.

**Augment Experiment Files**
The section on Reference Traces outlined the use of "Naming Schemes" for associating pairs of forward and reverse readings, and for assigning reference traces. The naming scheme must be loaded from pregap4’s File menu. "Augment Experiment Files" must be activated in order for the naming scheme to be applied. No parameters need be set.

**Quality Clip**
The reliability of the base calls varies with position along the sequence. Near to both ends the data is less reliable. The "Quality Clip" option trims the ends of the sequences by analysing their confidence values or accuracy estimates (if present) or the density of unknown bases in the sequence. By observing these "clip points" other processing programs will work more reliably.

**Reference Traces**
As explained above it is necessary to specify a reference trace (preferably one for each strand of the data if processing data from both strands). The Reference sequence can also be set here. Note that even if our suggestion to preload the reference traces into the gap4 database is followed, it is still necessary to specify them here for use by the mutation detection modules.

**Trace Difference**
This is the program which compares the patient and reference traces to search for possible mutations. It adds data to the experiment files to mark each predicted mutation, and this data will appear as tags in the gap4 database. It can also create a new trace file containing the difference of the reference and the sample. The numerical parameters control the sensitivity of the algorithms, and hence the ratio between the numbers of false positive and negative results.

**Heterozygote Scanner**
This is the program which compares the patient and reference traces to search for possible heterozygous bases. It adds data to the experiment files to mark each predicted heterozygous base, and this data will appear as tags in the gap4 database. The numerical parameters control the sensitivity of the algorithms, and hence the ratio between the numbers of false positive and negative results.

**Gap4 shotgun assembly**
In order to be able report the positions of mutations relative to the reference sequence, and to be able to compare sets of samples from patients, it is necessary to perform multiple sequence alignment on the data. This is termed "assembly" and is usually performed by gap4, although other programs can be operated via pregap4. If following the suggestion to preload the reference sequence to a temporary database for each batch, supply the name of this database here. Otherwise a new database should be named and created from this option. (If this strategy is adopted make sure that the reference sequence and the references traces are assembled!) The parameters that control the assembly process and are described elsewhere.
Note that pregap4 has the facility to save its configuration and parameter settings. This means that the current configuration will be set automatically next time the program is used (and hence the steps just described only need to be performed once). In addition pregap4 can be run non-interactively by typing a single line on the command line. Taking these two capabilities together, means that only one line need be typed in order to process all subsequent batches of data (assuming the file names are reused, which is easy to arrange.)

2.1.11 Discussion Of Mutation Data Processing Methods

At present pregap4 and gap4 clearly show their primary usage in the field of genome assembly, but versions tailored to mutation studies can be created once the requirements are agreed. Ideally all processing should be controlled by a single program which once configured for any project should require users to provide only the project name - all other file names and parameters could be preset, and all processing, including archiving and backup, performed automatically, leaving the data ready for visual inspection.

The automatic mutation and heterozygote detection programs work well on all the test data we have but now they require evaluation by external groups. Such analysis would enable us to improve the algorithms and to tune their parameters. At present we know that sometimes a base will be declared both as a mutation and as a heterozygous position when visual inspection shows that it is one or the other.

There is still much that can be done overall to improve the methods, but the text above summarises their status in July 2002. Although currently valuable for real scientific and clinical work they should perhaps be viewed as prototypes.
3 Preparing readings for assembly using pregap4

3.1 Organisation of the Pregap4 Manual

Pregap4 is a relatively simple program to use. It is also very flexible and extendable, and so much of the manual is taken up by explaining to programmers and system managers how it can be configured. The average user need not be concerned with these details.

The Introductory section of the manual is meant to give an overview of the program: what it is for, the files it uses and functions it performs, and how to use it. It is very important for all users to have a basic understanding of the files used by pregap4 and the processes through which it can pass their data (see Section 3.2.1 [Summary of the Files used and the Processing Steps], page 202). The next section of the Introduction (see Section 3.2.3 [Pregap4 Menus], page 210) tabulates the program’s menus. This is followed by an overview of the pregap4 user interface (see Section 3.2.2 [Introduction to the Pregap4 User Interface], page 206) which should give a clear idea of how to actually use the program, and concludes the introductory section.

More detail about how to define the set of files to be processed (see Section 3.3 [Specifying Files to Process], page 211) is followed by a section showing how to run pregap4 and giving examples of its use (see Section 3.4 [Running Pregap4], page 212). Next are sections on configuring the pregap4 user interface (see Section 3.5 [Configuring the Pregap4 User Interface], page 214).

The next part of the manual describes how to use the Configure Modules Window to select the modules to apply and to set their parameters (see Section 3.6 [Configuring Modules], page 216). This is one of the longest and most detailed parts of the manual in that it describes how to configure all the current possible modules, many of which will not be available at all sites, and several of which perform identical functions. Obviously, only the entries which describe the functions that are available at a site, are of interest.

One of the important tasks of pregap4 is to make sure that each reading’s Experiment file contains all the information needed by gap4 to ensure the accuracy of the final consensus sequence and to make the project proceed as efficiently as possible. Pregap4 provides several methods for sourcing this information. One of these, as for example employed at the Sanger Centre in the UK, is to encode some information about a reading in its reading name. Pregap4 contains flexible mechanisms to enable a variety of the “Naming schemes” or “Naming conventions” to be used as a source of information to augment the Experiment files (see Section 3.8 [Pregap4 Naming Schemes], page 236). Alternatively pregap4 can use simple text databases as an information source (see Section 3.10 [Information Sources], page 240), or the user can set up some Experiment file record types for use with a batch of readings (see Section 10.3.1 [Experiment file format record types], page 401).

The rest of the manual deals with increasingly complicated matters, and the average user should never need to consult these sections. First there is a section on adding an removing modules (see Section 3.11 [Adding and Removing Modules], page 243). This describes how to control the list of modules which appear in the Configure Modules Window. The package is usually shipped with this list set to contain more modules than are likely to be available at any one site and so it might be found useful to remove those that are not available.

The next two sections, as their names imply, are for programmers only (see Section 3.12 [Low Level Pregap4 Configuration], page 244) and (see Section 3.13 [Writing New Modules], page 261).

3.2 Introduction

Before entry into a gap4 database the raw data from sequencing instruments needs to be passed through several processes, such as screening for vectors, quality evaluation, and conversion of data
formats. Pregap4 is used to pass a batch of readings through these steps in an automatic way. It provides an interface for setting up and configuring the processing and for controlling the passage of the readings through each stage. The separate tasks are termed "modules" and each module is typically managed by a dedicated program. Pregap4 wraps all of these modules into a single easy to use environment, whilst maintaining the flexibility to select and extend the processing modules. It is an, as yet, unpublished replacement of the program pregap Bonfield, J.K. and Staden, R. Experiment files and their application during large-scale sequencing projects. DNA Sequence 6, 109-117 (1996).

3.2.1 Summary of the Files used and the Processing Steps

Gap4 stores the data for an assembly project in a gap4 database. Before being entered into the gap4 database the data must be passed through several steps via pregap4. The range of tasks that can be performed using pregap4 are shown schematically in the following figure.
The package can handle data produced by a variety of sequencing instruments, and also data
ertered using digitisers or that has been typed in by hand. One of the first steps is to convert trace
files, such as those of ABI, which are in proprietary format, to SCF files (see Section 10.1 [SCF
introduction], page 383).

Next, as originally put forward in Bonfield, J. K., and Staden, R. The application of numerical
estimates of base calling accuracy to DNA sequencing projects. Nucleic Acids Research 23, 1406-
1410 (1995) (see Section 1.2.5 [The use of numerical estimates of base calling accuracy], page 26),
if they are not already included in the files, base call confidence values are calculated, and are
normally stored in the reading’s SCF file.

Next the base calls are copied from the trace files to text files known as Experiment files (see
Section 10.3 [Experiment files], page 401).

Note it is also possible to enter sequence readings in the form of FASTA files for use at this stage
of the processing, in which case they will be automatically converted to Experiment file format.

All the subsequent processes operate on the Experiment files.

Experiment file format is similar to that of EMBL sequence entries in that each record starts
with a two letter identifier, but we have invented new records specific to sequencing experiments.
Gap4 can make use of information about readings which may not be contained within the raw data
files, such as sequencing chemistry and whether it is a forward or reverse reading. Gap4 will work
without this information, but at a reduced level. For instance knowing which forward and reverse
readings belong together allows gap4 to check the validity of assembly and for automatic ordering
of contigs.

One of pregap4’s next tasks is to augment the Experiment files to include data about the
chemistry, vectors, primers and templates used in the production of each reading, and if necessary
it can extract this information from external databases (see Section 3.10 [Information Sources],
page 240), or via local reading name conventions (see Section 3.8 [Pregap4 Naming Schemes],
page 236). Once the Experiment file for a reading contains all the necessary information the
remaining processing programs can be used in turn to analyse the data.

First the reading is marked at both ends to define the range of reasonable quality base calls (see
Section 3.6.8 [Quality Clip], page 220).

Then the reading is searched for the presence of sequencing vector at the 5’ end 3’ ends (see
Section 3.6.9 [Sequencing Vector Clip], page 221).

Next the sequence is checked for the presence of “cloning” vector, i.e. non-sequencing vectors,
such as those of BACs (see Section 3.6.11 [Cloning Vector Clip], page 223).

The final check of this type is to screen the reading for any vector that may have been missed
in the previous searches (see Section 3.6.12 [Screen for Unclipped Vector], page 223).

The next check is to screen the reading for any set of sequences which it may be contaminated
by, such as E. coli (see Section 3.6.13 [Screen Sequences], page 224).

Note that vector sequence files are normally stored in the package vectors directory/folder. If a
file of vector file names is used the vector sequences can also be stored in its directory/folder. Files
of file names and vector-primer files can also contain environment variables to define the location
of vector files.

Vector_primer files, vector sequence files and files of file names must be stored in plain text
files (see Section 10.5 [Vector_primer Files], page 415), (see Section 10.6 [Vector sequence format],
page 416).

Pregap4 is usually used non-interactively once the modules have been configured, but some
groups prefer (or have the time) to check the data by eye using the program trev (see Section 7.1
[Trev], page 283) at this stage.
Another option is to search the readings for families of known repeats (see Section 3.6.18 [Tag Repeats], page 226). This will tag any regions which are found to match known repeats.

Some groups are using the package for mutation studies and the final pregap4 option, prior to assembly is to use the tracediff and hetscan programs (see Section 2.1 [Introduction to mutation detection], page 185) to search the readings for mutations (see Section 3.6.21 [Trace Difference], page 229 and see Section 3.6.22 [Heterozygote Scanner], page 231).

Pregap4 can also be used to assemble the readings into a gap4 database (see Section 3.6.23 [Gap4 Shotgun Assembly], page 232), or to assemble the readings using an external assembly engine such as FAKII (see Section 3.6.26 [FakII Assembly], page 233), and then to enter that assembly into a gap4 database (see Section 3.6.28 [Enter Assembly into Gap4], page 234).

It is unlikely that any particular user will want to employ all of these options and one of pregap4’s modes of use is to enable users to configure the program for their work (see Section 3.6 [Configuring Modules], page 216). Not only can they select which tasks should be performed, and which of the alternative programs ("modules") should be used for them, but also the order in which they are applied. Although it is very rarely a problem, this high level of flexibility comes at a price in the current version of pregap4: pregap4 does not include code to check on the logicality of the configuration set by a user and will attempt to execute the modules in the order given. There are some users, who having read this section, will configure pregap4 to perform assembly before creating the Experiment files from the trace files. Pregap4 will attempt to do this and no data will be assembled as the files given to the assembly engine will be in the wrong format. This is just something to be aware of.

Pregap4 uses configuration files to remember the setup for each user or project. These files define which modules are activated and what their parameter settings are (see Section 3.7 [Using Config Files], page 235). These files, which can obviously save considerable amounts of time, are created automatically and can be saved from the Configure Modules Window once the configuration is complete.

The trace files are not altered, but are kept as archival data so that it is always possible to check the original base calls and traces. The trace files are used by gap4 to display traces and to compare the final consensus sequence with the original data, therefore they must be kept online for the lifetime of the project. To save disk space it is best to use SCF files and, if they were derived from a proprietary format such as that of ABI, to remove the originals.

Any changes to the data prior to assembly (and we recommend that none are made until readings can be viewed aligned with others) are made to the copy of the sequence in the Experiment file. For example the results of all the searching procedures outlined above are added as new records to each reading’s Experiment file. The reading data, in Experiment file format, is entered into the project database (see Section 1.16 [Gap Database Files], page 162), usually via one of the assembly engines. All the changes to the data made by gap4 are made to the copies of the data in the project database. Once the data has been copied into the gap4 database the Experiment files are no longer required.

During processing pregap4 uses temporary files. The number and nature of these files depends on the modules used. At the very least pregap4 will produce files containing the names of the input files and the result of their processing. Those that were processed successfully will be stored in a file with a name ending ".passed" and those that failed in one ending ".failed". The ".passed" file can be used as a file of input file names for assembly into gap4 (assuming that a pregap4 assembly module has not already been used).

While it is running, pregap4 will create files with a file name prefix defined by the user, and store them in an output directory of the user’s choice (see Section 3.3 [Specifying Files to Process], page 211).
When processing has finished pregap4 will produce a report containing information from each module and the final list of passed and failed sequences.

### 3.2.2 Introduction to the Pregap4 User Interface

Pregap4 provides interfaces to define the batch of data files to be processed, which modules are to applied to them; to configure the modules, and to start the processing. It also provides mechanisms for adding and removing modules, but this facility will be used far less often than the others.

Pregap4 supports two styles of windowing. The default method is a compact mode, with the alternative being "separate" mode - similar to gap4 and spin.

This is the "separate" window style. Here the main window is always visible, with commands in the main window bringing up new windows. In the picture above the configure window can be seen on top of the main window.

The second style is "compact" mode.
In the compact picture above the most common top level windows are "pages" in a tabbed notebook. The benefit is greatly reduced screen space and quicker controls, but the text output window is no longer permanently visible. The Window Style can be changed using the options menu (see Section 3.5.2 [Window Styles], page 215).

### 3.2.2.1 Introduction to the Files to Process Window

Pregap4 operates on batches of files. These files can be binary trace files (in ABI, ALF or SCF format), Experiment Files, or plain text, and do not need to all be in the same format. The Files to Process Window is used to define which files are to be processed. The "Files to Process" dialogue (see below) can be brought up from the File menu, or by pressing the appropriate tab when in compact_win mode.
On the left hand side of the figure is the current list of files to process. This list can be edited simply by clicking with the mouse and typing.

On the right side of the panel is the pregap4 output filename prefix, the output directory name, and several buttons. The filename prefix is used when pregap4 needs to create files. For example after processing there may be prefix.passed, prefix.failed files. All files will be created within the output directory.

The buttons allow selection of the files to process. The "Add files" button will bring up a file browser, which will allow one or more files to be selected. Pressing Ok on the file browser will then add the selected files to the "List of files to process" panel on the left side of the pregap4 window.

The "Add file of filenames" button may be used to select a list of files whose filenames have been written to a ‘file of filenames’.

The "Clear current list" button will remove all filenames from the list.

Both the "Add files" and "Add file of filenames" button append their selections to the list of files to process, so to replace the current list the "Clear current list" button must first be used.

The "Save current list to..." button may be used to produce a new file of filenames, containing the combined list of files to process.

### 3.2.2.2 Introduction to the Configure Modules Window

The "Configure Modules" dialogue is available from the Modules menu or, when using the compact window style, by pressing the Configure Modules tab.

As can be seen in the figure below, the left side of the display contains a list of the currently loaded modules. One module in this list will be highlighted. The right side of the display shows the configuration panel for this highlighted module and is module specific.
Chapter 3: Preparing readings for assembly using pregap4

The module list shown on the left consists of a series of module names and their status, and is termed the "enable status". The tick or cross at the left of the name indicates whether this module is enabled. The text to the right of the module name indicates whether the module has been given all the parameters needed for it to run. This will be one of "ok" (all configuration options have been filled in), "-" (no configuration options exist for this module), "edit" (further configuration is required) or blank (this module is disabled).

The "enable status" can be toggled by left clicking on the tick/cross to the left of the module name. The enable status can be written to the current Pregap4 configuration file using the "Save Module List" or "Save All Parameters" commands in the Modules menu. Left clicking anywhere on a module name in the module list will switch the pane on the right side of the window to display any available parameters for this module. Not all modules will have parameters to configure.

For modules that do have parameters, the top line of the configuration panel will contain two buttons labelled "Select params to save" and "Save these parameters". The "Select params to save" button will add check boxes next to each parameter. Clicking on these check boxes allows selection of individual parameters to save for this module. Once these have been selected pressing the "Save" will save only those selected to the pregap4 configuration file. Pressing the "Save these parameters" button will save all parameters for this module to the configuration file.

The bottom strip of the window is an "Information Line".

3.2.2.3 Introduction to the Textual Output Window

Pregap4 has a main text output window identical to that of gap4 and spin. It is used for showing textual results in the top section and error messages in the lower part. Full details of the user interface are given elsewhere (see [User Interface], page 373), but an example of the Text Output Window is given below.
3.2.2.4 Introduction to Running Pregap4

When pregap4 is started the user first needs to select the files to process. This is done using the "Files to Process" command (from the File menu).

The "Configure Modules" tab allows for the currently available modules to be enabled or disabled, and the module parameters edited accordingly.

Once all modules have been configured (so that none have edit listed next to their name) pregap4 is ready to begin processing. This is started by pressing "Run" or by selecting "Run" from the File menu.

When pregap4 has a setup that would be useful in the future "Save All Parameters (in all modules)" from the Modules menu can be used, and pregap4 will store all the module parameters to a configuration file ready for subsequent runs.

When processing has finished pregap4 will produce a report containing information from each module and the final list of passed and failed sequences.

If for any reason pregap4 fails a particular step in the processing, users are strongly recommended to correct whatever has caused the module to fail, clean up any files it has created, and then repeat the whole process. That is, until users have a good understanding of what happens at each stage of processing, it is better to repeat all the steps with the original list of files, than to try to guess which step to continue from.

3.2.3 Pregap4 Menus

The main window of pregap4 contains File, Modules, Information source and Options menus.
3.2.3.1 Pregap4 File menu

The File menu includes functions to set the files for processing, loading configuration files and naming schemes, including configuration components, starting processing and exiting.

- Set Files to Process (see Section 3.3 [Specifying Files to Process], page 211)
- Load New Config File (see Section 3.7 [Using Config Files], page 235)
- Load Naming Scheme (see Section 3.8 [Pregap4 Naming Schemes], page 236)
- Include Config Component (see Section 3.9 [Pregap4 Components], page 240)
- Save All Parameters (in all modules) (see Section 3.6 [Configuring Modules], page 216)
- Save All Parameters (in all modules) to: (see Section 3.6 [Configuring Modules], page 216)
- Save Module List (see Section 3.6 [Configuring Modules], page 216)
- Exit

3.2.3.2 Pregap4 Modules menu

The pregap4 Modules menu contains options for adding and configuring modules, and running pregap4.

- Add/Remove Modules (see Section 3.11 [Adding and Removing Modules], page 243)
- Configure Modules (see Section 3.6 [Configuring Modules], page 216)
- Select all modules
- Deselect all modules

3.2.3.3 Pregap4 Information source menu

The Information source menu contains options for specifying how the information required for the experiment files is to be obtained. These menu options can also be entered from the "Augment Experiment Files" module.

- Simple Text Database (see Section 3.10.1 [Simple text Database], page 240)
- Experiment File Line Types (see Section 3.10.2 [Experiment File Line Types], page 242)

3.2.3.4 Pregap4 Options menu

The Options menu contains options for setting fonts and colours and defining the style of the user interface.

- Set Fonts (see Section 3.5.1 [Fonts and Colours], page 215)
- Set Colours (see Section 3.5.1 [Fonts and Colours], page 215)
- Compact Window Style (see Section 3.5.2 [Window Styles], page 215)
- Separate Window Style (see Section 3.5.2 [Window Styles], page 215)

3.3 Specifying Files to Process

Pregap4 needs to be given a list of files to process. These files can be binary trace files (in ABI, ALF, SCF, CTF or ZTR format), Experiment Files, FASTA, or plain text. The files to process do not need to all be in the same format. FASTA files will be converted to Experiment files.
Refering to the figure above, the "Files to Process" dialogue can be brought up from the File menu, or just by pressing the appropriate tab when in compact_win mode.

On the left hand side we have the current list of files to process. This list can be edited simply by clicking with the mouse and typing as normal. This only edits Pregap4's temporary copy of this list and does not modify the contents of any file of filenames that the list was obtained from.

On the right side of the panel is the pregap4 output filename prefix, the output directory name, and several buttons. The filename prefix is used when Pregap4 needs to create files for its own use, both for temporary and not so temporary files. For example after processing there may be prefix.passed, prefix.failed files. The prefix defaults to 'pregap' until a file of filenames is loaded, in which case it switches to the last used file of filenames. All files will be created within the output directory, regardless of where the input files reside. The output directory defaults to the current directory or to the last used input directory.

The buttons allow selection of the files to process. The "Add files" button will bring up a file browser, which will allow one or more file to be selected. Pressing Ok on the file browser will then add the selected files to the "List of files to process" panel on the left side of the pregap4 window. The "Add file of filenames" button may be used to select a list of files whose filenames have been written to a 'file of filenames'. The list of files to process may be edited within pregap4, allowing new filenames to be added or removed. The "Clear current list" will remove all filenames from the list. Both the "Add files" and "Add file of filenames" button append their selections to the list of files to process, so to replace the current list the "Clear current list" button must first be used. Finally the "Save current list to..." button may be used to produce a new file of filenames, containing the combined list of files to process.

3.4 Running Pregap4

When the Run button or Run command (File menu) is used, pregap4 starts processing the files using the selected modules and their configurations. If the configuration is invalid an error message will be produced. For example the following may be written to the error window, and the configure modules panel will be selected with the problematic module automatically highlighted.
Fri 10 Jul 10:04:25 1998 Run: Module sequence_vector_clip needs configuring

Assuming that the configuration is correct, the processing will start and output will be sent to the output window as progress is made. The progress within each module is shown by a series of fullstops (.) for each correctly processed sequence, and an exclamation mark (!) for each failed sequence.

The text output window above shows the early processing stages of 20 sequences. When finished pregap4 will produce a report containing information from each module and the final list of passed and failed sequences. For example:

- Report Production -
  Passed files:
  xb54a3.s1.exp (xb54a3.s1SCF.gz) : type EXP
  xb54b12.r1L.exp (xb54b12.r1LSCF.gz) : type EXP
  xb54b12.r1.exp (xb54b12.r1SCF.gz) : type EXP
  xb54b12.s1.exp (xb54b12.s1SCF.gz) : type EXP
  xb54c3.s1.exp (xb54c3.s1SCF.gz) : type EXP

  Failed files:
  xb54g5.s1.exp (xb54g5.s1SCF.gz) 'screen_vector_clip: sequence too short'

- Report from 'Augment Experiment Files' -
  xb54a3.s1.exp : added fields SF CF SC SP TN ST PR SI CH.
  xb54b12.r1L.exp : added fields SF CF SC SP TN ST PR SI CH.
  xb54b12.r1.exp : added fields SF CF SC SP TN ST PR SI CH.
  xb54b12.s1.exp : added fields SF CF SC SP TN ST PR SI CH.
The list of passed and failed files are written to `prefix.passed` and `prefix.failed`, where `prefix` is the output filename prefix specified in the "Files to Process" panel. The reports are written to `prefix.report`. The passed and failed files contain the most recent filenames associated with each sequence. So if a sequence fails early on it could be listed as something like `xb54a3.s1SCF.gz` and if it fails later it will be listed like `xb54a3.s1.exp`. This is because it is the final filename which is important for later processing, such as for assembly into gap4.

A `prefix.log` file is also created containing a list of passed files, failed files, and the filename history for each file (the intermediates will still exist). The format of the passed section is "filename (file_type) PASSED". The format of the failed section is "filename (file_type) ERROR: error message". The format of the file history lines is a series of "filename (file_type)" segments separated by "<-", with the original filename listed to the right. Filenames containing Tcl meta-characters may be 'escaped' using curly braces or back slashes. (The Tcl `subst` command may be used to generate the original name.) An example of a log file follows. This was produced with the command line "pregap4 "Sample 671" WT5.exp zf89a2.s1.scf xb56e5.s1.scf".

```
[passed files]
ha59a6.s1.exp (EXP) PASSED
WT5.exp (EXP) PASSED
xb56e5.s1.exp (EXP) PASSED

[failed files]
zf89a2.s1.exp (UNK) ERROR: screen_vector_clip: sequence too short

[passed file history]
ha59a6.s1.exp (EXP) <- ha59a6.s1.scf (SCF) <- {Sample 671} (ABI)
WT5.exp (EXP)
xb56e5.s1.exp (EXP) <- xb56e5.s1.scf

[failed file history]
zf89a2.s1.exp (UNK) <- zf89a2.s1.scf
```

Some modules may also keep their own separate records, such as an assembly log. Where this is the case, it will be explained in the help specific to that module.

After running pregap4 it is time to either assemble the data (if this was not done using pregap4) or to edit it. If the data has already been assembled with Pregap4 then you will need to start up gap4 and use 'Open Database'. Otherwise one of the gap4 assembly functions should be used, with the `filename_prefix.passed` file. For more information on this see the Gap4 manual.

### 3.5 Configuring the Pregap4 User Interface
3.5.1 Fonts and Colours

The pregap4 Options menu contains options for modifying the fonts and colours used. These options are common to many programs and so are documented elsewhere. See Section 9.7 [Font Selection], page 381. See Section 9.5 [Colour Selector], page 378.

3.5.2 Window Styles

Pregap4 supports two styles of windowing. The default method is a compact mode, with the alternative being "separate" mode - similar to gap4 and spin.

![Screenshot of Pregap4 interface]

This is the "separate" window style. Here the main window is always visible, with commands in the main window bringing up new windows. In the picture above the configure window can be seen on top of the main window.
The second style is "compact" mode.

In the compact picture above the most common top level windows are "pages" in a tabbed notebook.

The benefit is greatly reduced screen space and quicker controls, but the text output window is no longer permanently visible.

To switch styles select the "Compact Window Style" and "Separate Windows Style" commands from the Options menu.

3.6 Configuring Modules

The "Configure Modules" dialogue is available from the Modules menu or, when using the compact window style, by pressing the Configure Modules tab.

This dialogue contains the main interface through which most of the user’s interaction with pregap4 will be performed. The left side of the display contains a list of the currently loaded modules. One module in this list will be highlighted. The right side of the display shows the configuration panel for this highlighted module.
The module list shown on the left consists of a series of module names and their status, and is termed the "enable status". The [ ] and [x] strings at the left of the name indicates whether this module is enabled; crossed boxes are enabled modules. The highlighting is another indication of whether the module is enabled. The "General Configuration" module is mandatory and cannot be disabled. The text to the right of the module name indicates whether the module has been given all the parameters needed for it to process. This will be one of "ok" (all configuration options have been filled in), "-" (no configuration options exist for this module), "edit" (further configuration is required") or blank (this module is disabled).

The "enable status" can be toggled by left clicking on the "[ ]" to the left of the module name. The enable status can be written to the current Pregap4 configuration file using the "Save Module List" or "Save All Parameters" commands in the Modules menu. Left clicking anywhere on a module name in the module list will switch the pane on the right side of the window to display any available parameters for this module. Not all modules will have parameters to configure.

For modules that do have parameters, the top line of the configuration panel will contain a button labelled "Save these parameters". This button will save all parameters for this module to the configuration file. Note that this is not the same as the "Save all parameters" option in the main Modules menu, as this saves all parameters in all modules.

3.6.1 General Configuration

Description
This is a mandatory module. It is always the first module executed and will not appear in the "Add/Remove Modules" list. Its purpose is to set general parameters which affect several other modules. At present it contains just two items.

Option: Get entry names from trace files
Many trace formats include storage for a sequence "sample name". This option controls whether or not the sample name should be used instead of deriving the name from the filename. If "No" is answered to this question then the sequence sample name will be
generated by removing the filename suffix; for example *xb55a2.s1.ztr* will become *xb55a2.s1*.

### 3.6.2 Estimate Base Accuracies

**Description**

This module analyses the traces at each base call to estimate a confidence value for the called base. It does this by simply looking at the area underneath the trace for the called base and dividing this by the highest area under the trace for the three uncalled bases. This is a very simplistic statistic which should ideally only be used for measuring the average reliability of the entire sequence rather than any individual base. If another program (eg Phred, or ATQA) is available then this should be used in preference. From the 2002 release the eba values are normalised to the phred scale (this was achieved by comparing the original eba values and phred values for 4.6 million base calls of Sanger Centre data).

There are no adjustable parameters for this module.

### 3.6.3 Phred

Phred is not included as part of the Staden Package. It is available from Phil Green. [http://www.genome.washington.edu/UWGC/analysistools/phred.htm](http://www.genome.washington.edu/UWGC/analysistools/phred.htm)

**Description**

Phred is an ABI base caller. Ewing, B. and Green, P. 1998. Base-Calling of Automated Sequencer Traces Using Phred. II. Error Probabilities. Genome Res. 8, 186-194. It will analyse the chromatogram data to produce new base calls. For each base it assigns a confidence value indicating how likely this base call is to be correct. These confidence values are significantly more reliable than those produced by eba and they are compatible with the Phrap assembly program and the gap4 consensus algorithm.

Phred can process either ABI or SCF files, but pregap4 will automatically convert all input to SCF format first. This means that the phred pregap4 module will be able to process any supported trace format.

There are no adjustable parameters for this module.

### 3.6.4 ATQA

ATQA is not included as part of the Staden package. It is available from its developers, Daniel H. Wagner, Associates, at [http://www.wagner.com/](http://www.wagner.com/)

**Description**

The ATQA program estimates confidence values for each called base in a lane file. A confidence value corresponds to the probability that the associated base call is incorrect by the formula

\[
\text{score} = -10 \times \log_{10}(\text{probability of error}).
\]

(This is the same log scale used by Phred.) In fact, the ATQA program computes four confidence values for each called base. The first three values correspond to the probabilities of substitution, insertion, and deletion errors, respectively. The fourth value is a combined score representing the probability that the called base is an error...
of any sort. Currently, only the combined confidence value is used by Staden package software.

Unlike Phred, the ATQA program does not produce base calls. Rather, it assigns confidence values to each base call in a lane file based on features of the trace data. The current version of the ATQA program is tuned to base calls made by the ABI base caller and to trace data from the ABI 377 sequencer.

Although ATQA can read ABI files, it will not create SCF files in such circumstances. However pregap4 will always convert any non SCF trace files into SCF format before running ATQA, so an explicit conversion is not required.

### 3.6.5 Trace Format Conversion

**Description**

This converts files between the various supported trace formats. At present it can read ABI, ALF, SCF, CTF and ZTR formats, and can write SCF, CTF and ZTR. Of these formats, ZTR typically represents the smallest size and is fast due to its own internal compression routines.

The Trace Format Conversion may also be used to apply some simple editing methods to the traces. These include down-scaling (to reduce file size), background subtraction, and amplitude normalisation.

**Option: Output format**

This selects the format for the output trace files. If the output format is the same as the input format then the input files will not be overridden. Instead new files will be produced with names based on the input names, generated by replacing (for example) ".scf" with "..scf". The available output format choices are ZTR, CTF and SCF.

**Option: Downscale sample range**

These select whether to reduce the scale used to store the amplitudes, and if so to what range. ABI files typically range from 0 to 1600 (which is approximately 11-bit data). Shrinking this down to 0 to 255 (8-bit) will usually be visually comparable as the trace displays in Gap4 and Trev are typically smaller than 255 pixels high, although if the Y scale is increased differences will still be detectable. The purpose of this is to further reduce file size.

**Option: Subtract background**

This attempts to eliminate the trace background by a simple technique of deducting the lowest of the four amplitudes from all of the four amplitudes. This is an overly crude method which should only be used when the preprocessing software included on the sequencing manufacturer’s instruments has not been used.

**Option: Normalise amplitudes**

This uses a sliding window to compute the average single strengths. From this it scales the data to try and provide, on average, more uniform peak heights along the trace. Again this is a very simplistic method and so it is not advisable unless their is a problem with the sequencing manufacturer’s own software.

**Option: Delete temporary files**

When pregap4 can determine that a trace file is neither the original input or the final output then it is considered to be a temporary file which may be suitable for deletion. An example would be using Phred with ABI files and then converting to ZTR. Phred
produces SCF files and so we have ABI to SCF to ZTR, in which the SCF files may be safely deleted.

### 3.6.6 Initialise Experiment Files

**Description**

This module creates an Experiment File from a trace file (of any format). It uses the `init_exp` program to write ID, EN, LN, LT, AQ and SQ Experiment File line types. This module is mandatory for many subsequent modules, such as vector clipping/screening and assembly.

There are no adjustable parameters for this module.

### 3.6.7 Augment Experiment Files

**Description**

This module adds further data to the Experiment File, with the additional information typically obtained from external sources. Such information could be the data required by the vector clipping program, or template information needed by gap4.

The parameters for this module may be configured by using the "Simple Text Database" (see Section 3.10.1 [Simple text Database], page 240) or "Experiment File Line Types" (see Section 3.10.2 [Experiment File Line Types], page 242) dialogues. These both allow setting of the Experiment File records to be written during the Augment stage.

### 3.6.8 Quality Clip

**Description**

This module determines where the sequence quality is too poor to use for reliable assembly. It supercedes the Uncalled Base Clip module. This uses the `qclip` program which reads and writes to Experiment Files. Its default quality evaluation is based on the range of values produced by the Estimate Base Accuracies module (quality value 70, averaged over 100 bases). For use with phred, try lower values such as quality value 15 averaged over 50 bases. When quality values are not available it will use the same method as the Uncalled Base Clip module; to analyse the base calls and count the number of undetermined bases within a given window of sequence. Both 5' and 3' ends may be quality clipped.

For the confidence mode of clipping the method starts from the point of highest average quality, and then steps outwards in both directions until the average quality is below a defined threshold.

For the sequence mode of clipping the method starts from a defined position and steps outwards in both directions until the number of uncalled bases within a given window length exceeds a predefined threshold. For more details see the `qclip` documentation (see Section 11.13 [qclip], page 436).

Note that the Phrap assembly algorithm works best without quality clipping and it can make use of the full length of readings (due to the use of the Phred confidence values).

**Option: Clip mode**

This may be one of "by sequence" or "by confidence". The "by sequence" mode is equivalent to the Uncalled Clip module. The "by confidence" mode uses Phred-scaled confidence values to determine the quality for clipping. This does not work with eba confidence values.
Option: Minimum extent
   The lowest allowable 5’ clip position.

Option: Maximum extent
   The largest allowable 3’ clip position.

Option: Minimum length
   If after quality clipping the good portion of a sequence is shorter than the specified
   length, then this file will be rejected with the message "qclip: Sequence too short".

Option: Window length
   The window length over which the confidence will be averaged. This option is only
   relevant for the "clip by confidence" mode.

Option: Average confidence
   The minimum average confidence (over ‘window length’ bases) for sequence to be ac-
   cepted as good quality. This option is only relevant for the "clip by confidence" mode.

Option: Start offset
   The base number to start the 5’ and 3’ good quality searches from. This option is only
   relevant for the "clip by sequence" mode.

Option: 3’ window length
   The window length in which to count uncalled bases. This option is only relevant for
   the "clip by sequence" mode.

Option: 3’ number of uncalled bases
   The maximum allowed count of uncalled bases in a single window length. This option
   is only relevant for the "clip by sequence" mode.

Option: 5’ window length
   The window length in which to count uncalled bases. This option is only relevant for
   the "clip by sequence" mode.

Option: 5’ number of uncalled bases
   The maximum allowed count of uncalled bases in a single window length. This option
   is only relevant for the "clip by sequence" mode.

3.6.9 Sequencing Vector Clip

Description
   This module uses the vector_clip program to identify and mark the sequencing vector
   (those used to produce templates for sequencing, eg m13mp18 or puc18). To achieve
   this task it needs to know information about the vector including the cut site position
   and the position of the primer site relative to the cut site. See Section 5.8 [Defining
   the Positions of Cloning and Primer Sites for Vector_Clip], page 273.

Option: Use Vector-primer file
   Vector_clip may be told to search through a series of vectors and primers held within
   an external file. Alternatively we can request that it looks only at one specific, known,
   vector. This question is to determine which of the two mutually exclusive methods
to use. In general it is still important for the Experiment File to contain primer and template data. The Vector-primer module can be used to add the primer and sequencing vector information to the Experiment File but not the template name.

**Option: Vector-primer filename.**
This is only used if the "Use Vector-primer file" question was answered with "Yes". Each input sequence will be compared against each vector-primer pair to find the best match. This provides a simple way of comparing against multiple vectors or comparing against both forward and reverse primers of a single vector. For further details on creating this vector-primer file, see Section 5.6 [Vector Primer file format], page 272..

**Option: Select vector-primer subset**
This is used in conjunction with the vector-primer filename to indicate which of the vector-primer pairs listed in this file should be used. Initially this is set to all vector-primer pairs, but efficiency will be greatly increased if just the required subset is selected. (Internally pregap4 will then temporarily produce a new vector-primer filename each time vector_clip requires one, containing just the selected items.) To select more than one vector-primer pair use the standard listbox mouse bindings: single left click to pick an item; click and drag to select a range; and control left click to toggle a single item. The selected list will be saved to the pregap4 configuration file whenever all the parameters for this module are saved.

**Option: Max primer to cut-site length**
This parameter is only used when a vector-primer file is defined. The sequence stored in the vector-primer file may be considerably longer than we expect to see at the start of the sequences being analysed. By defining the maximum length of sequence we expect to see, vector_clip may be more sensitive and slightly faster.

**Option: Vector file name**
This, and the following two options, are only used if the "Use Vector-primer file" question was answered with "No". The vector file name should be the name of a file containing just the vector bases or white space, in a plain text format.

**Option: Cut site**
The cut site specified as a base count from the start of the vector file.

**Option: Primer site**
The primer site specified as a base offset from the cut site. e.g. for m13mp18 forward primers the value is 41. If, instead of the usual single value, two values are specified separated by a slash, then this gives the values for the universal forward and reverse primers (for example "41/-24"). Only use this format if the PR (primer type) experiment file line type is known AND will be specified in the experiment file. If the PR record is not specified in the experiment file, the primer site position will be set to zero, and the vector clipping is unlikely to work correctly. (PR values do not have to be known if they can be derived using naming schemes such as those used by the Sanger Centre). If the primer site indicates a custom primer sequence then the primer site is taken to be 0.

**Option: Percentage minimum 5' match**
Option: Percentage minimum 3' match
Both ends of the sequence are checked using a dynamic programming algorithm to find the optimal alignment. An end is marked as vector if the percentage match is at least as high as this supplied parameter.
Option: Default 5’ position  
This specifies the value to use for marking the 5’ sequencing vector if none is detected. Specifying this as -1 will cause the absolute value given for the primer site (which is specified as relative to the cut site).

3.6.10 Cross_match  
Cross_match is not included as part of the Staden Package. It is available from Phil Green. http://www.genome.washington.edu/UWGC/analysistools/swat.htm  
Description  
This uses the cross_match program to search for sequencing vector. (Future versions may also check for other cloning vectors.) This allows for searching of multiple vector files. However as cross_match does not make use of primer and cut site information the vector detection is inherently less sensitive than vector_clip (see Chapter 5 [Screening against Vector Sequences], page 267).

Option: FASTA vector file name  
This specifies a fasta format file of one or more sequencing vector sequences.

Option: Minimum match length  
Minimum length of matching word for SWAT comparison.

Option: Minimum score  
Minimum SWAT score.

3.6.11 Cloning Vector Clip  
Description  
This module searches for non "sequencing" vectors used in the shotgunning process, eg for Cosmid or YAC. Any fragment in any orientation of this vector could be present so there is no need for the cut sites to be known. The vector_clip program is used for this task (see Chapter 5 [Screening against Vector Sequences], page 267).

Option: Vector file name  
The filename containing the vector sequence. At present this should be a file containing a single plain text sequence containing just the bases or white space.

Option: Max probability  
For each match its probability of occurring by chance is calculated. Any match with a probability lower than ‘Max probability’ is accepted.

3.6.12 Screen for Unclipped Vector  
Description  
This module may be used to identify undetected segments of sequencing vector or to detect recombinations. After searching and marking sequencing vector, any further strong matches to the sequencing vector indicate a possible problem. This module uses the vector_clip program (see Chapter 5 [Screening against Vector Sequences], page 267). Note that this module requires the Sequencing Vector Clip module to be used before screening, otherwise all sequences containing unclipped vector will be falsely rejected.
Option: Minimum length of match
If a match of at least this length is found then the sequence currently being processed will be rejected.

3.6.13 Screen Sequences

Description
This module can perform very fast matches between the sequences to process and one or more screen sequences. Any sequence containing a significant match is rejected. An example of use for this module is to reject sequences prior to assembly that appear to be contaminated with E. coli. This uses the `screen_seq` program (see Section 11.14 [Screen_seq], page 439).

Option: Screen single sequence
This is yes/no question used to determine whether the screen sequence filename is the filename of a single sequence or a filename of a file containing a series of sequence filenames. To compare just one file select "Yes".

Option: Screen sequence file (of filenames)
This is either the filename of a single sequence or the filename of a file of filenames, depending on the answer to the previous question. The sequence files must be in plain text format containing just the bases or white space.

Option: Maximum screen sequence length
The maximum length of any individual screen sequence.

Option: Minimum match length
Any fragment containing an exact match longer than this length will be rejected.

3.6.14 Blast Screen

Description
This module uses the `blastall` program to compare all the input sequences against a prebuilt blast database of screen sequences. It is not possible to compare against a subset of the database - to do this build a new blast database using `formatdb`. This module is an alternative to the Screen Sequences module which uses the `screen_seq` program.

Blast may be used for either completely rejecting sequences or for simply tagging the matching segments, or for both. If you wish to tag with several tag types, then several instances of the Blast screen module need to be used.

Blast is not included as part of the Staden Package. It is available from the NCBI.

Option: BLAST database
This is the filename of the BLAST database to screen against, with the `.nhr`, `.nin` and `.nsq` suffixes removed.

Option: E value
This specifies the ‘E value’ used by blast when determining which hits should be considered as real.

Option: Match fraction
This is the total percentage of the sequence which much have a blast match somewhere in the BLAST database searched in order to reject this sequence. Segments of the input
sequence that match multiple components in the BLAST database are only counted once when computing this percentage, but the locations of the matches in the BLAST database do not need to be consecutive.

If you wish to accept everything, but still want to tag the matches, then set the match fraction to greater than 1.0.

**Option: Tag type**

The default for this is `<none>` which indicates no tagging is required. Otherwise this should be a 4 letter tag type (such as `REPT`) known to gap4.

### 3.6.15 Interactive Clipping

**Description**

This module invokes the `trev` program to view the raw chromatogram files. The user can then adjust the quality and vector clip positions if desired. The `trev` window will contain Next and Previous buttons to skip from trace to trace. The Reject buttons allows a trace to be rejected, in which case it is added to the failure file with the message "interactive clip: manually rejected".

There are no adjustable parameters for this module.

### 3.6.16 Extract Sequence

**Description**

This module uses the `extract_seq` program to extract the sequence information from binary trace files, Experiment files, or from the old Staden format plain files. The output contains the sequences split onto lines of at most 60 characters each, in plain or fasta format. The input files are passed unchanged onto subsequent modules.

**Option: Output only the good sequence**

When reading an experiment file or trace file containing clip marks, output only the good sequence which is contained within the boundaries marked by the `QL`, `QR`, `SL`, `SR`, `CL`, `CR` and `CS` line types.

**Option: Consider cosmid as good sequence**

When the `Output only the good sequence` option is specified this controls whether the cosmid sequence should be considered good.

**Option: Output in fasta format**

Specifies that the output should be in fasta format rather than plain text.

**Option: Output in one file only**

If this option is selected then the output from every sequence is sent to one file. This is best used with the `Output in fasta format` option selected, and is useful for feeding into BLAST searches, for example. The file to write to is specified in the `File name` field.

If this option is unselected then the output is sent to separate files, one per sequence. The output files have the same name as the input files, except with an extra suffix specified in the `File name suffix` field.

### 3.6.17 RepeatMasker

Description
This module uses the RepeatMasker program. This is a program which searches for a comprehensive set of repeat sequences. Any matches which are found will be tagged with a comment indicating the type of repeat. These tags will then be visible from within gap4. Full documentation is available from the author of RepeatMasker, or from typing RepeatMasker -h.

Option: Repeat library
This specifies the directory containing the library of repeat sequences. Only one library directory may be specified. The library "<default>" will let RepeatMasker use its own default library.

Option: RepeatMasker cutoff
This specifies the cutoff score for RepeatMasker. The documentation with RepeatMasker states that a cutoff of 250 will guarantee no false positives.

Option: Gap4 tag type
When a repeat is found a tag will be added to the Experiment File. This specifies the tag type to use. It should be one of the tag types available to Gap4, but other tag types may be used if desired (they will be coloured as is COMMENT tags in gap4).

Option: Types of repeat to screen against
The default setting of RepeatMasker is to search for primate repeats, however it may be told to search for other repeat families or to restrict its search to only ALU primate repeats. The full list of options here are Alu only, Rodent only, Simple only, Mammalian excluding primate/rodent, and no low complexity. These are as defined in the RepeatMasker documentation. It is not known what effect enabling mutually exclusive options will have.

3.6.18 Tag Repeats
Description
This module uses the repe program to identify and mark known repetitive elements within the sequences. An example usage is to tag all ALU fragments. This information may be used by the gap4 assembly algorithm to improve the assembly by initially ignoring matches between two ALU fragments which may otherwise produce incorrect assemblies. If available, we recommend using RepeatMasker instead of this module.

Option: Repeat file name
This is the filename of a file of filenames, each of which contain a single repeat to search for. The format of these individual files is plain text consisting of just the nucleotides and white space.

Option: Repeat score
This is the minimum score for classifying a matched segment as a repeat.

Option: Tag type
This is the gap4 tag type to use for identifying this repeat segment. It is not possible to choose different tag types for different repeats, although the tag comments contain the match score and match filename.

3.6.19 Mutation Detection
Description

Superceded by the newer modules: (see Section 3.6.21 [Trace Difference], page 229) and (see Section 3.6.22 [Heterozygote Scanner], page 231).

This module compares each sequence chromatogram against a "wild type" or reference chromatogram to detect point mutations. The mutations are detected by aligning and subtracting each trace from the wild type trace to produce a "difference trace". The difference trace is then analysed to identify point mutations which are written back to the Experiment File and MUTN tags. This uses the trace_diff program Bonfield, J.K., Rada, C. and Staden, R. Automated detection of point mutations using fluorescent sequence trace subtraction. Nucleic Acids Res. 26, 3404-3409 (1998).

Obviously the reference traces should be as similar as possible to the ones being compared against it. It should be prepared by sequencing the wild type from the same primer, and using the same chemistry as the readings being screened. One good way to produce a reference trace is to run the wild type sequence on the gel along with the other samples. It is also possible to get gap4 to produce a consensus trace. This requires using pregap4 twice. Firstly process the sequences through pregap4 with all the appropriate options except with the mutation detection module disabled. Assemble these sequences into gap4. Within gap4, for each contig start up the Contig Editor and select Save Consensus Trace from the command menu. This will produce a trace which is the average of the traces in that contig. Then delete the gap4 database and reprocess the sequences using Pregap4, this time using mutation detection to compare against the consensus trace.

Option: Wild type file (+ve strand)
Option: Wild type file (-ve strand)
These are the filenames of the chromatogram for the wild type sequence on each strand. These may be in any allow trace format (SCF, ZTR, ABI, CTF or ALF). In the augment stage, these are represented in the WT line type using plus_filename|minus_filename notation.

Option: Start position
Option: End position
These define the range within each sequence in which to identify mutations. The algorithm works better on good quality data so including very bad sequence may give errors.

Option: Score
This a threshold used to determine when a peak in the difference trace is considered to be a mutation. The higher the value the more stringent the test.

Option: Alignment band width
The trace alignment is performed by firstly doing a sequence alignment on the text sequences contained in the two files. This parameter specifies the band width for this alignment. Smaller values give quicker alignments, but only work if the alignment is sufficiently close to the main diagonal.

Option: Other arguments
This allows for any other arguments to be passed to the trace_diff program. See the trace_diff documentation for more details.

The module above is superceded by the newer modules: (see Section 3.6.21 [Trace Difference], page 229) and (see Section 3.6.22 [Heterozygote Scanner], page 231).
3.6.20 Reference Traces and Reference Sequences

Description
This module specifies the reference traces and reference sequences used by the two mutation detection modules (see Section 3.6.21 [Trace Difference], page 229 and see Section 3.6.22 [Heterozygote Scanner], page 231). The left and right clip points for each trace can also be specified.

A reference trace should be as similar as possible to the ones being compared against. It should be prepared by sequencing the wild type from the same primer and using the same chemistry as the readings being screened. One good way to produce a reference trace is to run the wild type sequence on the gel along with the other samples.

If the input files have been sequenced from both strands, reference traces from each strand may be specified here. Note that in order for pregap4 to choose the appropriate wild type trace it needs to know the strand for each input sequence. This is specified by the PR record in the experiment file which is typically generated using a naming convention (see Section 3.8 [Pregap4 Naming Schemes], page 236) If pregap4 cannot determine the strand, or if only one reference trace is specified, then each input sequence will be compared against the +ve strand reference trace.

The reference data supplied in this module, when entered with gap4 shotgun assembly, will add REFS and REFT notes (see Section 1.15 [Notes], page 159) to the gap4 database. A reference sequence is used to number bases in the Contig Editor (see Section 1.6.12 [Reference sequences and traces], page 83) and in reporting the positions of mutations (see Section 1.6.7.9 [Report Mutations], page 72.)

Option: Reference Trace (+ve strand)
Option: Reference Trace (-ve strand)
These are the filenames of the chromatogram for the reference trace on each strand. These may be in any allowable trace format (ZTR, SCF, ABI, CTF or ALF). The filenames are entered into the experiment file as WT records by the "Augment Database" phase of pregap4, so this module must also be enabled.

Option: Clip left
Option: Clip right
These values determine which region of the reference trace (in bases) is used for mutation detection. This can be used to exclude poor quality regions, or restrict the range over which mutation detection occurs. Restricting the range will also speed up the algorithms. If you specify -1 for all values, the entire trace is used. i.e. No clipping occurs. If the range specified is too small, the mutation detection algorithms may report an error, since there must be a useful overlap between the sequences in order to process them.

Option: Reference Sequence
This specifies the reference sequence, which is typically an annotated EMBL entry. This field is optional.

Option: Start base number
If a reference sequence was specified this indicates which base number it will start counting from within Gap4's contig editor. It also defines the positions of mutations, as output by the Report mutations function of gap4 See Section 1.6.7.9 [Report Mutations], page 72..

Option: Circular
Option: Sequence length
If the reference sequence is defined to be circular then the length needs to be known too. When the base number reaches the sequence length the next base in the sequence
will be renumbered to base 1. This may be useful if the circular reference sequence needs to be chopped to form a linear sequence at a different position than the standard numbering. (For example this is typical when sequencing the mitochondrial variable loop, which by standard conventions contains base number 1.)

Note that it is possible (though no longer recommended) to use gap4 to produce a consensus trace. This requires using pregap4 twice. Firstly process the sequences through pregap4 with all the appropriate options except with the mutation detection modules disabled. Assemble these sequences into gap4. Within gap4, for each contig start up the Contig Editor and select Save Consensus Trace from the command menu (available only in expert mode). This will produce a trace which is the average of the traces in that contig. Then delete the gap4 database and reprocess the sequences using Pregap4, this time using mutation detection to compare against the consensus trace. Best results are usually obtained by first deleting pads in the consensus sequence. You should inspect the resulting consensus trace carefully to ensure there are no discontinuities introduced as a result of the pad deletions.

3.6.21 Trace Difference

Description

This module compares each sequence chromatogram against a "wild type" or reference chromatogram to detect point mutations. The mutations are detected by aligning and subtracting each trace from the wild type trace to produce a "difference trace". The difference trace is then analysed to identify point mutations which are written back to the Experiment File as MUTA tags. The basic idea is explained in the paper Bonfield, J.K., Rada, C. and Staden, R. Automated detection of point mutations using fluorescent sequence trace subtraction. Nucleic Acids Res. 26, 3404-3409 (1998).

This implementation is the second version of the algorithm. The previous version used basecalls to do trace alignment. This led to problems when bases were called in error (often the case around mutations). The new algorithm ignores the basecalls completely and aligns the trace signals themselves, avoiding such problems. This is much more computationally intensive, but it has proved to be fast enough for interactive use.

If the input files have sequenced from both strands then two wild type sequences may be given. In order for pregap4 to choose the appropriate wild type trace it needs to know the strand for each input sequence, which is typically generated using the naming convention. A simple naming scheme is provided with pregap4 (in the lib/pregap4/naming_schemes directory) called "mutation_detection.p4t". This can be loaded from the pregap4 file menu. It assumes that trace names have an 'f' or 'r' suffix, denoting the forward and reverse strands respectively. If you need something more complex, then you’ll have to create and load your own naming scheme. If pregap4 cannot determine the strand, or if only one wild type is specified, then each input sequence will be compared against the +ve strand wild type.

The reference or wild type traces for tracediff are specified in the see Section 3.6.20 [Reference Traces module], page 228.

Option: Sensitivity

This threshold is used to determine when an above/below baseline double peak in the difference trace is considered to be a mutation. It is specified in standard deviations from the mean over the analysis window. The higher the value, the more stringent the test. This value is reduced dynamically by the algorithm in the presence of mutations since small mutations near larger ones can often be missed with a uniform sensitivity setting. It’s likely that some experimentation with this parameter will be required for optimal mutation detection in your data.
Option: Noise threshold

This threshold is used to filter out low level noise during the analysis phase. It is specified as a percentage of the maximum peak-to-peak trace difference value. A high threshold will lead to fewer false positives but you run the additional risk of missing low level mutations.

Option: Analysis window length

Analysis of the trace difference is done over a local region to counter the effects of non-stationarity in the trace signal. The analysis region is defined by a short window whose length is specified in bases. The window is asymmetric in that it’s located to the left of the base it’s positioned on. This avoids measurement problems when mutations are encountered. The window size is a tradeoff. If it’s too big, low level mutations may be missed. If it’s too small, there may be insufficient data to give unbiased measurements leading to many false positives.

Option: Maximum peak alignment deviation

The centres of each individual half-peak of a double peak above and below the baseline must align reasonably well for them to be considered to be real mutations. The amount of half-peak alignment deviation allowable is specified in bases by this parameter, usually as a fraction of one base.

Option: Maximum peak width

During analysis, the width of each peak is measured to avoid problems caused by gel artifacts. These often appear as broad peaks that overlay many bases. The maximum peak width is specified in bases. A lower value will lead to fewer false positives, but you run the additional risk of missing smeared mutations towards the end of a trace.

Option: Complement bases on reverse strand tags

After mutation detection and after readings have been assembled into a GAP4 database, GAP4 displays both forward and reverse readings in a single direction in the contig editor. This makes it much easier to compare sequences and traces in both directions simultaneously. When the corresponding traces are displayed, any reverse strand traces are complemented automatically such that the bases are interchanged. In this case, the original mutation tag generated by tracediff will then be of the wrong sense, so if checked, this option complements the tag base labels to match the complemented trace displayed by GAP4.

Option: Write difference traces out to disk

After trace difference analysis, the generated traces are normally discarded and not written to disk. Checking this option lets you save the trace difference files to the same directory as the original traces. The .ZTR trace format is used for this purpose. The original filename is retained and a "_diff.ztr" suffix is appended.

3.6.22 Heterozygote Scanner

Description

This module compares each input sequence chromatogram against a wild type or reference chromatogram and itself to detect heterozygote mutations. Heterozygote mutations are detected by examining the chromatogram for double overlapping peaks. A mutation "mask" is established by a set of double peak parameters. Comparisons are made against this mask to determine if the double peak in question is a valid heterozygote mutation.
Most of these parameters are user definable since no one set of parameters will be suitable for all data. The default parameters have been chosen to maximise heterozygote detection and at the same time, minimise the number of false positives reported. Some experimentation may be required to optimise these for your own data. After chromatogram analysis has been completed, mutation tags are written back to the Experiment File as \texttt{HETE} tags.

Both the wild type and the input traces are scanned for mutations. Any mutation appearing on the wild type and the input trace is ignored by the Heterozygote Scanner. This helps to cut down on the number of false positives reported. The reference or wild type traces for hetscan are specified in the see Section 3.6.20 [Reference Traces module], page 228.

**Option: Maximum peak alignment deviation**

The centres of each individual half-peak of a double peak must align reasonably well for them to be considered to be real heterozygotes. The amount of half-peak alignment deviation allowable is specified in bases by this parameter, usually as a fraction of one base.

**Option: Minimum amplitude for peak 1/2**

Low level noise in a trace can often look like perfect double peak heterozygotes to the heterozygote scanner. These options setup a mask for the peak amplitudes as a percentage of the maximum trace value. Anything below these percentages isn’t considered to be a valid heterozygote. Peak 1 refers to the highest amplitude peak, whereas peak 2 refers to the 2nd highest amplitude peak.

**Option: Minimum peak amplitude ratio**

The ratio of peak 1 to peak 2 amplitude is used to help identify true heterozygotes from noise and gel artifacts. This parameter forms a mask such that any double peak whose amplitude ratio is less than this value is rejected by the heterozygote scanner. Normally you’d expect the ratio to be in the region of 0.5, but trace preprocessing can distort these figures somewhat.

**Option: Complement bases on reverse strand tags**

After mutation detection and after readings have been assembled into a GAP4 database, GAP4 displays both forward and reverse readings in a single direction in the contig editor. This makes it much easier to compare sequences and traces in both directions simultaneously. When the corresponding traces are displayed, any reverse strand traces are complemented automatically such that the bases are interchanged. In this case, the original mutation tag generated by hetscan will then be of the wrong sense, so if checked, this option complements the tag base labels to match the complemented trace displayed by GAP4.

### 3.6.23 Gap4 Shotgun Assembly

**Description**

This module assembles the processed sequences into gap4 using gap4’s own assembly engine. Note that this is incompatible with use of "Enter assembly into Gap4", which should only be used for external (to gap4) assembly engines.

**Option: Gap4 database name**

**Option: Gap4 database version**

The name and version of the database to assemble into.
**Option: Create new database**
This is a toggle to define whether the specified gap4 database should be created or appended to. Be warned that at present creating a new database will overwrite existing one of the same name, in the same directory, without any warnings.

**Option: Minimum exact match**
**Option: Maximum number of pads**
**Option: Maximum percentage mismatch**
These control the main assembly parameters within gap4. For more details see Section 1.7.1 [Normal shotgun assembly], page 94.

### 3.6.24 Cap2 Assembly

Cap2 is not included as part of the Staden Package. It is available from Xiaoqiu Huang (huang@mtu.edu).

**Description**
This module uses the *cap2* program to perform shotgun assembly. Output will be placed in the *fofn*.assembly directory, where *fofn* is the filename prefix listed in the "Files to Process" panel. The output is in a format suitable for directed assembly within gap4. This can also be performed by using the "Enter Assembly into Gap4" module.

There are no adjustable parameters for this module.

### 3.6.25 Cap3 Assembly

Cap3 is not included as part of the Staden Package. It is available from Xiaoqiu Huang (huang@mtu.edu).

**Description**
This module uses the *cap3* program to perform shotgun assembly. Output will be placed in the *fofn*.assembly directory, where *fofn* is the filename prefix listed in the "Files to Process" panel. The output is in a format suitable for directed assembly within gap4. This can also be performed by using the "Enter Assembly into Gap4" module. Cap3 differs from Cap2 in that it can make use of confidence values (in the range supplied from *phred*) and constraints.

**Option: Auto-generate constraints**
When enabled, this uses the reading direction (forward / reverse primers), the template name and the insert size, to produce a file containing data to constrain how the readings may be assembled.

### 3.6.26 FakII Assembly

FakII is not included as part of the Staden Package. It is available from Susan Miller (susanjo@cs.arizona.edu).

**Description**
This module uses the *FakII* suite of programs to perform shotgun assembly. Output will be placed in the *fofn*.assembly directory, where *fofn* is the filename prefix listed in the "Files to Process" panel. The output is in a format suitable for directed assembly within gap4. This can also be performed by using the "Enter Assembly into Gap4" module.
Option: E limit  
Option: D limit  
Option: O threshold  
These parameters control the graph component of FakII, which is used to find the initial overlaps between sequences.

Option: Auto-generate constraints  
When enabled, this uses the reading direction (forward / reverse primers), the template name and the insert size, to produce a file containing data to constrain how the readings may be assembled.

Option: E rate  
Option: O threshold  
Option: D threshold  
These parameters control the assemble component of FakII, which is used for determining the best construction of sequences from the overlap graph.

Option: Assembly number  
This allows for non optimum assemblies to be chosen. The optimum assembly is assembly number 1, with the next optimum being number 2, and so on.

3.6.27 Phrap Assembly  
Phrap is not included as part of the Staden Package. It is available from Phil Green. http://www.genome.washington.edu/UWGC/analysistools/phrap.htm  
Description  
This module uses the phrap program to perform shotgun assembly. Output will be placed in the fofn.assembly directory, where fofn is the filename prefix listed in the "Files to Process" panel. The output is in a format suitable for directed assembly within gap4. This can also be performed by using the "Enter Assembly into Gap4" module. Phrap can make use of the confidence value information written by the phred program to produce better assemblies. Phrap also uses the full length of the sequence and will ignore any quality clipping. It is still necessary to clip sequencing vector.

Option: Minimum exact match  
Minimum length of matching word for SWAT comparison.

Option: Minimum SWAT score  
Minimum SWAT score.

Option: Other phrap arguments  
Any other phrap command line arguments.

3.6.28 Enter Assembly into Gap4  
Description  
This module is used to enter assemblies into gap4 which have been generated externally to gap4 (ie all assembly engines except "Gap4 shotgun assembly"). This is achieved by using the gap4 "Directed Assembly" function. The assembly is read from the fofn.assembly directory, where fofn is the filename prefix listed in the "Files to Process" panel.
Option: Gap4 database name
Option: Gap4 database version

The name and version of the database to assemble into.

Option: Create new database

This is a toggle to define whether the specified gap4 database should be created or appended to. Be warned that at present creating a new database will overwrite existing one of the same name, in the same directory, without any warnings.

Option: Post-assembly quality clipping
Option: Lowest (average) quality to use

This can be used to direct gap4 to run the "Quality Clip" function after entering the assembly. This performs quality clipping by identifying segments where the average quality is below a particular threshold. This should only be necessary if quality clipping was not performed earlier (eg because Phrap was used for assembly), and even then it is usually better to use difference clipping instead.

Option: Post-assembly difference clipping

This can be used to direct gap4 to run the "Difference Clip" function after entering the assembly. This identifies ends of readings where the alignment between readings and consensus is bad and marks these ends as hidden data. This is primarily designed for use after the Phrap assembly engine, which sometimes leaves poorly aligned end fragments.

3.6.29 Email

Description

This module can be used to send an E-mail indicating that the processing of Pregap4 has reached a given point. This may be of use when running pregap4 in batch mode, where the GUI is not visible. Typically the email module is placed at the end of the module list to indicate that pregap4 has (almost) finished, however it may be used elsewhere in the module list if desired.

Option: Email address
The email address to send a message to.

Option: Email program
The mail agent used for sending the message.

Option: Program arguments
The arguments (except for the email address) to the mail agent. These could include options for setting the email subject.

3.6.30 Old Cloning Vector Clip - Obsolete

Description

This is an older version of the Cloning Vector Clip module. It still uses the vector_clip program to perform this task, but does not use the newer probabilistic model for analysing matches. It is still present as an option for people who have tuned the parameters for their data and are happy with this. The probability mode is recommended (see Chapter 5 [Screening against Vector Sequences], page 267).
Option: Vector file name
The filename containing the vector sequence. At present this should be a file containing a single plain text sequence containing just the bases or white space.

Option: Word length
Option: Number of diagonals
Option: Diagonal score
The searching method involves hashing words to quickly identify matches and then combining these words along the best and neighbouring diagonals to produce an overall score which is compared against the diagonal score to determine whether this is vector sequence. The score is normalised from 0 (no match) to 1.0 (perfect match). For full details on this see the vector_clip manual.

3.6.31 ALF/ABI to SCF Conversion - Obsolete

Description
This module converts ABI and ALF files to SCF format using the makeSCF program. SCF format is not required by programs such as gap4, but it is considerably smaller and has been designed to give high compression ratios.

Option: SCF bit size
This selects the data size for the chromatogram data. An 8 bit value can store 256 possible values, which is typically good enough for display purposes. If Y scaling is required (for instance because the signal strength diminishes significantly along the length of the trace), or further computational analysis of the trace is required, a 16 bit data size should be chosen. As the majority of the trace file is the sample data, using 8 bit data typically saves about half of the disk space.

This module may also be used for converting 16-bit SCF files to 8-bit SCF files.

3.7 Using Config Files

Pregap4 uses configuration files to remember the setup for each user or project. These files define which modules are activated and what their parameter settings are. The files, which can obviously save considerable amounts of time, are created automatically and can be saved from the Configure Modules Window once the configuration is complete.

The "Load New Config File" option, available from the File menu, may be used to switch to a new (existing) configuration file. Pregap4 will display a file browser window to enable selection of another configuration file. Once chosen, Pregap4 will discard the existing configuration and use the new one. From this point onwards, any modifications and saving in Pregap4 will be to the new configuration file.

3.8 Pregap4 Naming Schemes

The "Load Naming Scheme" command is in the File menu. It will bring up a dialogue requesting the pathname of a naming scheme file. The browse button will automatically bring up the file browser in the pregap4 naming scheme directory, however naming schemes can be loaded from elsewhere if desired. The "Save to config file" query determines whether the component is also copied to the current pregap4 configuration file to make this component the default for subsequent pregap4 runs.

The use of naming schemes within pregap4 is specifically for extracting information from a reading name in order to supply parameters to other pregap4 modules or to gap4. For example
a naming scheme may be used to indicate where both the forward and reverse primers have been used to generate two sequences, which gap4 can then use for checking assembly and suggesting possible contig joins.

Currently only two naming schemes are supplied with pregap4, both of which are from the Sanger Centre. To create your own naming schemes please see Section 3.8.3 [Writing Your Own Naming Scheme], page 238.

### 3.8.1 Old Sanger Centre Naming Scheme

**Filename**  
sanger_names_old.p4t

**Description**  
This scheme extracts information from sequence names by assuming that they adhere to the old-style Sanger Centre naming scheme. The information extracted consists of the template name, primer type and chemistry information. The format of a reading name is as follows.

```
<template_name>.<strand><primer><conditions><repetition>
```

In the above, *strand* and *primer* are each one character long and are defined according to the following tables. *Conditions* can be 0, 1 or 2 characters indicating none, one or two sequencing conditions. *Repetitions* is optional and is used purely for creating unique names when resequencing a template with the same strand, primer and conditions as a previous sequencing reaction.

<table>
<thead>
<tr>
<th>Strand</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>s, f</td>
<td>Forward, single stranded template.</td>
</tr>
<tr>
<td>r</td>
<td>Reverse, single stranded template.</td>
</tr>
<tr>
<td>p</td>
<td>Forward, double stranded template.</td>
</tr>
<tr>
<td>q</td>
<td>Reverse, double stranded template.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Universal primer (end of insert).</td>
</tr>
<tr>
<td>2, 3, etc</td>
<td>Custom primer.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>Dye terminator chemistry.</td>
</tr>
<tr>
<td>1</td>
<td>Long gel</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a, b, c, ...</td>
<td>Any letter except l and t</td>
</tr>
</tbody>
</table>

For example "U16F10.p1t" is a forward dye-terminator sequence from the double stranded template named U16F10 using the universal primer.

**Configuration section**

```
[naming_scheme]
```
Configuration elements
PR_com, TN_com, CH_com.

3.8.2 New Sanger Centre Naming Scheme

Filename sanger_names_new.p4t

Description
This scheme extracts information from sequence names by assuming that they adhere to the new-style (~1997) Sanger Centre naming scheme. This is explained clearly on the Sanger Centre’s web pages at http://www.sanger.ac.uk/Software/sequencing/

The information extracted consists of the template name, primer type and chemistry information. The format of a reading name is as follows.

<template_name>.<strand><primer><chemistry>

The strand, primer and chemistry fields are each one character long and are mandatory. They are defined by the following tables.

<table>
<thead>
<tr>
<th>Strand</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>p</td>
<td>Forward, double stranded template.</td>
</tr>
<tr>
<td>q</td>
<td>Reverse, double stranded template.</td>
</tr>
<tr>
<td>r</td>
<td>Reverse, single stranded template.</td>
</tr>
<tr>
<td>s</td>
<td>Forward, single stranded template.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Universal primer (end of insert).</td>
</tr>
<tr>
<td>2</td>
<td>Custom primer.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>standard (ABI) terminator</td>
</tr>
<tr>
<td>d</td>
<td>dRhodamine terminator</td>
</tr>
<tr>
<td>p</td>
<td>standard (ABI) primer</td>
</tr>
<tr>
<td>e</td>
<td>energy transfer primer</td>
</tr>
<tr>
<td>b</td>
<td>big dye primer</td>
</tr>
<tr>
<td>c</td>
<td>big dye terminator</td>
</tr>
<tr>
<td>l</td>
<td>licor</td>
</tr>
</tbody>
</table>

For example "U16F10.p1t" is a forward standard (ABI) terminator sequence from the double stranded template named U16F10 using the universal primer.

Configuration section
[naming_scheme]
Configuration elements
PR_com, TN_com, CH_com.

3.8.3 Writing Your Own Naming Schemes

The naming schemes are defined in the "component" files. At present two examples exist; both are naming schemes taken from the Sanger Centre. It is possible to define your own naming scheme, or indeed any other component. A component is basically just a file which you want to add (in its entirety) to the user’s pregap4 configuration file. Typically these files end in the extension ‘.p4t’.

The naming schemes are defined by use of three variables: ns_name, ns_regexp and ns_lt.

ns_name is simply a text name for the naming scheme.

ns_regexp is a regular expression which will be matched against each sequence identifier. The bracketed segments are assigned to Tcl variables which can be referenced as $1, $2, $3 etc.

ns_lt is an array indexed by Experiment File line types. The contents of a particular array element is either a string containing the value for that line type or the word subst followed by a substitution list of the following format:

```
subst {string {pattern replacement} ... default replacement}
```

In addition to this we need a bit of preamble stating that the following component is part of the pregap4 naming scheme section. This can be done by making sure the first line of the component file is [naming_scheme].

A completely new example naming scheme may be, in English, as follows:

The reading identifier will consist of the template name, followed by a full stop, followed by two characters to determine the primer type and position, a single character to determine the chemistry, and any extra characters needed to create a unique name. Forward and reverse readings from the same "insert" or "template" will share the same template name. This in turn allows for gap4 to know the relative positions, orientations and distances of two such readings and hence will allow it to point out possible problems.

Putting this more specifically: a template name is any string of alpha-numerics (a-z, 0-9 and underscore). The primer type could be defined as:

```
uf universal forward primer item ur universal reverse primer
cf custom forward primer
cr custom reverse primer
```

The chemistry can be defined as:

```
p Dye-Primer
P Big dye-primer
t Dye-Terminator
T Big dye-terminator
```

For example fred.ufp, fred.urp and bert.cfT are all valid names.

The above variable definitions may seem complex so we shall work through the example naming scheme. Firstly we need to define the regular expression. To new users this can be complex, but is described in great detail in many places (try the Unix "grep" manual page). In the shortest form: dot (.) matches any character; square brackets delimit a set of characters, any one of which is allowed (or if it starts with ^ it is the complement set - any except those listed). Following a
character or set with + indicates one or more copies of the preceeding expression, * is for zero or more copies, and ? is for zero or one copy.

So to define our example names we would start our component file with:

```
[naming_scheme]
set ns_name "Example naming scheme"
set ns_regexp {([~.]*)\.(..)(.).*}
```

The backslash in the above text is to state that we want to match a real full stop character instead of the "any character" that regular expressions usually regard full stop as meaning. The `ns_regexp` will store the three bracketed segments in `\$1`, `\$2` and `\$3`.

The first segment is the template name. To use this we simply add:

```
set ns_lt(TN) \{$1\}
```

The next segment is the primer type. The primer type is defined for gap4 as a single digit number. 0 is for unknown, 1 is universal forward primer, 2 is universal reverse primer, 3 is custom forward primer, and 4 is custom reverse primer. So we wish to map `uf` to 1, `ur` to 2, `cf` to 3, `cr` to 4, and anything else to 0. This is done with the following command:

```
set ns_lt(PR) {subst \{$2\} {uf 1} {ur 2} {cf 3} {cr 4} 0}
```

The final segment is the chemistry. At present gap4 only distinguishes between dye-primer and dye-terminators, although our naming scheme also "knows about" big dyes. So we wish to map both `p` and `P` to chemistry type 0, and `t` and `T` to chemistry type 1. Anything else we'll also assume is dye-primer. In much the same way that the regular expressions work, we can use square brackets in our patterns to say "any of these letters". So the command for this is:

```
set ns_lt(CH) {subst \{$3\} {[pP] 0} {[tT] 1} 0}
```

The final line to add to the component file is `set_name_scheme`. This is a pregap4 command which tells it that you have finished defining the naming scheme. So the completed component file is simply:

```
[naming_scheme]
set ns_name "Example naming scheme"
set ns_regexp {([~.]*)\.(..)(.).*}
set ns_lt(TN) \{$1\}
set ns_lt(PR) {subst \{$2\} {uf 1} {ur 2} {cf 3} {cr 4} 0}
set ns_lt(CH) {subst \{$3\} {[pP] 0} {[tT] 1} 0}
set_name_scheme
```

### 3.9 Pregap4 Components

A "Component" in pregap4 is a predefined section of a pregap4 configuration file. It will generally be used to add on complex configurations which are not easily created using the GUI. Currently there are only two predefined components, both of which specify a naming scheme and so are easiest loaded using the Load Naming Convention function; see Section 3.8 [Load Naming Convention], page 236.
The "Include Config Component" command in the File menu is used to load a component. The "browse" button will bring up a file browser listing the default pregap4 component directory, however components can be loaded from elsewhere if desired. The "Save to config file" query determines whether the component is also copied to the current pregap4 configuration file to make this component the default for subsequent pregap4 runs.

Note that a component may have a configuration section listed within it. If this is present the component will replace any configuration with the same section name.

3.10 Information Sources

The "Information Sources" menu contains options to obtain information from external data sources, such as a text database. This does not include information encoded inside a reading name convention. At present there are only two options - Simple Text Database and Experiment File Line Types - although it is hoped that a link up to a robust relational database could also be included here.

3.10.1 Simple Text Database

This option allows interrogation of a very simple format text database with one line per sequence. The sequence identifier is the first word of a line with one or more additional columns of information relating to specific information about that sequence. All columns in the database file must have the same format and only one database file may be used at any one time.

For example, we may wish to store the primer type, primer site, template name and the number of strands on the template for each sequence. This corresponds to the PR, SP, TN and ST Experiment File line types. We could then create a text database looking something like the following:

```
#I D P R S P T N S T
xb54a3.s1 1 41 xb54a3 1
xb54b12.s1 1 41 xb54b12 2
xb54b12.r1 2 -24 xb54b12 2
xb54b12.r1L 2 -24 xb54b12 2
```

(The first line, starting with # is just a comment. Pregap4 does not use this; it is purely so that we know which information is in which column.)

We can then direct pregap4 to extract the information from each of these four columns for each reading being processed and to store this information in the Experiment File. This information can then be utilised by the vector clipping and assembly modules.
Chapter 3: Preparing readings for assembly using pregap4

The Simple Text Database interface consists of an entry box to specify the database file name, add and delete buttons, and a line type selector for each column in the database (excluding the reading name column). The above picture contains the database setup for extracting the primer type, primer position, template name and number of strands as described in the above example.

The "Add column" button adds a new line type selector at the bottom of the window. This contains an option menu which can be clicked to choose a new Experiment File line type and a label indicating the column number. The "Delete column" button removes the bottom-most line type selector.

The "Ok" button will accept this configuration and will also write the details to the current pregap4 configuration file. To disable a previously setup Simple Database Configuration press delete until there are no line types listed and then press Ok once more.

The simple text database does not need to include a record for every reading and special characters can be used to encode names so that readings produced in similar ways can be grouped. For example, if the first 6 letters of the name encode a "plate" name, and all the sequences on that plate have been sequenced using the same vector then we could create a database file as follows.

```
#I D S F S C S P
6abz91* m13mp18.seq 6249 41/-24
6aca68* puc18.seq 248 40/-28
6aca69* puc18.seq 248 40/-28
6aca70* puc18.seq 248 40/-28
6acb21* m13mp18.seq 6249 41/-24
6acd49* puc18.seq 248 40/-28
6acd51* puc18.seq 248 40/-28
```

The sequence identifier (ID) is searched for using a pattern matching rule (as dictated by the Tcl `string match` command). The pattern matching uses special characters as follows:

* Matches any sequence of characters in the reading identifier, including an empty string.

? Matches any single character in the reading identifier.

[chars] Matches any character in the set given by chars. If a sequence of the form r-v appears in chars, then any character between r and v, inclusive, will match (rstuv).

\x Matches the single character x. This provides a way of avoiding the special interpretation of the characters *?[]\ in the reading identifier.

3.10.2 Experiment File Line Types

A list of Experiment File line types may be viewed and edited using this option (see Section 10.3.1 [Experiment file format record types], page 401). A table of the Experiment File line types is displayed along with their current values. A brief description of the line type underneath the mouse cursor is displayed in the Information Line at the bottom of the window. The table consists of three columns. The first is a label identifying the line being edited. The second column is an option menu from which either Value or Command may be selected. The third column is the current value or command.
Line type values will be used for every sequence. The Augment Experiment Files module will add this line type to each Experiment File. This is suitable for specifying information which is constant across an entire batch, such as insert size (SI) or operator (OP). The Line type commands are executed each time the Augment Experiment Files module adds that line to the Experiment File. Hence the commands are used for information which may change from sequence to sequence. This table should be used for editing line type values, but we do not recommend that you use it for editing commands (although it is useful to know which commands have been set).

In the above example the PR, SP and ST commands were generated using the Simple Text Database interface, whilst the SC and SF values come from the Sequencing Vector Clip module parameters and the SI value was typed in by hand using this table.

The "OK (in memory)" and "OK (and save)" buttons will accept the currently displayed values and commands. The "OK (in memory)" button will use these settings for the current pregap4 runs. The "OK (and save)" button will use them for the current session and all subsequent pregap4 sessions as it saves the information to the pregap4 configuration file.

3.11 Adding and Removing Modules

This section details how to select newly written modules in Pregap4, or how to change the order of existing modules.

It is for system managers and advanced users only.

Pregap4 has a default set of modules to use. Any module within this list may be enabled or disabled. If you only need to screen a set of experiment files using blast or screen_seq it may be tempting to use the Add/Remove Modules screen (from Modules menu) to remove everything else. This is not necessary; just disable the unwanted modules. The real purpose of Add/Remove Modules is to define the contents and order of the list that appears in the Configure Modules screen. This may be required if you create your own modules, or if you wish to never use certain modules. (Removing them from the list instead of simply disabling them will speed up starting Pregap4.)
It is possible for a module to be used more than once. For example if you wish to use blast to screen against several databases then this control may be used to add two "Blast screen" items to the Configure Modules screen. Note though, that this is not applicable to many modules. For example it is not possible to screen against multiple vectors by simply using multiple Sequencing Vector Clip modules (rather this should be done using a file of vector-primer information). No error checking is performed with the Add/Remove Modules screen.

A pregap4 module is a specific piece of Tcl/Tk code that interfaces between pregap4 (by providing a run procedure and an optional GUI for configuration) and an external program to do the main work (as Tcl itself is generally too slow for anything except the most simple of operations). The exact specification of a module can be found elsewhere (Section 3.13 [Writing New Modules], page 261).

All modules must end in `.p4m`. Pregap4 uses a module search path to search for files with this suffix. The module search path is a space separated list. By default it will be set to `$STADENROOT/lib/pregap4/modules`. It may be adjusted temporarily within the program, or permanently by setting the MODULE_PATH variable within your `.pregap4rc` or run-specific configuration files. For example:

```
set MODULE_PATH "$env(STADLIB)/pregap4/modules ."
```

The two lists shown in the dialogue represent the current modules to use (on the left) and the total list of known modules. Modules may be added to the left (to use) list by clicking any mouse button on the right hand list, dragging the mouse cursor to a location within the left list, and then release the mouse button. To remove a module from the ‘to use’ list simply drag and drop from left to right. This mechanism also allows for changing the order of modules within the left list.

The order of modules is vitally important and in the current version of Pregap4 the validity of the order is not checked. Common sense should prevent most problems. For instance it is pointless to assemble and enter into gap4 before vector clipping. The best source of information on the
possible orderings comes from the documentation for each individual module. Some modules are
directly incompatible with each other as they perform the same or mutually exclusive tasks. For
example it is only possible to use one of the assembly methods.

Once the modules have been selected press "Apply" to reinitialise Pregap4. If you wish to
make your newly selected list the default for subsequent Pregap4 runs use the "Save Module List"
command in the Modules menu.

3.12 Low Level Pregap4 Configuration

Most users will never need to configure pregap4 at this level. However by understanding the
methods that pregap4 uses you will see how to tailor Pregap4 in a more flexible manner.

The pregap4 configuration file consists of several sections. Each section is started with
"[section_name]". The main pregap4 sections are [module_list], [global_variables], and
one section per module named [::module_name].

3.12.1 Low Level Global Configuration

The [module_list] section contains definitions of the MODULE_PATH and MODULES variables. The MODULE_PATH is a space delimited list of directory names in which to search for the pregap4 modules (*.p4m files). The MODULES variable is the default list of modules to list in the configuration window. The order of modules in this list determines the order that they will be executed in. The default [module_list] section is as follows:

[module_list]
set MODULE_PATH "$env(STATLIB)/pregap4/modules ."
set MODULES {
  phred
  atqa
  convert_trace
  eba
  compress_trace
  init_exp
  augment_exp
  quality_clip
  trace_clip
  sequence_vector_clip
  cross_match_svec
  cloning_vector_clip
  screen_vector
  screen_seq
  blast
  interactive_clip
  repeat_masker
  tag_repeats
  trace_diff
  gap4_assemble
  cap2_assemble
  cap3_assemble
  fakii_assemble
  phrap_assemble
  enter_assembly
  email
The [global_variables] section defines the values for each of the Experiment File line types. These are currently primarily used by the Augment Experiment Files module, but may also be used by the vector clipping and mutation detection modules. The default [global_variables] section is blank.

As an example, the following section defines the sequencing vector file for each sequence to be m13mp18.vector with 6249 and 41 as the cut site and primer site. Each sequence has a primer type of 1 (forward universal primer) and the template name is derived from the sequence name by taking the segment of the string preceding the full stop.

```
[global_variables]
set SF m13mp18.vector
set SC 6249
set SP 41
set PR 1
proc TN_com {} { global lines; return [lindex [split $lines(ID) .] 0] }
```

### 3.12.2 Low Level Component Configuration

Pregap4 Components may each contain their own configuration section. Where several components are mutually exclusive, such as components describing naming conventions, it makes sense to give each component the same configuration section. This will ensure that loading a new component will overwrite the old one. At present the only defined components both create a [naming_scheme] section.

Components may redefine items which could appear in other configuration sections. In this case the last definition of that setting will take priority. For instance if a component defines the TN_com procedure and this is also defined in the [global_variables] section then the component will only take priority if it is after the global section in the configuration file.

Components may also be used to define parameters for modules. Once again the components need to be listed after the module definitions being overridden. To define module components in this way, use the `module` command. An example follows.

```
module tag_repeats {set repeat_file repeats.list}
```

### 3.12.3 Low Level Module Configuration

Each module has its own configuration section named after the module name. The configuration section will be "[:module_filename_prefix]" where `module_filename_prefix` is the filename of the module with the .p4m extension removed. For example, the `init_exp.p4m` module has a configuration section of [:init_exp].

Each module is loaded into its own namespace, also named after the module in the same manner. Thus in the above example the Initialise Experiment Files module uses the namespace ::init_exp. This means that all local variables within that module will be within that name space and will not clash with identical named variables in other modules. When pregap4 reads the configuration file any configuration section starting in double colon is taken to be a name space and the following configuration is executed in that namespace. So the following example enables the Initialise Experiment Files module, but disables the Estimate Base Accuracies module.

```
[::init_exp]
set enabled 1
[::eba]
```
In the following sections the variables, inputs and outputs of each module are listed. Every module has an enabled local variable. This may be either 0 for disabled or 1 for enabled. Disabled modules are still listed in the configuration panel, although they will not be executed.

The tables in each section below list the module filename, the local variables and a very brief description of their valid values, the files used or produced by this module, the possible sequence specific errors that can be produced (which will be written to the failure file as the reason for failure), and the format of any SEQ lines in the module report. Other information may also be reported, but the SEQ lines are easily recognisable to facilitate easy parsing of results.

3.12.3.1 General Configuration

Filename init.p4m

Local variables
use_sample_name 0/1

Files (none)

Errors init: Unreadable or nonexistent file
init: Unknown file type

Report (None)

3.12.3.2 ALF/ABI to SCF Conversion

Filename to_scf.p4m

Local variables
bit_size 8/16

Files Creates the SCF files if required.

Errors makeSCF: makeSCF error message

Report SEQ seqid: created from old seqid

3.12.3.3 Estimate Base Accuracies

Filename eba.p4m

Local variables (none)
Chapter 3: Preparing readings for assembly using pregap4

Files
Modified SCF files with new confidence values.

Errors
eba: *eba error message*

Report
(none)

3.12.3.4 Phred

Filename
phred.p4m

Local variables
(none)

Files
- `fofn.tmp` — temporary
- `fofn.scf_dir` — temporary directory
- `phred.log` — phred log file

Createss the SCF files if required.

Errors
phred: *phred error message*

Report
SEQ seqid: created from old seqid
SEQ seqid: re-base-called

3.12.3.5 ATQA

Filename
atqa.p4m

Local variables
(none)

Files
Updates the confidence values within SCF files.

Errors
ATQA: *ATQA error message*

Report
SEQ seqid: confidences recalculated by ATQA.

3.12.3.6 Trace Format Conversion

Filename
convert_trace.p4m

Local variables
- `output_format`: ZTR/CTF/ZTR
- `down_scale`: 0/1
down_scale_range integer
subtract_background 0/1
normalise 0/1
del_temp_files 0/1

Files Creates Trace files.

Errors convert_trace: convert_trace error message

Report SEQ seqid: created from old seqid

3.12.3.7 Initialise Experiment Files

Filename init_exp.p4m

Local variables (none)

Files Creates Experiment files.

Errors init_exp: init_exp error message

Report SEQ seqid: created from old seqid

3.12.3.8 Augment Experiment Files

Filename augment_exp.p4m

Local variables (none)

Global variables All the XX and XX_com variables that define the two letter Experiment File line types.

Files Updates Experiment files.

Errors (none)

Report SEQ seqid: added fields field names

3.12.3.9 Uncalled Base Clip
Chapter 3: Preparing readings for assembly using pregap4

Filename uncalled_clip.p4m

Local variables
- offset integer
- min_extent integer
- max_extent integer
- right_win_length integer
- right_num_uncalled integer
- left_win_length integer
- left_num_uncalled integer

Files Modifies Experiment files

Errors clip: clip error message

Report SEQ seqid: clipped using 'clip'

3.12.3.10 Quality Clip

Filename quality_clip.p4m

Local variables
- clip_mode sequence/confidence
- window_length integer
- conf_val integer
- min_extent integer
- max_extent integer
- min_length integer
- offset integer
- right_win_length integer
- right_num_uncalled integer
- left_win_length integer
- left_num_uncalled integer

Files Modifies Experiment files

Errors qclip: qclip error message qclip: Sequence too short (length=%d)

Report SEQ seqid: clipped using 'qclip'
3.12.3.11 Sequencing Vector Clip

Filename sequence_vector_clip.p4m

Local variables

min_5_match float
min_3_match float
def_5_pos integer
update_exp_file 0/1
use_vp_file 0/1
vp_file filename
vector_list string
vp_length integer

Global variables

SP
SP_com
SC
SC_com
SF
SF_com

Files

fofn.tmp — temporary
fofn.svec_passed
fofn.svec_failed

Errors

sequence_vector_clip: No SF, SC or SP information
sequence_vector_clip: vector_clip error message
sequence_vector_clip: lost file

Report

SEQ seqid: passed
SEQ seqid: failed (reason)
SEQ seqid: lost

3.12.3.12 Cross_match

Filename cross_match_svec.p4m

Local variables

minmatch integer
minscore integer
vector_file filename
gap_size  integer
tag_type  string

Global variables
SF
SF_com

Files
fofn.tmp — temporary
fofn.tmp.log — temporary
fofn.tmp.screen — temporary

Errors
cross_match: cross_match error message
cross_match: entirely vector

Report
SEQ seqid: masked start to end
SEQ seqid: no matches

3.12.3.13 Cloning Vector Clip

Filename  cloning_vector_clip.p4m

Local variables
word_length  integer
probability  float
update_exp_file  0/1

Global variables
CF
CF_com

Files
fofn.tmp — temporary
fofn.cvec_passed
fofn.cvec_failed

Errors
sequence_vector_clip: No CF information
sequence_vector_clip: vector_clip error message
sequence_vector_clip: lost file

Report
SEQ seqid: checked
SEQ seqid: failed (reason)
SEQ seqid: lost

3.12.3.14 Old Cloning Vector Clip
Filename  old_cloning_vector_clip.p4m

Local variables

```plaintext
word_length  integer
num_diags    integer
diag_score   float
update_exp_file  0/1
```

Global variables

```plaintext
CF
CF_com
```

Files

```plaintext
fofn.tmp — temporary
fofn.cvec_passed
fofn.cvec_failed
```

Errors

```plaintext
sequence_vector_clip: No CF information
sequence_vector_clip: vector_clip error message
sequence_vector_clip: lost file
```

Report

```plaintext
SEQ seqid: checked
SEQ seqid: failed (reason)
SEQ seqid: lost
```

3.12.3.15 Screen for Unclipped Vector

Filename  screen_vector.p4m

Local variables

```plaintext
min_match  integer
update_exp_file  0/1
```

Global variables

```plaintext
SF
SF_com
```

Files

```plaintext
fofn.tmp — temporary
fofn.screenvec_passed
fofn.screenvec_failed
```

Errors

```plaintext
sequence_vector_clip: No CF information
sequence_vector_clip: vector_clip error message
sequence_vector_clip: lost file
```
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3.12.3.16 Screen Sequences

Filename: screen_seq.p4m

Local variables

- min_match: integer
- max_length: integer
- screen_mode: single/fofn
- screen_file: filename

Files
- fofn.tmp — temporary
- fofn.screenseq_passed
- fofn.screenseq_failed

Errors
- screen_seq: screen_seq error message
- screen_seq: lost file

Report
- SEQ seqid: passed
- SEQ seqid: failed (reason)
- SEQ seqid: lost

3.12.3.17 Blast Screen

Filename: blast.p4m

Local variables

- database: filename_prefix
- match_fraction: float
- e_value: float
- tag_type: string

Files
- fofn.blast

Errors
- blast: match fraction fraction

Report
- SEQ seqid: total match length = match_length (fract=match_fraction)
3.12.3.18 Interactive Clipping

Filename  
interactive_clip.p4m

Local variables
(none)

Files  
May modify Experiment files.

Errors  
interactive_clip: manually rejected

Report  
SEQ seqid: accepted
SEQ seqid: rejected

3.12.3.19 Extract Sequence

Filename  
extract_seq.p4m

Local variables
(none)

Files  
User specified

Errors  
extract_seq: extract_seq error message

Report  
(none)

3.12.3.20 Tag Repeats

Filename  
tag_repeats.p4m

Local variables
repeat_file filename
score float
tag_types string

Files  
fofn.tmp — temporary
fofn.tagrep_free
fofn.tagrep_repeat
fofn.tagrep_log

Errors  
tag_repeats: lost file

Report  
SEQ seqid: no repeat found
SEQ seqid: repeat found (details)
3.12.3.21 RepeatMasker

Filename  repeat_masker.p4m

Local variables

- **tag_type**: string
- **alu_only**: 0/1
- **simple_only**: 0/1
- **no_primate_roden**: 0/1
- **rodent_only**: 0/1
- **no_low_complexity**: 0/1
- **library**: directory
- **cutoff**: integer

Files

- **fofn.fasta.cat**
- **fofn.fasta.masked**
- **fofn.fasta.masked.log**
- **fofn.fasta.out**
- **fofn.fasta.out.xm**
- **fofn.fasta.tbl**

Errors  (none)

Report  SEQ seqid: Repeat repeat name

3.12.3.22 Mutation Detection

Filename  trace_diff.p4m

Local variables

- **score**: float
- **start**: int
- **end**: int
- **band_width**: int
- **other_args**: string
- **update_exp_file**: 0/1

Global variables

- **WT**
- **WT_com**
Files

May modify Experiment files.

Errors

trace_diff: No wildtype information
trace_diff: trace_diff error message

Report

SEQ seqid: mutations at positions
SEQ seqid: no mutations

3.12.3.23 Gap4 Shotgun Assembly

Filename gap4_assemble.p4m

Local variables

database_name string
database_version character
create 0/1
min_match integer
max_pads integer
max_pmismatch float
enter_all 0/1

Files

database_name.database_version
database_name.database_version.aux

Errors

gap4_assemble: failed with code error_code

Report

SEQ seqid: failed (assembly error code)
SEQ seqid: failed (filename contains spaces)
SEQ seqid: skipping - not in correct format
SEQ seqid: assembled

3.12.3.24 Cap2 Assembly

Filename cap2_assemble.p4m

Local variables

(none)

Files

fofn.tmp — temporary
fofn.assembly/fofn
fofn.assembly/cap2_stdout
fofn.assembly/cap2_stderr
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**Errors**
cap2_s: cap2_s error message
cap2_s: rejected

**Report**
SEQ seqid: rejected
SEQ seqid: assembled
SEQ seqid: failed (filename contains spaces)

### 3.12.3.25 Cap3 Assembly

**Filename** cap3_assemble.p4m

**Local variables**

<table>
<thead>
<tr>
<th>Local variable</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>generate_constraints</td>
<td>0/1</td>
</tr>
</tbody>
</table>

**Files**
fofn.tmp — temporary
fofn.assembly/fofn
fofn.assembly/cap3_stdout
fofn.assembly/cap3_stderr
fofn.con
fofn.con.results
fofn.cap3_info
fofn.contigs.qual

**Errors**
cap3_s: cap3_s error message
cap3_s: rejected

**Report**
SEQ seqid: rejected
SEQ seqid: assembled
SEQ seqid: failed (filename contains spaces)

### 3.12.3.26 FakII Assembly

**Filename** fakii_assemble.p4m

**Local variables**

<table>
<thead>
<tr>
<th>Local variable</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>graph_e_limit</td>
<td>float</td>
</tr>
<tr>
<td>graph_o_threshold</td>
<td>float</td>
</tr>
<tr>
<td>graph_d_limit</td>
<td>float</td>
</tr>
<tr>
<td>assem_number</td>
<td>integer</td>
</tr>
<tr>
<td>assem_e_rate</td>
<td>float</td>
</tr>
<tr>
<td>assem_o_threshold</td>
<td>float</td>
</tr>
</tbody>
</table>
assem_d_threshold float
generate_constraints 0/1

Files
fofn.tmp — temporary
fofn.assembly/fofn
fofn.assembly/graph_stderr
fofn.assembly/graph.dat
fofn.assembly/constraints_stderr
fofn.assembly/constraints.ascii
fofn.assembly/constraints.dat
fofn.assembly/assemble_stderr
fofn.assembly/assemble.dat
fofn.assembly/write_exp_file_stdout
fofn.assembly/write_exp_file_stderr

Errors fakii: rejected

Report SEQ seqid: assembled
SEQ seqid: rejected

3.12.3.27 Phrap Assembly

Filename phrap_assemble.p4m

Local variables
minmatch integer
minscore integer
other_args string

Files fofn.tmp — temporary
fofn.assembly/fofn
fofn.assembly/phrap_stdout
fofn.assembly/phrap_stderr
fofn.contigs
fofn.contigs.qual
fofn.singlets
fofn.phrap_log

Errors phrap: singlet
phrap: failed

Report SEQ seqid: assembled
SEQ seqid: singlet
SEQ seqid: failed
3.12.3.28 Enter Assembly into Gap4

Filename enter_assembly.p4m

Local variables

- database_name: string
- database_version: character
- create: 0/1
- quality_clip: 0/1
- quality: integer
- difference_clip: 0/1

Files
- fofn.assembly/fofn — reads
- fofn.assembly/* — reads
- database_name.database_version
- database_name.database_version.aux

Errors (none)

Report SEQ seqid: failed

3.12.3.29 Email

Filename email.p4m

Local variables

- email_program: string
- email_args: string
- email_address: string

Files (none)

Errors (none)

Report (none)

3.12.3.30 Shutdown

Filename shutdown.p4m

Local variables (none)
3.13 Writing New Modules

3.13.1 An Overview of a Module

A pregap4 module is a single file containing a series of functions with predefined interfaces. Pregap4 uses these functions to communicate with module.

This section is for system managers and programmers only.

The module itself is written using the Tcl/Tk language. A definition of this language is outside the scope of this manual, however several books exist on the subject. Each modules executes inside a Tcl "namespace". This means that modules may make use of global variables and global function names without fear of clashing with other modules. Indeed the use of specific function names and global variables is of considerable importance for designing a new module.

3.13.2 Functions

The basic structure of a module is that it has a series of known functions which pregap4 expects to use. Some of these functions are mandatory, whilst others will only be called by pregap4 if they have been defined.

\textit{name} \hspace{1cm} \text{Mandatory.}
Arguments: none
Returns: The textual name of the module.
This function is used to query a human readable name for the module (eg "ALF/ABI to SCF Conversion"). This name is used in the module list at the left side of the pregap4 window.

\textit{init} \hspace{1cm} \text{Optional.}
Arguments: None
Returns: None
This sets up any data structures needed for this module. It can be used for providing defaults for global variables when they are not known (eg they have no settings in the system or user pregap4rc files) and for setting up any other data structures required.

\textit{run} \hspace{1cm} \text{Optional.}
Arguments: A Tcl list of files to process
Returns: A new Tcl list of files for subsequent processing.
This is the main work horse. It is optional, however in all but the most esoteric cases, it will be needed.
The single argument is a Tcl list of sequence names. These are either filenames on disk or identifiers used for fetching data from a database. The module should loop through the sequences which it can process (which may not be all of them, depending on the known information and file types).

When finished, it needs to return a new list of files. If a file has been rejected by this module (e.g., it is completely sequencing vector) then this sequence name should be omitted from the returned list. However, do make sure that all failed files have an error string attached to them by setting the file_error(seq name) array element.

**shutdown**  Optional.

Arguments: A Tcl list of files to process

Returns: A new Tcl list of files for subsequent processing.

Deallocates any data structures that have been setup during the init or run stages. Most modules will not need this function. As with the run module, the returned value should be the list of passed files, which is generally the same as the list passed into this function.

A special module, which is always included by pregap4, is the shutdown.p4m module. This is always the last module to have shutdown called. It produces the reports for pregap4 and does some general house keeping.

**create_dialogue**  Optional.

Arguments: A tk pathname

Returns: None

This creates a dialogue controlling the parameters for this module. The tk pathname passed into this function should be the root for all components of this dialogue. (Note though that this is not a toplevel window, but a subwindow of the main pregap4 dialogue.)

**check_params**  Optional.

Arguments: None

Returns: A variable name or a blank string.

This checks that this module has valid answers to all of its mandatory questions. If this is the case, a blank string is returned, otherwise the first variable name which needs a value is returned.

**process_dialogue**  Optional.

Arguments: A tk pathname

Returns: 0 for failure, 1 for success

This is executed in all modules before the run functions are executed. It’s purpose is to extract any information from user editable entries or checkboxes ready for the run function to utilise. It may also be used to check that the data entered is valid.
The return code is used to indicate whether this module has sufficient data to execute. If 0 is returned pregap4 will beep and make sure that the dialogue 'tab' for this module is displayed. Further processing then stops until the 'Run' button is pressed again. For instance if a module needs to know the sequencing vector to screen against, then this should check if the value has been entered or can be obtained via a command. If so it returns 1.

configure_dialouge path mode
Optional.
Arguments: A tk pathname, the configure mode
Returns: None
If this function is present pregap4 will add a button to the top of the module dialogue inviting the user to save the parameters for this module to the configuration file. In early releases of pregap4 (2000.0 and before) a "Select parameters to save" button was also available. To maintain compatibility with older modules the "mode" parameter is still used. If you wish the module to be backwards compatible with old pregap4 releases then this needs to be checked to make sure that it contains "save_all". If it does not then no action should be taken. In the 2001 release and newer the "mode" parameter will always contain "save_all" so no check is required. To save the dialogue information this function should use the pregap4 mod_save and glob_save functions.

3.13.3 Module Variables

mandatory
The existence of this variable (set to anything) states that this module cannot be disabled.

hidden
The existence of this variable states that its name shall not appear in the module list (although it will still be used).

report
The contents of this variable are displayed at the end of the pregap run by the shutdown.p4m module.

3.13.4 Global Variables

Several global variables exist which may need to be updated within the modules. For successful operation it is required to update these when applicable.

file_type
This is a Tcl array indexed by file name. It is initialised by the General Configuration module to be one of ABI, ALF, EXP, PLN, SCF or UNK.

file_error
This is a global array indexed by the current file name. If a file has been rejected by a module (ie not returned from the run function) then the appropriate array element must be filled with a reason. Typically the format for this reason will start with the module name followed by a colon. For example "makeSCF: unknown file type".

file_id
This is a global array, indexed by filenames, containing the sequence identifiers (which are often different to the sequence filenames). It is initialised by the General Configuration module.
Chapter 3: Preparing readings for assembly using pregap4

file_orig_name
This is a global array holding any original filename for each currently processed file. It is initialised by the General Configuration module such that each file points to its own filename.

When creating and returning a new file (such as when switching from SCF files to Experiment Files in the Initialise Experiment Files module) it is required that the arrays are all updated correctly. This involves creating new array elements for each of the above four arrays. The file_type array element, indexed by a new name should contain the new file type (eg set file_type(seq10.exp) EXP). The file_error array element should be set to a blank string. The file_id should inherit the sequence identifier from the original file (eg set file_id(seq10.exp) $file_id(seq10.scf)). The file_orig_name array element should point to the old filename (not the original filename pointed to by the old filename). In this way file_orig_name could be considered as a list of the intermediate files generated for each final sequence file.

3.13.5 Builtin Functions

Apologies, but this section of documentation is still unfinished.

The full definition of these functions may be found in the Tcl code for Pregap4 itself. It is recommended that you use the Unix grep utility to find the definitions and example uses.

3.13.6 An Example Module

The best examples are the existing modules. Try looking at the Compress Trace Files module as an example. This may be found in '$STADENROOT/lib/pregap4/modules/compress_trace.p4m'.

Chapter 4: Marking poor quality and vector segments of readings

4 Marking poor quality and vector segments of readings

Introduction to read clipping

For most assembly routines to work well it is necessary to present them with data of reasonable quality. Generally sequences produced by machines suffer from having poor quality data at one or both ends and so methods are needed to define where the data is too poor to use. Some base callers include an increased number of "N" symbols in the sequence in doubtful regions and so these can be searched for. In the ideal situation base accuracy estimates or confidence values for each base call will be available, and then these can be searched to find where the average confidence value becomes too low for reliable assembly. The program qclip (see Section 11.13 [qclip], page 436) performs both type of analysis.
5 Screening Against Vector Sequences

For most assembly engines to work well it is necessary to present them with data of good quality and which contains only the target sequence. One pre-assembly task is to locate and mark all segments of readings which contain vectors used in their production. In our package this task is performed by vector_clip which compares batches of readings against vector sequences. Sequence readings are stored in experiment file format (see Section 10.3 [Experiment File], page 401) and, for the majority of projects each experiment file should contain the data required by vector_clip: the file names of the vectors to screen against, and, for the sequencing vector, the position of the cloning and primer sites. See Section 5.6 [Vector_Primer files], page 272 for an alternative and simpler method of defining vector data for vector_clip. The program pregap4 (see Section 3.2 [Pregap4], page 202), contains modules for creating experiment files from trace files, and for adding data about the vectors used. When vector_clip runs it adds records to the reading’s experiment file to denote the start and end of any segments which are found to match the vectors.

For conventional sequencing projects there are two types of vector for which readings will need to be screened: the sequencing vector, and, for cases where, say, whole cosmids or BACs have been shotgunned, the cloning vector. These two screening tasks are different. When screening for the sequencing vector we may expect to find data to exclude, both from the primer region and, when the insert is short, from the other side of the cloning site. It is also a wise precaution to check for rearrangements of the sequencing vector. When screening out cosmid vector we may find that either the 5’ end, or the 3’ end, or the whole of the sequence is vector. Also for the cloning vector search we need to compare both strands of the sequence.

In order to filter out readings that contain the sequences of contaminant DNA such as E. coli, a separate program screen_seq should be used (see Chapter 6 [Screening for known possible contaminant sequences], page 279).

A further type of search is required for a new method that is being developed at MRC HGMP, Hinxton, UK. This new method (M. Starkey, personal communication) is an application of a technology described as "molecular indexing" Unrau, P. and Deugau, K.V. (1994) Non-cloning amplification of specific DNA fragments from whole genomic DNA digests using DNA indexers. Gene 145, 163-169. It produces sequences with a primer at their 3’ ends which need to be found and removed.

Some groups are using transposons to produce random start points for sequencing reactions, and vector_clip contains an experimental search procedure for dealing with the data generated by such methods.

Vector_clip is usually run as part of the pregap4 process (see Section 3.2 [Pregap4], page 202) and will usually be called three times: the first to locate and mark the sequencing vector; next to check for vector rearrangements; and finally to locate and mark cosmid vector segments.

Vector_clip operates on batches of readings using files of file names: one input file and two output files - one for the names of the readings that pass and one for those that fail. The program also modifies the reading files.

In earlier versions of vector_clip all the information needed about the vector (i.e. its name, location on disk, the cloning and primer sites used) for each reading was expected to be stored in the reading’s experiment file (See Section 10.3 [Experiment File], page 401.) but, as is explained in the next paragraph, the newest version employs an alternative method for providing data about sequencing vectors. For notes on defining the cloning and primer sites, see Section 5.8 [Defining the Positions of Cloning and Primer Sites for Vector_Clip], page 273.

The 1999.0 release of the package contained an experimental new method of providing vector_clip with data about the vectors to search for. Using feedback from the trial period we have simplified the method and improved the algorithm.
The new method uses files containing, not the complete vector sequences, but the segments of sequence between the primers and the cloning site. These files are termed "vector_primer" files see Section 5.6 [Vector_Primer files], page 272, and the vector_primer mode of vector_clip uses the data in these files to search for the vectors.

The vector_primer file can contain the data for up to (at present) 100 vector and primer combinations, although it would not be efficient to compare each reading against an unnecessarily large number of records. When vector_clip finds a match to one of the vectors defined in the vector_primer file it can not only mark the matching segment in the reading, but also adds the name of the file containing the vector sequence, and the primer type to the readings experiment file. The vector file name can then be used by vector_clip in its search for vector rearrangements, and the primer type can be used by gap4 in its analysis of read pairs (Note, however, that for read pair analysis, gap4 still needs to know which readings came from the same template, so that data must be added to the Experiment file in some other way).

A big advantage of the vector_primer file method, is that it simplifies the task of providing vector_clip with data. In addition, the task of creating the vector_primer files is simplified in that the -V option in vector_clip removes the necessity for the records in the vector_primer file to contain precisely the sequence between the primer and the cloning site Section 5.7 [Vector_Primer File Notes], page 273.

Vector_primer files are also used by the search for transposon data.

If setting up these programs seems a little daunting, it is important to realise that the majority users need not concern themselves with the details of vector_clip and the creation of experiment files for their readings; or if they do, these configuration operations are only performed once per project, and are made relatively easy by the use of pregap4.

5.1 Algorithms

For locating sequencing vector the program uses a dynamic programming algorithm and two percentage matches as cutoffs - one for the 5' end and another for the 3' end. Both searches include the poor quality data at the ends of the readings. This mode writes the SL and SR records in experiment files.

If the users selects the vector_primer file mode of vector_clip the program searches the 5' end of each reading for all of the forward and reverse sequence segments in the primer_vector file and notes the one which matches best. If this one is above the user defined threshold the 5' clip point will be set and the experiment file will be modified accordingly. The program then compares the rest of the reading with all of the segments in the vector_primer file to find the one which matches best. Again if the user defined threshold is reached the experiment file will be modified accordingly. If the best 5' and 3' matches come from different records in the vector_primer file a warning message is printed. If a 5' match is found it will be used to determine the file name of the vector sequence and the primer type. If only a 3' match is found it will be used to determine these items. If no match is found no PR record is written. This mode writes the SL, SR SF and PR records in experiment files. If the vector file name is missing from the vector_primer file record, the SF record is not written.

For locating cloning vector two algorithms are available, both of which use hashing. The original method needs a "Word length" (word_length), the "Number of diagonals to combine" (num_diags) and a "Cutoff score" (diagonal_score). The word length is the minimum number of consecutive bases that will count as a match. The algorithm treats the problem like a dot matrix comparison. First it finds all matches of length word_length; then it locates the diagonal with the highest normalised score. Then it adds the scores for the adjacent diagonals (num_diags). If the combined score is at least "diagonal_score" the experiment file is updated to indicate the location of the vector sequence. The score represents the proportion of a diagonal that contains matching words, and the maximum
score for any diagonal is 1.0. This mode writes the CS records in experiment files. If the whole reading is cloning vector this mode writes a PS record containing "all cloning vector",

A newer method also hashes using "word length" consecutive bases and accumulates the hits for each diagonal, but instead of using a score cutoff, it decides if there is a match using a probability threshold "P" supplied by the user. For each length of diagonal vector_clip calculates "E" the score that would be expected for probability "P", and then compares it with the observed score "O". If for any diagonal O>=E a match is declared and expressed as 100(O-E)/E. This new method is an attempt to overcome the problem that even though the scores on diagonals are normalised to lie in the range 0.0 to 1.0 the scores are still a function of the diagonal length. The probability P hence allows vector_clip to use a different cutoff score for each length of diagonal. Tests have shown that the probability based algorithm is very much more reliable than the older one. By default the program still uses the old algorithm, the probability based one being switched on by the user specifying a probability cutoff (option -P). It is strongly recommended that the probability based method is used and for our data we have found that a probability of 0.0000000000001 or 1.0e-13 gives good results. This mode writes the CS records in experiment files. If the whole reading is cloning vector this mode writes a PS record containing "all cloning vector".

The search for "vector rearrangements" uses a simple algorithm which looks only for a match of length "minimum match". All readings that contain a string of characters of at least this length that match a segment of the vector sequence exactly will be classed as "vector rearrangements" and their names will not be written to the file of passed file names. This mode writes a PS record containing "vector rearrangement" in experiment files if a match is found. Note that if a reading's Experiment file does not contain an SF (i.e. name of sequencing vector file) the vector rearrangements search does not fail the reading: its name goes into the pass file.

The search for transposon generated data is somewhat complicated, as is explained below.

The transposon ends must be stored in a vector_primer file. The vector sequence file should be named in the SF record. Numerous scores are required.

First get the transposon end sequences from the vector_primer file. Then get the vector sequence and rotate it around the cloning site. Next use dynamic programming to search with both of the transposon end sequences and note the highest score. If above score L reset SL. Now use hashing to search the 20 bases after SL for a match to any part of the vector, on both strands. If the best match is above score l, use dynamic programming to try to align from the match point to the cloning site. If the alignment score is >= score R reset SL. If the previous two steps fail to find a match to vector we assume that the transposon inserted into the target DNA and not the vector. The reading could hence run into vector at its 3' end so we use dynamic programming to search from SL onwards, for the sequences either side of the cloning site (we do not know the orientation of the transposon (and hence the read) relative to the vector). If we find a match >= score R reset SR.

5.2 Options

Usage: vector_clip [options] file_of_filenames
Where options are:
- [-s mark sequencing vector] [-c mark cloning vector]
- [-h hgmp primer] [-r vector rearrangements]
- [-w word_length (4)] [-n num_diags (7)]
- [-d diagonal score (0.35)] [-l minimum match (20)]
- [-L minimum % 5' match (60)] [-R minimum % 3' match (80)]
- [-m default 5' position] [-t test only]
- [-M Max vector length (100000)] [-P max Probability]
- [-v vector_primer filename] [-i vector_primer filename]
Options:

- **s** Mark sequencing vector. Searches for 5' primer, 3' running into vector.
- **c** Mark cloning vector. Searches both strands for cloning vector.
- **h** Hmp primer. Searches 3' end for a primer.
- **i vector_primer filename** Mark transposon data.
- **r** Vector rearrangements. Searches for sequencing vector rearrangements.
- **t** Test only. Does not change the experiment files, displays hits.

### 5.3 Parameters (defaults in brackets)

- **-L** minimum percentage match 5' end (60) sequencing vector searches and transposon search
- **-R** minimum percentage match 3' end (80) sequencing vector searches and transposon search
- **-m** minimum 5' position allows a minimum 5' end cutoff to be set if a sufficiently good match is not found (i.e. it is really a default 5' cutoff position). If a value of -1 is used the program will set the cutoff to be the distance between the primer and the cloning site.
- **-v vector-primer-pair filename** sequencing vector search using vector-primer-pair file
- **-V vector_primer length** the length of the sequence stored in the vector_primer file to use for the 5' search
- **-w** word_length (4) cloning vector search hash length
- **-P** probability cloning vector search, (a score less likely than P is a match)
- **-n** num_diags (7) cloning vector search, old score based algorithm: number of diagonals to combine
- **-d** diagonal score (0.35) cloning vector search, old score based algorithm
- **-l** minimum match (20) sequencing vector rearrangements and transposon search minimum match length
- **-M** maximum vector length (100000) all algorithms, reset for vectors >100000 bases
- **-p** passed fofn file of file names for passed files
- **-f** failed fofn file of file names for failed files

**input fofn ...** input file of file names
5.4 Error codes

The following errors can occur.
1. Error: could not open experiment file
2. Error: no sequence in experiment file
3. Error: sequence too short
4. Error: missing vector file name
5. Error: missing cloning site
6. Error: missing primer site
7. Error: could not open vector file
8. Error: could not write to experiment file
9. Error: could not read vector file
10. Error: missing primer sequence
11. Error: hashing problem
12. Error: alignment problem
13. Error: invalid cloning site
14. Warning: sequence now too short (no message)
15. Warning: sequence entirely cloning vector (no message)
16. Warning: possible vector rearrangement (no message)
17. Warning: error parsing vector primer file
18. Warning: primer pair mismatch!
19. Aborting: more than X entries in vector primer file

5.5 Examples

Screen for sequencing vector using 5' cutoff of 70%, a 3' cutoff of 90% and default 5' primer position of 30. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -s -L70 -R90 -m30 -pfiles.pass -f files.fail files.in
```

Screen for sequencing vector using 5' cutoff of 60%, a 3’ cutoff of 80% and default 5’ primer position of 30. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail. This shows that the default search is for sequencing vector.

```
vector_clip -m30 -pfiles.pass -f files.fail files.in
```

Screen for sequencing vector using 5' cutoff of 60%, a 3’ cutoff of 80% and a vector-primer-pair file called vpfile. Only the 20 bases closest to the cloning site will be used for the 5’ search. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -v vpfile -V20 -pfiles.pass -f files.fail files.in
```

Screen transposon data using 5’ cutoff of 80%, a 3’ cutoff of 85%, a match length of 10 and a vector-primer-pair file called vector primer file. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -i vector_primer_file -L 80 -R 85 -l 10 -pfiles.pass \ 
-f files.fail files.in
```
Screen for cloning vector using the old algorithm with a word length of 4, summing 7 diagonals and diagonal cutoff score of 0.4. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -c -w4 -n7 -d0.4 -p files.pass -f files.fail files.in
```

Screen for cloning vector using the probability based algorithm with a word length of 4 and probability cutoff of 1.0e-13. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -c -P 1.0e-13 -p files.pass -f files.fail files.in
```

Screen for 3’ primer using a cutoff of 75%. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -h -R 75 -p files.pass -f files.fail files.in
```

Screen for sequencing vector rearrangements using a cutoff of 20 bases. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -r -l 20 -p files.pass -f files.fail files.in
```

### 5.6 Vector Primer file format

The vector primer files store the data for each vector/primer pair combination as a single record (line) and up to 100 records can be contained in a file. The items on each line must be separated by spaces or tabs (only the file name can contain spaces) and a newline character ends the record. It is important to realise that the format has been simplified since the first version of the method appeared in release 1999.0 and any files created for the 1999.0 release will need to be edited!

The items in a record are:

- `name`
- `seq_r`
- `seq_f`
- `file_name`

`name` is an arbitrary record name. `seq_r` is the sequence between the reverse primer and the cloning site. `seq_f` is the sequence between the forward primer and the cloning site. `file_name` is the name of the file containing the complete vector sequence.

An example file containing two entries (for m13mp18, and a vector called f1) is shown below. "\"" symbols have been used to denote wrapped lines and so it can be seen that the first record is shown on two lines and the next on 1.

```
m13mp18 atacgaattcgagtcggtacctc ggggatcctctagagtcgacctgcaggcatgcaagcttggc \ /pubseq/tables/vectors/m13mp18.seq
f1 CCGGGAATTCGCGGCCGCTCGACT CTAGACTCGAGTTATGCATGCA af_clones_vec
```

Note that the segments can be longer (or shorter) than the sequences between the primer and the cloning site. The `-V` option of `vector_clip` allows the user to specify that a fixed number of bases closest to the cloning site be used for any particular run, and so the same record in the vector primer file could be used for several primers as long as the cloning site was the same. If it is necessary to get the sequence segments precisely defined refer to the figure below. This contains an annotated section of the m13mp18 vector around the SmaI site, to see how it corresponds to the first record in the vector primer file. The primers shown are the 16mer reverse(-21) and the 17mer forward(-20), and the vector primer record is the sequence between the primers with a space at the cloning site, followed by a file name.
5.7 Vector_Primer File Notes

There are several consequences of using vector_primer files to specify the sequencing vector details. Please read a description of the vector_primer file algorithm in the algorithms section Section 5.1 [Vector_clip algorithms], page 268.

Firstly, to get the vector segments of readings marked correctly it is not necessary to include the relevant data in their experiment files.

Secondly, because vector_clip compares all the primer-vector pairs in the primer_vector file it would be inefficient to include very large numbers of records in these files. Instead it would be better to have a master vector_primer file which contained all the combinations used in the lab and then to copy the relevant ones to project specific files.

Thirdly, even though vector_clip can write the PR record (primer type) into the experiment file if it finds a match, gap4 still needs the template name data in order to do read pair analysis.

Finally note that the -V option for vector_clip means that the segments of sequence in the vector_primer file need not be made exactly the right length when the files are created: it matters only that the cloning site is correctly specified and that there is sufficient length of sequence on either side. For example, vector_primer files could be created in which all records included 40 bases from either side of the cloning sites. The -V option allows the alignment to be limited to the segment of sequence closest to the cloning site. For example, -V 20 specifies that at most 20 bases around the cloning site are used.

5.8 Defining Cloning and Primer Sites for Vector_Clip

Vector sequences should be stored in simple text files with up to 80 characters of data per line. Sequencing vectors are those vectors such as m13 used to produce templates for sequencing. All other vectors, such as cosmids vectors, that are used to purify and grow the DNA prior to it being subcloned into sequencing vectors are termed "cloning vectors". It is important that the files containing cloning vector sequences which are used by vector_clip are arranged so that the cloning site follows the last base in the file. For example (where X is the cloning site):

```
start of file
acatacatacatatatata
acatagatagatacaga
.
.
.
```
Cloning Vector File Base Ordering

In order for vector_clip to search readings for segments of sequencing vectors it either needs to use a vector_primer file or it needs to know the positions of the cloning site and primers. If not using a vector_primer file, each reading’s experiment file should contain SC and SP records, and also a primer type record (PR). The following section explains the numbering system used with an example for m13mp18, and then describes how to use spin (see Section 8.2 [Introduction], page 293) to work out the values for other vector, cloning site, and primer combinations.

The position of the cloning site depends on the ordering of the bases in the particular vector sequence file being used. That is, as the sequences are circular, the file may be arranged to start at any base and still give the same circular sequence. Vector_Clip must be told the correct position of the cloning site, then, relative to that, the position of the first base that will be included in the reading. i.e. the relative position of the first base 3’ of the primer.

Below we use EMBL entry M13MP18 as an example. The figure includes a double stranded listing of 120 characters of m13mp18 around the SmaI site at 6249, and some of the restriction sites. Between the restriction sites and the sequence we have added lines to explain the numbering used by vector_clip. The numbers below the row of “+” symbols show positive positions (to the right of the SmaI cloning site), and the numbers below the “-” symbols show negative positions (to the left of the cloning site). Below these lines we show the sequences of the 16mer reverse primer "r(-21)" which is at relative position -24, and the 17mer forward primer "f(-20)" which is at relative position 41.

The positions of SmaI site and forward and reverse primers for M13MP18

```
EcoRI
  .  TaqI
  .   .  SacI
  .   .   .  XmaI
  .   .   .  .HpaII
  .   .   .  .AsuC2I
  .   .   .  .SmaI
  .   .   .   .  BamHI
  .   .   .   .  MboI
  .   .   .   .  Sau3AI
  .   .   .   .  XhoII
  .   .   .   .   .  PspN4I
  .   .   .   .   .  XbaI
  ++         +10+       
---20--------10--------123456789012
r(-21) 432109876543210987654321
  aacagctatgaccatg
acacaggaacagctatgaccatgattacgaattcgagctcggtacccggggatcctcta
  6210 6220 6230 6240 6250 6260
tgtgtcctttgctgatactggtactaatgcttaagctcgagccatgggcccctaggagat
  HinfI
  .  SalI
  .  .  AccI
```
5.9 Finding the Cloning and Primer Sites

The problem addressed here is how to work out the positions of the cloning and primer sites for vector clip. The numbers can be worked out from listings of the vector sequences but once you know how, it is far easier to use the restriction enzyme search in spin (see Section 8.2 [Introduction], page 293) to do it, and that is what we explain here. Some familiarity with spin will help. To use the restriction enzyme search in spin it is necessary to have created a file containing the definitions of the sequences to search for (see Section 10.4 [Restriction enzyme files], page 414) These files give each enzyme a name and a set of strings (with cut positions marked by "'"). The name is terminated by "/" and each string by "/". An extra "/" terminates all the data for each enzyme. For example "fred/aaa'ttt/gatc'a//" defines enzyme fred to have two recognition sequences aaattt and gatca with cut positions denoted by "'".

For our current purpose we treat the primer sequences as restriction enzymes which each have a single recognition sequence; making sure that the sequence is the sense of the primer that is present in the sequence being searched, and that the "cut positions" define the 3' ends (i.e. the end where the new sequence will start). Again if we use the m13mp18 vector, its SmaI cloning site and the 17mer (-20) forward and 16mer (-21) reverse primers as an example. The reverseprimer has the sequence 5'aacagctatgaccatg3' and the forward one is 5'gtaaaacgacggccagt3', and the SmaI site is ccc'ggg where "'" defines the cutsite.

The restriction enzyme file should contain the following:

\[f(-20)/'actggccgtcgttttac//\]
\[SmaI/CCC'GGG//\]
\[r(-21)/aacagctatgaccatg3'///\]

This names the 17mer forward primer as f(-20) and defines its recognition sequence as 'actggccgtcgttttac. Note that this is the complement of the primer (which is what appears in the sequence being searched) and that the "cut position" is defined by the "'" symbol. SmaI is named and defined in the next record by SmaI/CCC'GGG//. The 16mer reverse primer is named r(-21) and defined by r(-21)/aacagctatgaccatg3', and this time we search for the sequence of the primer and the "cut position" is again at the 3' end.

Having started spin and read in the m13mp18 sequence select "Restriction enzyme map" from the "search" menu. A dialogue will appear requesting "Select input source" and with "6 cutter..."
file" as the default. Select "personal" and give the name of the file for m13mp18 (a file containing the definitions shown above should be found in $STADTABL/m13mp18_primers). The names "f(-20), Smal and r(-21)" should appear in the selection box in the dialogue. Select all three and the graphical result should appear. Magnify the plot by hitting the "+50%" button and then scroll to the region around 6249 which should look as shown below.

This plot and the functionality of spin are sufficient to work out the numbers for vector_clip, but there is also a way of getting the values printed in the text output window (this is described later). To obtain the numbers from the plot first touch the line showing the Smal site, its position (6249) will be written in the information line at the bottom of the plot. This is the position of the cloning site and hence is the value for the SC record in the experiment file. Now click on the line for the Smal site and the information line will display "Select another cut": click on the line for the forward primer; the distance between the Smal site and the forward primer (41) will be displayed in the information line, and in the top right hand box of the plot. Being to the right of the cloning site, this gives a positive value for the experiment file SP record. Clicking on the Smal site, and then the line for the reverse primer, gives 24 which, being to the left, is a negative value for the SP record.

To get the numbers displayed in the text output window, select the "Output ordered on position" item from the "Results" menu of the "Restriction enzyme map" plot. For the example given here they will appear as shown below.
Finally it is also possible to work out the numbering by using the restriction enzyme search in the spin "sequence display" which can be selected from the "View" menu. It will appear as shown below.
6 Screening Readings for Contaminant Sequences

This section explains how to use the program screen_seq to filter out unwanted readings: i.e. how to search for and separate readings containing the sequences of extraneous DNA, such as vector or bacterial sequences. We have separated this task from that of locating and marking the extents of sequencing vector and other cloning vectors. There we require precise identification of the junction between the vectors and the target DNA. The filtering process described here is designed to spot strong matches between readings and a panel of possible contaminating sequences, and it splits readings into passes and fails. Readings that fail have a PS line containing the word "contaminant" and a "CONT" tag added to their experiment file.

Normal usage would be to compare a batch of readings in experiment file format against a batch of possible contaminant sequences stored in (at present) simple text files. Each batch is presented to the program as a file of file names, and the program will write out two new files of file names: one containing the names of the files that do not match any of the contaminant sequences (the passes), and the other those that do match (the fails). It is also possible to compare single readings and single contaminant files by giving their file names (i.e. it is not necessary to use a file of file names for single files).

Given the frequent need to compare against the full E. coli genome the algorithm is designed to be fast. Only one parameter is required: the minimum match length, min_match. All readings which contain a segment of sequence of length min_match which exactly matches a possible contaminant sequence are filtered out.

The search is conducted only over the clipped portion of the readings. On our aging Alpha machine it takes about 1 second to compare both strands of a reading against the 4.7 million bases of E. coli.

6.1 Parameters

- `-l` Length of minimum match (25).
  all readings with a match of this length are hits

- `-m` Maximum vector length.
  the length of the longest sequence to screen against (100000).

- `-i` Input file of reading file names.
  the file names of the readings to screen.

- `-I` Input file of single reading to screen.
  the file name of the reading to screen.

- `-s` Input file of sequence file names.
  the file names of the sequences to screen against.

- `-S` Input file name of single sequence to screen against.

- `-p` Passed output file of file names.
  for the names of the readings that do not match.

- `-f` Failed output file of file names.
  for the names of the readings that match.

- `-t` Test only mode.
  results are only written to stdout and the experiment files are not altered.
6.2 Limits

Screen_seq is currently set to be able to process a maximum of 10,000 readings and 5000 screening sequences in a single run. The maximum length of any screening sequence is 100,000 although this can be overridden by use of the -m parameter (set it to 5000000 for E. coli). At present the sequences to screen against must be stored in simple text files containing individual sequences, with no entry names, and <100 characters per line.

6.3 Error codes

The following errors can occur.
1. "Failed to open file of file names to screen against". Fatal failure to open the file of file names to screen against.
2. "Failed to open single file to screen against". Fatal failure to open the file to screen against.
3. "Failed to open file of file names to screen". Fatal failure to open the file of file names to screen.
4. "Failed to open single file to screen". Fatal failure to open the file to screen.
5. "Failed to open file of passed file names". Fatal failure to open the file of file names for readings that do not match.
6. "Failed to open file of failed file names". Fatal failure to open the file of file names for readings that match.
7. "Error: could not open vector file". An individual sequence file could not be opened.
8. "Error: could not read vector file". An individual sequence file could not be read.
9. "Error: could not hash vector file". An individual sequence file could not be prepared for comparison.
10. "Error: could not open experiment file". The file does not exist or is unreadable.
11. "Error: no sequence in experiment file".
12. "Error: sequence too short". The reading is shorter than the minimum match length.
13. "Error: could not write to experiment file". The disk is full or the file is write protected.
14. "Error: hashing problem". An error occurred in the comparison algorithm. Please report to staden-package@mrc-lmb.cam.ac.uk

Inconsistencies in the selection of options, such as selecting -I and -i, should also cause the usage message (shown below) to appear, and the program to terminate.

Usage: screen_seq [options and parameters]

Where options and parameters are:

[-l minimum match (25)] [-m Max vector length (100000)]
[-i readings to screen fofn] [-I reading to screen]
[-s seqs to screen against fofn] [-S seq to screen against]
[-t test only]
[-p passed fofn] [-f failed fofn]

6.4 Examples

Screen the readings whose names are stored in fofn against a batch of possible contaminant sequences whose names are stored in vnames. Write the names of the readings that pass to file p and those that fail to file f. Increase the maximum sequence length to 5000,000 characters and require a minimum match of 20.
screen_seq -i fofn -s vnames -p p -f f -120 -m5000000

Screen the single reading stored in xpg33.g1 against a batch of possible contaminant sequences whose names are stored in vnames. If the reading does not match write its name to file p, otherwise to file f. Increase the maximum sequence length to 5000,000 characters and require a minimum match of 20.

screen_seq -I xpg33.g1 -s vnames -p p -f f -120 -m5000000

Screen the readings whose names are stored in fofn against a single possible contaminant sequence stored in ecoli.seq. Write the names of the readings that pass to file pass and those that fail to file fails. Increase the maximum sequence length to 5000,000 characters and require minimum match of 20.

screen_seq -i fofn -S ecoli.seq -p pass -f fails -120 -m5000000
Chapter 7: Viewing and editing trace data using trev

7 Viewing and editing trace data using trev

7.1 Introduction

For some types of sequencing project it is convenient to view and edit the chromatogram data prior to assembly into a gap4 database (see Section 1.2 [Gap4 Introduction], page 3), and this is the function of the program trev.

Trev displays the original trace data, its base calls and confidence values, and it allows the sequence of the trace to be edited and the left and right cutoffs to be defined. Several file formats can be read in addition to our own Experiment Files (see Section 10.3 [Experiment File], page 401), and ‘SCF’ files (see Section 10.1 [scf], page 383). Any edits made are normally saved to Experiment files, not to the chromatogram files which we regard as archival data.

A typical display from trev is shown below. It includes the trace data, the original sequence, the edited sequence, the menu bar, and the name of the sequence being edited. The left cutoff region is shown shaded.

The trace can be scrolled using the scrollbar directly beneath the menubar. The trace can be magnified in the vertical and horizontal directions using the scale bars to the left of the trace.

The base numbers, original sequence, edited sequence, confidence values and the trace can each be switched on or off, and the font for the original and edited sequence is selectable.
The figure below shows the bases, edited bases, a histogram of the confidence values, the traces, and the Information Window which can be switched on from the View Menu.

Trev can be used to produce postscript files of the traces so that they can be printed. The colours, line widths, etc are configurable. An example is shown in the figure below.
Chapter 7: Viewing and editing trace data using trev

Note that we strongly recommend that readings are not edited prior to assembly as it is far better to edit them when their alignment with other readings can be seen.

7.2 Opening trace files

Trace files can be opened either on the command line or from within Trev. In both cases it is possible to open several traces at once. In this case trev will add Next File, Previous File and Goto File buttons to allow quick navigation between traces.

On the command line, this is simply done by specifying several files. With the "Open" dialogue from within trev multiple files may be selected by dragging with the left mouse button or using shift+left button and control+left button to extend regions or to toggle loading of individual files.

7.2.1 Opening a trace file from the command line

usage: trev [-{ABI,ALF,EXP,SCF,PLN,Any}] [-edits value] [-editscf] [-xmag value] [-ymag value] [-restrict] [tracefilename ...]

-ABI, -ALF, -EXP, -SCF, -PLN, -Any
Optional. Defaults to Any. These define the possible input trace formats available. Currently these are 'ABI', 'ALF', experiment (see Section 10.3 [Experiment File], page 401), 'SCF' (see Section 10.1 [scf], page 383), plain ASCII text or 'any' in which case the program attempts to establish the file format from information contained within the trace file.

-edits value
Optional. Defaults to 1. If value is 1, the trace sequence can be edited. If value is 0, no edit line is displayed in Trev and the sequence may not be edited.

-editscf
Optional. By default writing to SCF is disabled for safety and reasons of preference (we feel that all edits should be contained within an associated Experiment File thus leaving the original trace file intact). Specifying -editscf allows writing to SCF files.
-pregap_mode
Optional. Only used by Pregap4. This adds a Reject button to Trev and disables certain file operations. This argument should only be used by programs that run Trev as subprocesses for processing batches of files.

-restrict
Optional. Restricts the use of the trace editor to a single file by disabling the ability to open another file from within Trev. The main use of this option is for calling Trev from within scripts.

-xmag value
Optional. Defaults to 150. Specifies the magnification along the X axis of the trace. Larger values represent higher magnifications.

-ymag value
Optional. Defaults to 10. Specifies the magnification along the Y axis of the trace. The value should be between 10 and 100 with 10 showing all the trace and 100 being the largest magnification.

7.2.2 Opening a trace file from within Trev

To open a trace file select the "Open..." command from the File menu. This brings up a file browser from where the trace name can be selected. See Section 9.6 [File Browser], page 379. The format of the trace file should be selected from the row of Format buttons. Currently these are 'ABI', 'ALF', Experiment File (see Section 10.3 [Experiment File], page 401), 'SCF' (see Section 10.1 [SCF File], page 383), plain ASCII text or 'any' in which case the program attempts to establish the file format from information contained within the trace file. Opening an experiment file opens the trace file named within the experiment file. Double clicking on the trace name will open this trace file.

If a trace file is already open, it is closed before the new one is opened. If the previous trace has been edited, but not saved, a dialogue box is displayed, asking if you wish to save the file before loading a new file. Selecting "Yes" will automatically save the file to its current filename. Selecting "No" will discard any changes that have been made.

7.3 Viewing the trace

The trace can be scrolled using the scrollbar directly beneath the menubar. The trace can be magnified both in the vertical and horizontal directions using the two scales to the left of the trace.

The base numbers, original sequence, edited sequence, confidence values and the trace can each be switched on or off by using the check buttons in the "Display" option of the View menu.

The font for the original and edited sequence can be chosen from three sizes, selectable by using the Font submenu of the View menu.

The figure below shows the bases, edited bases, a histogram of the confidence values, the traces, and the Information Window which can be switched on from the View Menu.
7.3.1 Searching

Selecting the "Search..." command in the View menu brings up a window into which a text string can be entered. Pressing the "Next" button positions the cursor at the start of the next piece of sequence that matches the string specified in the text box. Pressing "Previous", finds the previous match. The search is case insensitive.

7.3.2 Information

The comments from the SCF file of the trace can be displayed using the "Information" option in the View menu.

7.4 Editing

7.4.1 Setting the left and right cutoffs

Poor data at the left and right ends of the trace can be marked using the "Left Quality" and "Right Quality" options in the Edit menu. Alternatively a keyboard shortcut for editing the cutoff is to press Control L or Control R to edit left or right cutoff respectively. To select the left cutoff, choose the "Left Quality" option from the menu. Then click the left mouse button at the required position in the trace display. The region from the start of the sequence to this position will be highlighted in grey. To select the right hand cutoff, choose the "Right Quality" option in the Edit menu and click the required position in the trace display. The region between the left boundary and the end of the sequence will be highlighted. To prevent accidentally changing the cutoffs once these have been selected, choose the "Sequence" option in the Edit menu.

If vector sequence has been marked trev will also display these in a similar fashion to the quality cutoffs except in a peach colour. These cutoffs can be changed by selecting "Left Vector" and...
"Right Vector" in the same fashion as editing the quality cutoffs. Where both quality and vector
cutoffs coincide trev draws the regions by striping between both peach and grey.

7.4.2 Editing the sequence

If the ability to edit has not been disabled, there will be two windows showing the trace sequence.
The original sequence is displayed in the upper window. The window below this, which contains
the blue cursor, is the editing window. To edit this sequence, select the "Sequence" option in the Edit
menu. The editing cursor is positioned by clicking with the left mouse button within the display.
Bases are deleted to the left of the cursor using the delete key of the keyboard. Additional bases
are inserted to the left of the cursor. Only A, a, C, c, G, g, T, and t are allowed. It is recommended
that edits are entered in lower case to distinguish them from the original bases.

7.4.3 Undoing clip edits

It is often easy to accidently forget which editing mode you are in and adjust a quality or vector
clip point by mistake. Trev keeps track of all clip edits and hence these may be "Undone" by
selecting "Undo Clipping" from the Edit menu. This will remove the last clip edit. It is not yet
possible to undo sequence edits.

7.5 Saving a trace file

To save a trace file to a different file name or format choose the "Save As..." command from
the File menu. Select the format the file is to be saved in using the Format buttons. The output
formats are CTF, SCF, ZTR, experiment and plain text. Type a new name into the Selection box
or select an existing name from the list of file names. Experiment format traces can be saved to
their existing name using the "Save" option in the File menu.

7.6 Processing multiple files

When several trace files are specified on the command line to Trev, it will add Previous File,
Next File, and Goto File buttons. The Previous File and Next File simply step through the specified
trace files. The Goto File button will bring up a scrollable list of all the trace files specified. Clicking
on any trace filename in this list will jump to that file.

If Trev was brought up from Pregap4, or the -pregap_mode command line switch was used,
Trev will also display a Reject button. This may be used to indicate to Pregap4 that the trace file
shown is not worthy of any clipping at all and should be sent to the Pregap4 "failed" file.

7.7 Printing a trace

The Print option is available via the File menu, as shown below.
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It produces a PostScript file which you must then send to the printer yourself.

All sizes given in the dialogues explained below should be in PostScript points (72pt = 1inch).

Defaults and available options are specified in the file tk_utilsrc. These can be changed by copying the relevant line from tk_utilsrc into a file called .tk_utilsrc in your home or working directory, and then altering the settings as desired.

Note that it is not yet possible to include the histogram of confidence values in the postscript output.

7.7.1 Page options

7.7.1.1 Paper options

Currently available page sizes:

A4  
    (842 x 595)

A3  
    (1191 x 842)

US Letter  
    (792 x 612)

Please note that the page size and orientation options do not determine the paper format that your printer will use. This must be set externally to trev.
7.7.1.2 Panels

Traces are printed width-ways across the page. When the right-hand margin of the page is reached, printing continues below the current section and from the left-hand side. A 'panel' is one page-width's worth of trace (minus margins).

The trace and the sequence and sequence number information are printed entirely within the given height of the panel, and the separation gives the amount of space that is left between panels. Thus they, together with the page height and top and bottom margins, determine how many panels will be printed per page.

7.7.1.3 Fonts

All fonts listed should be available to most PostScript printers. Most printers will default to Courier if a selected font is not recognised.

7.7.2 Trace options

7.7.2.1 Title

The title is printed in the top left hand corner of every page. The default is the name of the trace file.

7.7.2.2 Line width and colour

The defaults are those used by the trev display. The colours shown in the selection dialogue may not correspond exactly to those printed, depending on the capabilities of your printer. Different colours will usually be printed using grey-scales on black and white printers.

7.7.2.3 Dash pattern

Dash pattern is in PostScript dash format:

dash_1 gap_1... dash_n gap_n offset

'dash_n' and 'gap_n' are the lengths of dashes and the gaps between them. The dash pattern starts at dash_1, continues to gap_n, then starts again at dash_1, until the whole line has been drawn. If n = 0, i.e. no values are given for 'dash' and 'gap', the result is a normal unbroken line.
Offset must be given, and is the distance into the dash pattern at which the pattern should be started. The dash pattern is not demonstrated by the example line on the \texttt{ps\_trace\_setup} dialogue.

\subsection*{7.7.2.4 Print bases}

Allows a subsection of the trace to be printed.

The 'Visible' button sets the region to that currently displayed in the main \textit{trev} window. If the display is altered, the print base settings will not change unless 'Visible' is pressed again. The whole sequence is printed if the start position is greater than the end position. The OK button will not work if the start or end positions given are outside the range of the sequence.

\subsection*{7.7.2.5 Print magnification}

The X and Y scales are taken from the \textit{trev} display, and cannot be set independently for PostScript output.

\subsection*{7.7.3 Example}

The segment of output displayed below indicates the effects of the settings given in the example dialogue screen dumps shown above. NB: the page has been clipped to save space. The section shown is the top part of an A4 page.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{example.png}
\end{figure}

\subsection*{7.8 Quitting}

To exit \textit{trev}, select the "Exit" command from the File menu. If the sequence has been edited but not saved, a dialogue box is displayed, asking if you wish to save the file before quitting. Selecting "Yes" will automatically save the file to its current filename. Selecting "No" will discard any changes that have been made.
8 Analysing and comparing sequences using spin

8.1 Organisation of the Spin Manual

The Introductory section of the manual gives an overview of the functions (see Section 8.2.1 [Summary of the Spin Functions], page 293), the menus (see Section 8.2.4 [Spin Menus], page 306) and the user interface (see Section 8.2.3 [Introduction to the Spin User Interface], page 295). The Introduction to the user interface includes a range of screen dumps which give an overview of what spin can do, and taken as a whole, the introduction should contain sufficient information to enable users to start using the program.

The next section describes in turn each of the main functions (see Section 8.3 [The Spin Functions], page 308). This is followed by a detailed description of the spin user interface (see Section 8.6 [The Spin User Interface], page 353). Next is a section describing how users can control the results from the functions, and how they can be manipulated once they have been obtained (see Section 8.7 [Controlling and Managing Results], page 365). The final part of the manual describes how to read sequences into spin and the kinds of manipulations which can be performed on them to prepare them for analysis (see Section 8.8 [Reading and Managing Sequences], page 367).

8.2 Introduction

Spin is an interactive and graphical program for analysing and comparing sequences. It contains functions to search for restriction sites, consensus sequences/motifs and protein coding regions, can analyse the composition of the sequence and translate DNA to protein. It also contains functions for locating segments of similarity within and between sequences, and for finding global and local alignments between pairs of sequences. To help assess the statistical significance of comparisons the program can calculate tables of expected and observed score frequencies for each score level. Most analytical functions which operate on single sequences add their graphical results to a "SPIN Sequence Plot" that is associated with the sequence being analysed. (An exception is the restriction enzyme search which produces its own separate window.) Most functions which compare pairs of sequences add their results to a "SPIN Sequence Comparison Plot". The SPIN Sequence Plot and the SPIN Sequence Comparison Plot each have associated sequence display windows: the Sequence Display and the Sequence Comparison Display. These allow the text of the sequences to be viewed and use cursors to show the corresponding positions in the graphical displays. The graphical plots can be zoomed, and cursors or crosshairs can be used to locate the positions of the individual results. Plots can be superimposed.

8.2.1 Summary of the Spin Single Sequence Functions

Spin's main single sequence analytical functions are accessed via the Statistics, Translation and Search menus. The "Statistics" menu contains options to count and plot the base composition (see Section 8.3.3 [Plot Base Composition], page 308) and also to count the dinucleotide frequencies (see Section 8.3.2 [Dinucleotide Frequencies], page 308).

The "Translation" menu contains options to set the genetic code (see Section 8.3.5 [Set Genetic Code], page 312), translate to protein (see Section 8.3.6 [Translation], page 313), find open reading frames and write the results in either feature table format or as fasta format protein sequence files (see Section 8.3.7 [Find Open Reading Frames], page 314), and to calculate codon tables.

The "Search" menu contains a variety of different searching techniques. "Protein genes" has four methods for finding protein genes (see Section 8.3.11.3 [Codon Usage Method], page 325) (see Section 8.3.11.5 [Author Test], page 330), (see Section 8.3.11.4 [Positional base Preferences], page 329) (accessed as a subcomponent of the Codon Usage Method), and (see Section 8.3.11.6 [Uneven
Positional base Frequencies], page 333). There is also a method to search for tRNA genes (see Section 8.3.11.8 [tRNA Search], page 335). It is also possible to perform subsequence or string searches (see Section 8.3.9 [String search], page 320) and restriction enzyme searches (see Section 8.3.8 [Restriction enzyme search], page 316). There are searches for start (see Section 8.3.11.1 [Start Codon Search], page 323) and stop codons (see Section 8.3.11.2 [Stop Codon Search], page 323), splice junction searches (see Section 8.3.11.7 [Splice Site Search], page 334), and general motif searches using weight matrices (see Section 8.3.10 [Motif Search], page 321).

8.2.2 Summary of the Spin Comparison Functions

This section outlines the functions obtained from the Comparison menu. All produce graphical and textual output. Using a score matrix, the "Find similar spans" function compares every segment of one sequence with all those of the other and reports those that reach a user defined score. The segments are of a fixed length (span) set by the user (see Section 8.4.1 [Finding Similar Spans], page 337). To look for short matching segments of any length, and allowing gaps, a local dynamic programming routine can be used (see Section 8.4.5 [Aligning Sequences Locally], page 347). The fastest routine for locating segments of similarity (and generally only suitable for DNA sequences) finds all identical subsequences (or words) (see Section 8.4.2 [Finding Matching Words], page 340). For a quick global comparison of sequences using a combination of the Matching Words and Matching Spans algorithms the "Find best diagonals" algorithm can be used (see Section 8.4.3 [Finding the Best Diagonals], page 342). Global alignments can be produced and plotted using a dynamic programming algorithm (see Section 8.4.4 [Aligning Sequences Globally], page 343).
8.2.3 Introduction to the Spin User Interface

Spin has several main displays. The first is a top level window from which all the main options are selected and which receives textual results. Most analytical functions which operate on single sequences add their graphical results to a "SPIN Sequence Plot" that is associated with the sequence being analysed. (An exception is the restriction enzyme search which produces its own separate window.) Most functions which compare pairs of sequences add their results to a "SPIN Sequence Comparison Plot". The SPIN Sequence Plot and the SPIN Sequence Comparison Plot each have associated sequence display windows: the Sequence Display and the Sequence Comparison Display. These allow the text of the sequences to be viewed and use cursors to show the corresponding positions in the graphical displays.

Spin is best operated using a three button mouse, but alternative keybindings are available. Full details of the user interface are described elsewhere (see [User Interface], page 373), and here we give an introduction based around a series of screenshots.

The main window (shown below) contains an Output Window for textual results, an Error window for error messages, and a series of menus arranged along the top (see Section 8.2.4 [Spin menus], page 306). The contents of the two text windows can be searched, edited and saved. Each set of results is preceded by a header containing the time and date when it was generated.

As can be seen the main menu bar contains File, View, Options, Sequences, Statistics, Translation, Comparison, Search and Emboss menus. In general most functions add their graphical results to a "SPIN Sequence Plot", but those obtained from the Comparison menu add their results to a "SPIN Sequence Comparison Plot".
8.2.3.1 Introduction to the Spin Plot

Most of the spin functions display their results in a two-dimensional plot called a "spin plot" (see Section 8.6.1 [Spin plot], page 354). Sets of matches from a single invocation of a function are termed "a result". Each result is plotted using a single colour which can be configured via the results manager (see Section 8.7.1 [Result manager], page 365).

The figure shown below shows a spin plot window containing the results of a gene search method based on codon usage, superimposed on a search for stop codons (see Section 8.3.11.3 [Codon Usage Method], page 325). Each plot window contains a cross hair. Its x position is shown in sequence base numbers in the left hand box above the plot, and the y coordinate, expressed using the score values of the gene search, is shown in the right hand box.
At the right hand side of each panel is a set of square boxes with the same colours as the lines drawn in the adjacent plot. These icon-like objects represent individual results and allow the user to operate on them. For example at the right of the middle panel is a pop-up menu containing the items: "Information", "List results", "Configure", "Hide" and "Remove". (see Section 8.7.1 [Result manager], page 365).

These icons can also be used to drag and drop the results to which they correspond. This is activated by pressing the middle mouse button, or Alt left mouse button, over the box and then moving the cursor over the spin plot to the new location or anywhere outside the spin plot (see Section 8.6.1.4 [Drag and drop], page 357)

Each spin plot window also contains a cursor that denotes the position of the cursor in the Sequence display window (see Section 8.6.2 [Spin Sequence Display], page 360). The user can move a cursor by clicking and dragging with the middle mouse button, or Alt left mouse button. This will move the cursor in the sequence display and all other cursors displayed that relate to the sequence.

The graphical results can be zoomed and scrolled in both x and y directions. Zooming is achieved using the X and Y scale bars at the top left hand corner of the plot. The individual plots can be scrolled in y using the scroll bars attached to their right hand edge. The sequence can be scrolled using the scroll bar at the base of the plot.

To illustrate further uses of the program we include some more screen dumps below.
The figure above shows the results of a search for restriction enzymes (see Section 8.3.8 [Restriction enzyme search], page 316).

The figure above is a plot of the base composition of a sequence.

The figure above shows the way in which the results of weight matrix searches for motifs are plotted (see Section 8.3.10 [Motif Search], page 321).
The figure about shows the way in which the results of searches for splice junctions are plotted. The donor and acceptor predictions are separated and a different colour is used for each reading frame (see Section 8.3.11.7 [Splice Site Search], page 334).

The figure above shows a method for finding protein coding regions which does not distinguish reading frame or strand (see Section 8.3.11.6 [Uneven Positional base Frequencies], page 333).
The figure above shows how results from the tRNA gene search function are displayed in the Output window (see Section 8.3.11.8 [tRNA Search], page 335).

8.2.3.2 Introduction to the Spin Sequence Display

Spin also has a sequence display window in which the user can view the sequence in textual form. This window allows the user to scroll along the sequence. Users can view one or both strands, can switch on displays of the encoded amino acids in up to six reading frames, can switch on a display of the restriction enzyme sites, and can perform other simple subsequence or string searches to locate features in the sequence. In the figure shown below the user has switched on a three phase translation on the top strand, double stranded sequence, and a restriction enzyme search.
The sequence cursor can be under the control of the graphics cursor i.e. the cursor in the sequence viewer can be moved by the user dragging the cursor in the graphics window. Similarly the cursor in the graphics plots can be moved by the sequence viewer cursor.
8.2.3.3 Introduction to the Spin Sequence Comparison Plot

All of the spin comparison functions display their results as points or lines in a two-dimensional plot called a "Spin Sequence Comparison Plot" (see Section 8.6.3 [Spin Sequence Comparison Plot], page 362). Sets of matches from a single invocation of a comparison command are termed "a result". Each result is plotted using a single colour which can be configured via the results manager (see Section 8.7.1 [Result manager], page 365).

The diagram above shows the results of a "Find similar spans" search (olive) (see Section 8.4.1 [Finding Similar Spans], page 337), and a "Find matching words" (red) (see Section 8.4.2 [Finding Matching Words], page 340).

At the right hand side is a set of square boxes with the same colours as the dots drawn in the adjacent plot. These icon-like objects represent individual results and allow the user to operate on them. For example clicking with the right mouse button brings up the pop-up menu beneath the "matching words" result contains the results menu for this result (see Section 8.7.1 [Result manager], page 365). These icons can also be used to drag and drop the results to which they correspond. This is activated by pressing the middle mouse button, or Alt left mouse button, over the box and then moving the cursor over the Spin Sequence Comparison Plot to the new location or anywhere outside the Spin Sequence Comparison Plot (see Section 8.6.3.4 [Drag and drop], page 364).

Crosshairs can be turned on or off using the check button labelled "crosshairs". The x and y positions of the crosshairs are indicated in the two boxes to the right of the check box.
Each sequence displayed in a Spin Sequence Comparison Plot will have a cursor of a particular colour. In the picture above, the sequence on the horizontal axis has a vertical blue cursor whereas the sequence on the vertical axis has a horizontal olive green cursor. In general, the same sequence displayed in several Spin Sequence Comparison Plots will have a cursor of the same colour. The user can move a cursor by clicking and dragging with the middle mouse button, or with Alt left mouse button. This will move all other cursors displayed that relate to the sequence, whether they are in different Spin Sequence Comparison Plots or within the sequence display.

Plots can be enlarged either by resizing the window or zooming. Zooming is achieved by holding down the control key and right mouse button and dragging out a rectangle. This process can be repeated. The Back button will restore the plot to the previous magnification.

To illustrate further uses of the program we include some more screen dumps below.

The picture above shows the results after performing a "Find similar spans" comparison between the three reading frames of two DNA sequences, producing nine superimposed sets of results.
Local alignment searches join similar segments with lines. The above screen dump shows such an analysis in which genomic DNA containing 7 exons and is compared to its corresponding cDNA.
The above screen dump shows a global alignment of the same pair of sequences.

### 8.2.3.4 Introduction to the Spin Sequence Comparison Display

A sequence comparison display is associated with a single set of results and can be invoked by bringing up a pop up menu for the required result, either from the Results manager (see Section 8.7.1 [Result manager], page 365), the Results menu in the Spin Sequence Comparison Plot, or the coloured square icon on the right of the plot.

The horizontal sequence is drawn above the vertical sequence and the central panel indicates characters which are identical. The buttons (< >) and (<< >>) scroll the sequences. Pressing the Lock button forces the sequences to scroll together. Movement of the sequences is also controlled by the scrollbars or by moving the corresponding cursor in the Spin Sequence Comparison Plot. The black cursors in the sequence display correspond to the position of the cursor in the Spin Sequence Comparison Plot. The sequences can be made to 'jump' to the nearest match in those results by pressing the "Nearest match" or "Nearest dot" buttons.
8.2.4 Spin Menus

The main window for spin contains File, View, Options, Sequences, Statistics, Translation, Search, Comparison and Emboss menus.

8.2.4.1 Spin File Menu

The File menu includes sequence reading, saving and management options.

- Load sequences (see Section 8.8.2 [Reading in sequences], page 368)
- Save (see Section 8.8.3.10 [Save], page 371)
- Change directory
- Sequence manager (see Section 8.8.3 [Sequence manager], page 369)
- Exit

8.2.4.2 Spin View Menu

The View menu contains options to give access to the Results Manager, and to the Sequence Display.

- Results manager (see Section 8.7.1 [Result manager], page 365)
- Sequence display (see Section 8.6.2 [Sequence display], page 360)

8.2.4.3 Spin Options Menu

The Options menu contains options for configuring spin and its functions.

- Change protein score matrix (see Section 8.5.5 [Changing the score matrix], page 351)
- Set protein alignment symbols (see Section 8.5.6 [Set protein alignment symbols], page 353)
- Configure maximum number of matches (see Section 8.5.2 [Changing the maximum number of matches], page 351)
- Configure default number of matches (see Section 8.5.3 [Changing the default number of matches], page 351)
- Hide duplicate matches (see Section 8.5.4 [Hide duplicate matches], page 351)
- Set fonts
- Colours

8.2.4.4 Spin Sequences Menu

The Sequences menu contain options for manipulating the sequences currently loaded into spin. All these operations are also obtainable from a pop up menu in the sequence manager (see Section 8.8.3 [Sequence manager], page 369).

- Horizontal (see Section 8.8.3.1 [Change the active sequence], page 369)
- Vertical (see Section 8.8.3.1 [Change the active sequence], page 369)
- Set range (see Section 8.8.3.2 [Set the range], page 369)
- Copy (see Section 8.8.3.3 [Copy sequence], page 370)
- Complement sequence (see Section 8.8.3.5 [Complement sequence], page 370)
- Interconvert t and u (see Section 8.8.3.6 [Interconvert t and u], page 370)
- Translate sequence (see Section 8.8.3.7 [Translate sequence], page 370)
- Scramble sequence (see Section 8.8.3.8 [Scramble sequence], page 370)
• Sequence type (see Section 8.8.3.4 [Sequence type], page 370)
• Rotate sequence (see Section 8.8.3.9 [Rotate sequence], page 371)
• Save (see Section 8.8.3.10 [Save sequence], page 371)
• Delete (see Section 8.8.3.11 [Delete sequence], page 371)

8.2.4.5 Spin Statistics Menu

The Statistics menu contains the spin functions for analysing and plotting the composition of sequences.
• Count sequence composition (see Section 8.3.1 [Count Sequence Composition], page 308)
• Plot base composition (see Section 8.3.3 [Plot Base Composition], page 308)
• Count dinucleotide frequencies (see Section 8.3.2 [Dinucleotide Frequencies], page 308).

8.2.4.6 Spin Translation Menu

The "Translation" menu contains options to set the genetic code, translate to protein, find open reading frames and to calculate codon tables.
• Set genetic code (see Section 8.3.5 [Set Genetic Code], page 312)
• Translate (see Section 8.3.6 [Translation], page 313),
• Find open reading frames (see Section 8.3.7 [Find Open Reading Frames], page 314)
• Calculate and write codon table to disk (see Section 8.3.4 [Calculate codon usage], page 309)

8.2.4.7 Spin Search Menu

The "Search" menu contains a variety of different searching and analysis techniques.
• Protein genes: Codon pref (see Section 8.3.11.3 [Codon Usage Method], page 325)
• Protein genes: Author test (see Section 8.3.11.5 [Author Test], page 330)
• Protein genes: Base bias (see Section 8.3.11.6 [Uneven Positional base Frequencies], page 333)
• tRNA genes (see Section 8.3.11.8 [tRNA Search], page 335)
• Search for string (DNA) (see Section 8.3.9 [Subsequence search], page 320)
• Restriction enzyme map (see Section 8.3.8 [Restriction enzyme search], page 316)
• Plot start codons (see Section 8.3.11.1 [Start Codon Search], page 323)
• Plot stop codons (see Section 8.3.11.2 [Stop Codon Search], page 323)
• Search for splice junctions (see Section 8.3.11.7 [Splice Site Search], page 334)
• Search using weight matrix (see Section 8.3.10 [Motif Search], page 321)

8.2.4.8 Spin Comparison Menu

The Comparison menu contains the spin analytical functions for comparing and aligning the sequences.
• Find similar spans (see Section 8.4.1 [Finding Similar Spans], page 337)
• Find matching words (see Section 8.4.2 [Finding Matching Words], page 340)
• Find best diagonals (see Section 8.4.3 [Finding the Best Diagonals], page 342)
• Align sequences (see Section 8.4.4 [Aligning Sequences Globally], page 343)
• Local alignment (see Section 8.4.5 [Aligning Sequences Locally], page 347)
8.3 Spin’s Analytical Functions

Spin contains both simple and sophisticated analytical functions, mostly producing graphical results. The following sections describe the functions, approximately in order of increasing complexity.

8.3.1 Count Sequence Composition

When a sequence is read into the program its composition is displayed in the Output Window to provide a simple check that the data has been read correctly. The values can also be requested from the "Statistics" menu, when a dialogue will allow subsections of the sequence to be analysed. The results are displayed as shown below.

```
------------------------------------------------------------------------
Wed 12 Nov 17:10:25 1997: sequence composition
------------------------------------------------------------------------
A 1966 (24.17%) C 1996 (24.54%) G 2185 (26.86%) T 1987 (24.43%) - 0 (0.00%)
------------------------------------------------------------------------
```

Or for protein sequences:

```
------------------------------------------------------------------------
Mon 14 Oct 17:11:04 2002: sequence composition
------------------------------------------------------------------------
Sequence MYSA_DROME: 1 to 2411
Protein
AA A B C D E F G H I K L M N
N 201 0 30 150 281 74 127 45 126 233 243 43 121
% 8.3 0.0 1.2 6.2 11.7 3.1 5.3 1.9 5.2 9.7 10.1 1.8 5.0
M 14287 0 3094 17263 36281 10891 7246 6171 14258 29865 27498 5642 13807
AA P Q R S T V W Y Z X * -
N 55 167 141 96 93 108 14 63 0 0 0 0
% 2.3 6.9 5.8 4.0 3.9 4.5 0.6 2.6 0.0 0.0 0.0 0.0
M 5341 21398 22022 8360 9403 10706 2607 10280 0 0 0 0
M 5341 21398 22022 8360 9403 10706 2607 10280 0 0 0 0
```

8.3.2 Count Dinucleotide Frequencies

This routine simply counts dinucleotide frequencies for the selected region of the sequence. It also calculates an expected distribution based on the base composition. The output looks like:

```
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th></th>
<th>C</th>
<th></th>
<th>G</th>
<th></th>
<th>T</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Obs</td>
<td>7.91</td>
<td></td>
<td>5.84</td>
<td></td>
<td>5.64</td>
<td></td>
<td>5.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.91</td>
<td></td>
<td>5.93</td>
<td></td>
<td>5.14</td>
<td></td>
<td>6.02</td>
<td></td>
</tr>
<tr>
<td>Obs</td>
<td>6.11</td>
<td></td>
<td>6.49</td>
<td></td>
<td>7.56</td>
<td></td>
<td>6.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.24</td>
<td></td>
<td>5.91</td>
<td></td>
<td>6.18</td>
<td></td>
<td>5.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Obs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expected</td>
<td></td>
<td></td>
<td>Expected</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.93</td>
<td></td>
<td></td>
<td>5.05</td>
<td></td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.49</td>
<td></td>
<td>5.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.38</td>
<td></td>
<td>6.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.59</td>
<td></td>
<td>6.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.22</td>
<td></td>
<td>6.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.56</td>
<td></td>
<td>6.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.14</td>
<td></td>
<td>6.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.86</td>
<td></td>
<td>5.97</td>
</tr>
</tbody>
</table>
```

8.3.3 Plot base composition

The composition of the sequence can be displayed graphically. A window is slid along the sequence one base at a time, and at each point the number of occurrences of each selected base
type is counted and plotted. Users can select which base types are counted, the size of the window used and the region of the sequence to analyse.

For example the A and T composition can be plotted by selecting base types A and T in the dialogue. As usual the values can also be listed in the text Output Window. Note that this "result", i.e. the base composition counts for every position along the sequence, will persist until the user explicitly removes it. To save memory delete results as soon as they are no longer required.

8.3.4 Calculate codon usage

Codon usage tables can be calculated and written to the Output Window, and written to disk. If required the values found can be added to the counts in a pre-existing codon table, or when written out to disk they can be concatenated with an existing codon table file. In the first case the existing file will be read and added to the values calculated for the region defined by the user. In the latter, the values calculated for the region defined by the user will be written immediately after those from the existing table, hence producing a pair of tables joined end to end. An example of this is shown at the end of this section, and a more typical result is shown below.
Referring to the figure of the dialogue below, the user can select the range and strand over which to count. Note that irrespective of the strand being counted, the positions in the sequence are always defined from the current 5' end. i.e. to count over bases 1 to 100 the user should set the Start position to 1 and the End position to 100. The values in the table can be expressed as observed counts or as percentages of usage for the cognate amino acid.

The table can be output as a single table (as shown above), or as a double table (shown below). The user can request that the counts from an existing table be read and added to the counts which
are about to be calculated, in which case the "File name" text window will be activated. If the user selects to output a double table, this dialogue will also be activated. To save the output in the selected form to a file, the user should fill in the "Save table to" text window.

Two of the protein coding search functions (see Section 8.3.11.3 [Codon Usage Method], page 325 and (see Section 8.3.11.5 [Author Test], page 330) work best using a double codon table. The top table should contain the codon usage for the coding regions and the bottom table the usage for non-coding regions. A typical double codon table of this sort is shown below.

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>F ttt</td>
<td>4 S tct</td>
<td>30 Y tat</td>
<td>5 C tgt</td>
</tr>
<tr>
<td>F ttc</td>
<td>35 S tcc</td>
<td>21 Y tac</td>
<td>15 C tgc</td>
</tr>
<tr>
<td>L tta</td>
<td>4 S tca</td>
<td>7 * taa</td>
<td>0 * tga</td>
</tr>
<tr>
<td>L ttg</td>
<td>24 S tcg</td>
<td>9 * tag</td>
<td>0 W tgg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>L ctt</td>
<td>71 P cct</td>
<td>1 H cat</td>
<td>17 R cgt</td>
</tr>
<tr>
<td>L ctc</td>
<td>39 P ccc</td>
<td>2 H cac</td>
<td>15 R cgc</td>
</tr>
<tr>
<td>L cta</td>
<td>0 P cca</td>
<td>14 Q caa</td>
<td>87 R cga</td>
</tr>
<tr>
<td>L ctg</td>
<td>4 P cgg</td>
<td>0 Q cag</td>
<td>18 R cgg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I att</td>
<td>33 T act</td>
<td>30 N aat</td>
<td>12 S agt</td>
</tr>
<tr>
<td>I atc</td>
<td>53 T acc</td>
<td>20 N aac</td>
<td>59 S agc</td>
</tr>
<tr>
<td>I ata</td>
<td>1 T aca</td>
<td>3 K aaa</td>
<td>23 R aga</td>
</tr>
<tr>
<td>M atg</td>
<td>32 T acg</td>
<td>0 K aag</td>
<td>117 R agg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>V gtt</td>
<td>30 A gct</td>
<td>71 D gat</td>
<td>58 G ggt</td>
</tr>
<tr>
<td>V gtc</td>
<td>22 A gcc</td>
<td>54 D gac</td>
<td>32 G ggc</td>
</tr>
<tr>
<td>V gta</td>
<td>7 A gca</td>
<td>6 E gaa</td>
<td>76 G gga</td>
</tr>
<tr>
<td>V gtg</td>
<td>5 A gcg</td>
<td>0 E gag</td>
<td>101 G ggg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>F ttt</td>
<td>10 S tct</td>
<td>8 Y tat</td>
<td>7 C tgt</td>
</tr>
<tr>
<td>F ttc</td>
<td>12 S tcc</td>
<td>2 Y tac</td>
<td>4 C tgc</td>
</tr>
<tr>
<td>L tta</td>
<td>6 S tca</td>
<td>4 * taa</td>
<td>7 * tga</td>
</tr>
<tr>
<td>L ttg</td>
<td>11 S tcg</td>
<td>3 * tag</td>
<td>4 W tgg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>L ctt</td>
<td>5 P cct</td>
<td>3 H cat</td>
<td>4 R cgt</td>
</tr>
<tr>
<td>L ctc</td>
<td>6 P ccc</td>
<td>1 H cac</td>
<td>4 R cgc</td>
</tr>
<tr>
<td>L cta</td>
<td>3 P cca</td>
<td>1 Q caa</td>
<td>9 R cga</td>
</tr>
<tr>
<td>L ctg</td>
<td>6 P cgg</td>
<td>3 Q cag</td>
<td>5 R cgg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I att</td>
<td>13 T act</td>
<td>6 N aat</td>
<td>7 S agt</td>
</tr>
<tr>
<td>I atc</td>
<td>7 T acc</td>
<td>0 N aac</td>
<td>3 S agc</td>
</tr>
<tr>
<td>I ata</td>
<td>12 T aca</td>
<td>5 K aaa</td>
<td>9 R aga</td>
</tr>
<tr>
<td>M atg</td>
<td>7 T acg</td>
<td>3 K aag</td>
<td>4 R agg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Codon</th>
<th>Usage</th>
<th>Codon</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>V gtt</td>
<td>6 A gct</td>
<td>2 D gat</td>
<td>8 G ggt</td>
</tr>
<tr>
<td>V gtc</td>
<td>2 A gcc</td>
<td>1 D gac</td>
<td>3 G ggc</td>
</tr>
<tr>
<td>V gta</td>
<td>5 A gca</td>
<td>1 E gaa</td>
<td>9 G gga</td>
</tr>
<tr>
<td>V gtg</td>
<td>4 A gcg</td>
<td>0 E gag</td>
<td>3 G ggg</td>
</tr>
</tbody>
</table>
To calculate such a table using SPIN the following steps are required. First calculate the codon usage for a typical coding segment and save the resulting table in table A. Then use the option again, but this time select to "Output double table", and type the name of table A into the "File name" text box. Next define the start and end points of a non-coding region, and save the results to double table B. The file containing double table B is now suitable for use by the protein gene searching functions.

8.3.5 Set genetic code

This function allows the user to change the genetic code used in all the options. The codes are defined as a set of codon tables stored in the directory tables/gcodes distributed with the package. The current list of codes and their codon table file names is shown at the end of this section.

The user interface consists of the dialogue shown below. The user selects the required code by clicking on it, and then clicking "OK" or "OK permanent". The former choice selects the code for immediate use, and the latter also selects it for future uses of the program.

When the dialogue is left the codon table selected will be displayed, as below, in the Output Window.

```
--- Standard ---
F ttt S tct Y tat C tgt
F ttc S tcc Y tac C tgc
L tta S tca * taa W tga
L ttg S tcg * tag W tgg
---
L ctt P cct H cat R cgt
L ctc P ccc H cac R cgc
L cta P cca Q caa R cga
L ctg P ccg Q cag R cgg
---
I att T act N aat S agt
I atc T acc N aac S agc
M ata T aca K aaa G aga
M atg T acg K aag G agg
---
V gtt A gct D gat G ggt
V gtc A gcc D gac G gcc
```
Chapter 8: Analysing and comparing sequences using spin

The following table shows the list of available genetic codes and the files in which they are stored for use by the package. They were created from genetic code files obtained from the NCBI.

<table>
<thead>
<tr>
<th>code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>code_1</td>
<td>Standard</td>
</tr>
<tr>
<td>code_2</td>
<td>Vertebrate Mitochondrial</td>
</tr>
<tr>
<td>code_3</td>
<td>Yeast Mitochondrial</td>
</tr>
<tr>
<td>code_4</td>
<td>Coelenterate Mitochondrial</td>
</tr>
<tr>
<td>code_4</td>
<td>Mold Mitochondrial</td>
</tr>
<tr>
<td>code_4</td>
<td>Protozoan Mitochondrial</td>
</tr>
<tr>
<td>code_4</td>
<td>Coelenterate Mitochondrial</td>
</tr>
<tr>
<td>code_5</td>
<td>Invertebrate Mitochondrial</td>
</tr>
<tr>
<td>code_6</td>
<td>Ciliate Nuclear</td>
</tr>
<tr>
<td>code_6</td>
<td>Dasycladacean Nuclear</td>
</tr>
<tr>
<td>code_6</td>
<td>Hexamita Nuclear</td>
</tr>
<tr>
<td>code_9</td>
<td>Echinoderm Mitochondrial</td>
</tr>
<tr>
<td>code_10</td>
<td>Euplotid Nuclear</td>
</tr>
<tr>
<td>code_11</td>
<td>Bacterial</td>
</tr>
<tr>
<td>code_12</td>
<td>Alternative Yeast Nuclear</td>
</tr>
<tr>
<td>code_13</td>
<td>Ascidian Mitochondrial</td>
</tr>
<tr>
<td>code_14</td>
<td>Flatworm Mitochondrial</td>
</tr>
<tr>
<td>code_15</td>
<td>Blepharisma Macronuclear</td>
</tr>
</tbody>
</table>

8.3.6 Translation - general

Translations of the sequence can be obtained in three ways. The first is an option available within the "Sequence manager" or the "Sequences" menu (see Section 8.8.3 [Sequence manager], page 369).

The second is an option in the Sequence display (see Section 8.6.2 [Spin Sequence Display], page 360) which enables the translation to be shown with the scrolling sequence.

The third, which has two methods of defining the segments to translate, is described here. The translations are written to the Output window, from where they can be saved to disk. A segment of a typical display is shown below.
Users select either to use a feature table to define the segments to translate or can simply enter a start and end position. In the latter case a six phase translation over that one segment is written out. If a feature table is used (and this assumes the sequence file was an EMBL entry complete with features), the CDS records from the table will be listed in the dialogue window and the user can select which ones should be used (see Section 8.8.1 [Use of feature tables in spin], page 367). The translations produced will be written in FASTA format ready to be saved to disk (which the next release of spin will do automatically!).

The user can also choose the line length, and whether one or three letter amino acid symbols are produced.

8.3.7 Find open reading frames

This function will find open reading frames greater than an specified length. The results can be output in two ways, either in feature table format
Chapter 8: Analysing and comparing sequences using spin

FT CDS 120..233
FT CDS 161..256
FT CDS 301..396
FT CDS 333..497
FT CDS 512..736
FT CDS 525..965
FT CDS 740..952
FT CDS 754..876
FT CDS 956..1789

or as a fasta file.

>120 120..233
VISENISLLKIGAKNHWLLLQQLKMMSGFCCNVNIY*
>161 161..256
EPSLAVKTIVKNGWFLCTCKCHLLNRYLFLD*
>301 301..396
ICCARTCAICDLKHALSPVFTRYLQFMMEQG*
>333 333..497
SEARFITSVYALFTVFDRTGLAESQLYALEKYNISPFGYLLFEITGAHRII*
>512 512..736
CLTLSSLKESFIRHAAYLEGSEKRDVCVARESKCESARSVTGGDSWIAVQPQRPLL
GRCNKRGPGSLA*
>525 525..965
ALIKVLLYDTRHTSKAGVKNMSVLSVRSNVARALLVQLQLVVIASGLFLSXLDFWGV
SISGGAVFVLFNLPMIFAWRHIQHTPAKRGAWTFAFGEAFKVLAMLVLLVVALAVKL
VFPLIVTWWLVLQLVAPEAVNKG*
>740 740..952
RFYDIICLAESPAGYTSERPGGLDILRIWRSFQSSGDGVGTGGVGGFNGILAADDYVG
AGGSDTGTGCH*
>754 754..876
YLPGVTRRJHQRKAGWPGHSKLAKJSKFWRCWICYWWRWF*
>956 956..1789
QQRVKIGMASENMTQDYICHGLHLNLQDDLRLRTSFLVDPQNNPATFWTINIDSMFFSVVL
GLLVFLVRSVAKATSGVPGQFQTISLIVGIFVNSVYKMDHYKSKLIAPALTIFVWVF
LMNMDLPLLIDLLPYIAENVGLPLARVVP6ADVNVLSMALGVFLILFYIKMKGIGG
FTKELTLQPFHNWAFIPVNLILEGVSLSKPSLGLRFLFGNYAGELIFILAGGPLLWWS
QWILVNPWAIYHFLITTLQAFIFMVLTVYLSMASEEH*

The user can select that start and end points over which to do the search, which strand to search (either the forward, reverse or both) and the minimum length of the open reading frame in codons. If the output is being written in fasta format, the name of file is also required.
8.3.8 Restriction enzyme search

The restriction enzyme map function finds and displays restriction sites found within a specified region of a sequence. Users can select the enzyme types to search for.

This figure shows a typical view of the Restriction Enzyme Map function in which the results for most enzyme types are shown as black vertical lines opposite the enzyme names, but in which some of the enzymes sites have been configured by the user to be drawn in different colours. If no result is found for any particular enzyme eg here AccIII, the line will still be drawn so that zero cutters can be identified. The results can be scrolled vertically (and horizontally if the plot is zoomed in). A ruler is shown along the base and the current cursor (the vertical black line) position is shown.
in the left hand box near the top right of the display. If the user clicks, in turn, on two restriction sites their separation in base pairs will appear in the top right hand box. Information about the last site touched is shown in the Information line at the bottom of the display.

8.3.8.1 Selecting Enzymes

Files of restriction enzyme names and their cut sites are stored in disk files. For the format of these files see Section 10.4 [Restriction enzyme files], page 414. Standard four-cutter, six-cutter and all-enzymes files are available and the users can use their own "personal" files. To create your own file of enzymes you may need to extract the information from the currently defined files. These are pointed to by the RENZYM.4, RENZYM.6 and RENZYM.ALL environment variables.

When the file is read the list of enzymes is displayed in a scrolling window. To select enzymes press and drag the left mouse button within the list. Dragging the mouse off the bottom of the list will scroll to allow selection of a range larger than the displayed section of the list. When the left button is pressed any existing selection is cleared. To select several disjoint entries in the list press control and the left mouse button. Once the enzymes have been chosen, pressing OK will create the plot.

8.3.8.2 Examining the Plot

Positioning the cursor over a match will cause its name and cut position to appear in the information line. If the right mouse button is pressed over a match, a popup menu containing Information and Configure will appear. The Information function in this menu will display the data for this cut site and enzyme in the output window.

It is possible to find the distance between any two cut sites. Pressing the left mouse button on a match will display "Select another cut" at the bottom of the window. Then, pressing the left button on another match will display the distance, in bases, between the two sites. This is shown in a box located at the top right corner of the window.
8.3.8.3 Reconfiguring the Plot

The plot displays the results for each restriction enzyme on a separate line. Enzymes with no sites are also shown. The order of these lines may be changed by pressing and dragging the middle mouse button, or Alt left mouse button, on one of the displayed names at the left side of the screen. For example the figure below shows the results seen above but after the coloured (i.e. non-black) rows of sites have been dragged and dropped to be vertically adjacent.

The results are plotted as black lines but users can select colours for each enzyme type by pressing the right button on any of its matches. A menu containing Information and Configure will pop up. Configure will display a colour selection dialogue. Adjusting the colour here will adjust the colour for all matches found with this restriction enzyme.

8.3.8.4 Printing the sites

From the Result manager (see Section 8.7.1 [Result manager], page 365), menu a pop-up menu for restriction sites results can be used to write the results in two forms to the the Output window - from here the results can be saved to a file. The two choices of format are "Output enzyme by enzyme" and "Output ordered on position", brief examples of which are shown below. The output also appears in an "Information" window. Note that these listings are also available from gap4.
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The restriction enzyme results output ordered "enzyme by enzyme". The enzymes sites are numbered and named and the actual cut site from the sequence is written, followed by the position of the cut, the fragment size, and finally a sorted list of fragment sizes. A list of zero cutters is written underneath.

### Matches found = 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
<th>Fragment lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApaLI</td>
<td>G’TGCAC</td>
<td>3506</td>
<td>3505 3505 4629 4629</td>
</tr>
</tbody>
</table>

### Matches found = 8

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
<th>Fragment lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoI</td>
<td>A’AATTC</td>
<td>1939</td>
<td>1938 1938 184</td>
</tr>
<tr>
<td>ApoI</td>
<td>G’AATTAT</td>
<td>2632</td>
<td>693 339</td>
</tr>
<tr>
<td>ApoI</td>
<td>A’AATTAT</td>
<td>2996</td>
<td>364 364</td>
</tr>
<tr>
<td>ApoI</td>
<td>G’AATTTC</td>
<td>3180</td>
<td>184 419</td>
</tr>
<tr>
<td>ApoI</td>
<td>A’AATTT</td>
<td>5283</td>
<td>2103 639</td>
</tr>
<tr>
<td>ApoI</td>
<td>G’AATTC</td>
<td>5702</td>
<td>419 693</td>
</tr>
<tr>
<td>ApoI</td>
<td>A’AATTC</td>
<td>6341</td>
<td>639 1455</td>
</tr>
<tr>
<td>ApoI</td>
<td>A’AATTC</td>
<td>7796</td>
<td>1455 1938 339 2103</td>
</tr>
</tbody>
</table>

### Matches found = 2

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
<th>Fragment lengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>AseI</td>
<td>AT’TAAT</td>
<td>1790</td>
<td>1789 435</td>
</tr>
<tr>
<td>AseI</td>
<td>AT’TAAT</td>
<td>2225</td>
<td>435 1789</td>
</tr>
</tbody>
</table>

### Zero cutters:

- Acc65I
- AccIII
- AcI
- ApaI
- AhdI
- Asp700I
- Asp718I
- AspEL
- AsuNHII
- AvrII

The restriction enzyme results output ordered on position. The enzymes sites are numbered and named and the actual cut site from the sequence is written, followed by the position of the cut, the fragment size, and finally a sorted list of cut sizes.
8.3.9 Subsequence search

Two subsequence or string searches are available. One, selected from the "Search" menu on the Output Window, produces both graphical and textual output, and the other, selected from the "Search" button in the Sequence display, moves the cursor to the position of the next match. Here we document the use of the first search, and the other is described in Section 8.6.2.1 [Sequence display string search], page 361.

As shown in the dialogue the user selects the range and strand over which the search should be performed, the search algorithm, the minimum percentage match, and the subsequence/string for which to search. The search algorithm allows either NC-IUB codes Cornish-Bowden, A. (1985) Nucl. Acids Res. 13, 3021-3030 or a literal search. The literal search will search for exact matches eg inputting a search string of "n" will search for the letter "n". The NC-IUB codes option can use any of the NC-IUB symbols shown in the figure below and the search is not case sensitive.

<table>
<thead>
<tr>
<th>NC-IUB SYMBOLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A,C,G,T</td>
</tr>
<tr>
<td>R (A,G)</td>
</tr>
<tr>
<td>Y (T,C)</td>
</tr>
<tr>
<td>W (A,T)</td>
</tr>
<tr>
<td>S (C,G)</td>
</tr>
<tr>
<td>M (A,C)</td>
</tr>
<tr>
<td>K (G,T)</td>
</tr>
<tr>
<td>H (A,T,C)</td>
</tr>
<tr>
<td>B (G,C,T)</td>
</tr>
<tr>
<td>V (G,A,C)</td>
</tr>
<tr>
<td>D (G,A,T)</td>
</tr>
<tr>
<td>N (G,A,C,T)</td>
</tr>
</tbody>
</table>
The matches are plotted as vertical lines at the match positions with the heights of the lines in proportion to their score. The matches are also written in the Output Window as shown below.

```
-----------------------------
Tue 19 Oct 11:52:50 1999: string search

Position 7837 score 9 percent match 90.000000
Percentage mismatch 10.0
1
string atrytayrat
::..::..:
atpase.seq atgctatgag
7837
```

### 8.3.10 Motif search

This option is used to search for motifs such as binding sites. The motifs are defined using weight matrices which are stored as files that need to be created beforehand. These matrices are usually calculated from alignments of trusted examples of the motif. The `make_weights` program can be used to create weight matrices from sets of aligned sequences (see Section 11.11 [Make_weights], page 432) We also plan to build up a library of matrix files which we will place in our ftp site.

An example weight matrix file is shown below. It consists of a title record; a record defining the motif length, an offset and the score range; 2 records which need to be present but which are ignored; 4 records defining the base frequencies calculated from the trusted examples.

An example weight matrix file is shown below. The first line gives the title ("Mount acceptors" in this example). The next line gives the motif length (18), the "mark position" (15), and the minimum and maximum scores (0.0 and 10.0). The "mark position" is an offset which is added to
the position of any matches reported by the search routine in spin. The next two lines are ignored by the programs. The first of them gives the matrix column positions, and the next gives the total counts in each column. The final lines (4 for DNA weight matrices) give the counts for each character type at each position in the motif. These counts are converted into weights that are used during the searches. Any position in a sequence which scores at least as high as the minimum score is reported as a match, and if the results are plotted they are scaled to fit the range defined by the minimum and maximum scores.

Mount acceptors

```
18 15 0.0 10.0
P -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3
N 113 113 113 113 113 113 113 113 113 113 113 113 113 113 113 113 113 113
T 58 50 57 59 67 56 58 49 47 66 64 31 34 0 0 11 41 31
C 21 28 34 25 29 33 35 32 42 40 33 25 74 0 0 23 28 41
A 17 11 11 18 7 17 12 23 15 3 10 29 5 113 0 24 21 21
G 17 24 11 11 10 7 8 9 9 4 6 28 0 0 113 55 23 20
```

Search results are plotted as log-odds and appear as shown below.

![Spin Sequence Plot](image)

The dialogue for the option is shown below.

![Search using weight matrix](image)

8.3.11 Gene finding

Chapter 8: Analysing and comparing sequences using spin

characteristic of protein coding regions, or RNA genes. Protein coding sequences produce particular amino acid sequences, often using preferred codons, and this leaves patterns in the sequence that can be used to distinguish them from non-protein-coding DNA. tRNA genes must produce stable cloverleaf structures and "standard" tRNAs must contain particular (conserved) bases at locations within the cloverleaf. These features can be used to locate tRNA genes, and probably other RNA genes could be sought in a similar way.

The methods described in the following sections are either "content" or "signal" searches and spin’s graphical presentation of results can be used to see if together they produce a consistent gene prediction.

8.3.11.1 Start codon search

This function plots the positions of all start codons using the default genetic code in all 3 reading frames. The positions can be listed to the Output Window. The start codons are plotted beneath the centre of the plot (in contrast to the stop codons which are plotted above the centre). If any of the gene search methods are currently being displayed, the start codons will automatically be plotted on top of the corresponding frame, otherwise they will be plotted in three separate plots. These plots can be dragged and dropped in the usual manner.

8.3.11.2 Stop codon search

Stop codons can be searched for on either (or both) strands of the sequence. The stop codons are displayed graphically, with a different colour used for each reading frame, and their positions can also be listed in the Output Window. If any of the gene search methods are currently being
displayed, the stop codons will automatically be plotted on top of the corresponding frame. As usual the graphical plots can be dragged and dropped to new locations.

In the example below we show the stop codon search results after they have been drawn on a plot containing results from a protein gene search method. Here we can see that the open reading frames coincide with the highest scoring segments from the protein gene prediction plots.
8.3.11.3 Codon usage method


The current method contains a number of improvements on the original one. We are trying to decide if each segment of the sequence is coding or non-coding. Each possibility is represented by a model consisting of a table of expected codon usage. The calculation finds the odds that each segment of the sequence fits either the coding or non-coding model, and the results are plotted as log odds.

The results for each reading frame are plotted in the graphics window with frame 1 in the top panel, frame 2 the middle and frame 3 in the bottom panel. Frame 1 is the frame of the first base in the active region. At each position along the sequence the program also plots a single dot for the reading frame with the highest score. These dots appear at the midpoints of the three panels and will form a continuous line if one reading frame is consistently the highest scoring.
The figure shown below shows a SPIN Sequence Plot containing the results of the codon usage method on a sequence from C. elegans. This sequence has strong codon usage bias and so produces clear results for the method. Here the results are for the standard codon usage employing only the codon usage table shown below and a window length of 67 codons (i.e. no table of codon usage for non-coding sequence was supplied, and no normalisation was performed on the coding table). Compare the results to the other screen dump shown later, which also uses a window of 67 codons.

Also visible in the figure are the cross hairs. Their x position is shown in sequence base numbers in the left hand box above the plot, and the y coordinate, expressed using the score values of the gene search, is shown in the right hand box. Each line in the window has its own colour and can be dragged and dropped to new locations to reorganise the plot. The cursor in the plot can be used to control the position of the cursor in the sequence display.
As can be seen in the dialogue below the user can define the size of the scan window in codons (note that the window length must be odd), the name of the file containing the codon usage table, and the region of the sequence to be analysed. The longer the window the smoother the plots but the more difficult it is to find the ends of the coding segments. The stronger the codon preference in the codon table the higher the discrimination between coding and non-coding (assuming the sequence being analysed has the same preferences as those of the table). Note also that the amino acid composition represented in the table will also influence the results.

The user should supply the name of a file containing two concatenated codon usage tables - the first being from coding sequence and the second from noncoding sequence. This double codon table can be calculated by spin using the Codon Usage function (see Section 8.3.4 [Calculate codon usage], page 309).

If the user gives the name of a file that contains only a single codon table the algorithm will assume that it is from coding sequence, and will generate a noncoding table that consists of the frequencies that would be expected if the sequence being analysed was random but had the same base composition as the codon table.

If no table is specified the program will generate a codon usage table corresponding to an average amino acid composition, and then derive a non-coding table from its base composition. This is equivalent to the "positional base preferences" method, and hence replaces it. More information about this method is given further down (see Section 8.3.11.4 [Positional base Preferences], page 329).

In addition the user can select to set the amino acid composition of the coding table to have an average amino acid composition, and/or to have no codon preference (i.e. for each amino acid the codon counts are equal, i.e. (TTT = TTC); (TTA = TTG = CTT = CTC = CTA = CTG); ...; (GGT = GGC = GGA = GGG)). In the latter case the search uses amino acid composition only.

The average amino composition used to normalise the values in the codon table is that described by McCaldon and Argos McCaldon and Argos (1988), Proteins 4, 99-122.

The dialogue also allows the user to control whether or not the positions of stop codons are included in the display.

Codon tables are scaled so that the sum of their values is 1000 and then any zero entries are set to 1/1000. Stop codons in the coding table are made to be neutral by setting them to the mean value for the table.

Example of the tables employed/calculated for an input coding table, no non-coding table, and normalise to average amino acid composition.
Table read in:

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>S</th>
<th>Y</th>
<th>C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ttt</td>
<td>03</td>
<td>03</td>
<td>07</td>
<td>05</td>
<td>09</td>
</tr>
<tr>
<td>ttc</td>
<td>035</td>
<td>035</td>
<td>015</td>
<td>015</td>
<td>05</td>
</tr>
<tr>
<td>tta</td>
<td>02</td>
<td>02</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>ttg</td>
<td>023</td>
<td>023</td>
<td>015</td>
<td>015</td>
<td>05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>P</th>
<th>H</th>
<th>R</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>tta</td>
<td>012</td>
<td>012</td>
<td>011</td>
<td>011</td>
<td>00</td>
</tr>
<tr>
<td>ttg</td>
<td>018</td>
<td>017</td>
<td>016</td>
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</tr>
<tr>
<td>tta</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>00</td>
</tr>
<tr>
<td>ttg</td>
<td>018</td>
<td>017</td>
<td>016</td>
<td>016</td>
<td>05</td>
</tr>
</tbody>
</table>

Program generates non-coding table from the base composition of the coding table:

<table>
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<tr>
<th></th>
<th>F</th>
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<th>Y</th>
<th>C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ttt</td>
<td>013</td>
<td>013</td>
<td>012</td>
<td>012</td>
<td>018</td>
</tr>
<tr>
<td>ttc</td>
<td>013</td>
<td>013</td>
<td>012</td>
<td>012</td>
<td>017</td>
</tr>
<tr>
<td>tta</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>00</td>
</tr>
<tr>
<td>ttg</td>
<td>018</td>
<td>017</td>
<td>016</td>
<td>016</td>
<td>05</td>
</tr>
</tbody>
</table>

<table>
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<th>P</th>
<th>H</th>
<th>R</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>tta</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>00</td>
</tr>
<tr>
<td>ttg</td>
<td>018</td>
<td>017</td>
<td>016</td>
<td>016</td>
<td>05</td>
</tr>
<tr>
<td>tta</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>012</td>
<td>00</td>
</tr>
<tr>
<td>ttg</td>
<td>018</td>
<td>017</td>
<td>016</td>
<td>016</td>
<td>05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>T</th>
<th>N</th>
<th>S</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>att</td>
<td>012</td>
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<td>011</td>
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</tr>
<tr>
<td>atc</td>
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<td>012</td>
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<td>012</td>
<td>00</td>
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<tr>
<td>ata</td>
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<td>011</td>
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</tr>
<tr>
<td>atg</td>
<td>016</td>
<td>016</td>
<td>016</td>
<td>016</td>
<td>00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>M</th>
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<th>R</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>att</td>
<td>016</td>
<td>016</td>
<td>016</td>
<td>016</td>
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</tr>
<tr>
<td>atc</td>
<td>016</td>
<td>016</td>
<td>016</td>
<td>016</td>
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</tr>
<tr>
<td>ata</td>
<td>016</td>
<td>016</td>
<td>016</td>
<td>016</td>
<td>00</td>
</tr>
<tr>
<td>atg</td>
<td>016</td>
<td>016</td>
<td>016</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>V</th>
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<th>D</th>
<th>G</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>018</td>
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<td>017</td>
<td>00</td>
</tr>
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<td>atg</td>
<td>024</td>
<td>024</td>
<td>023</td>
<td>023</td>
<td>00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>A</th>
<th>D</th>
<th>G</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>018</td>
<td>017</td>
<td>017</td>
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<td>018</td>
<td>018</td>
<td>017</td>
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</tr>
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<td>018</td>
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<td>024</td>
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<td>023</td>
<td>00</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th></th>
<th>V</th>
<th>A</th>
<th>D</th>
<th>G</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>018</td>
<td>018</td>
<td>017</td>
<td>017</td>
<td>00</td>
</tr>
<tr>
<td>atc</td>
<td>018</td>
<td>018</td>
<td>017</td>
<td>017</td>
<td>00</td>
</tr>
<tr>
<td>ata</td>
<td>018</td>
<td>018</td>
<td>017</td>
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<td>00</td>
</tr>
<tr>
<td>atg</td>
<td>024</td>
<td>024</td>
<td>023</td>
<td>023</td>
<td>00</td>
</tr>
</tbody>
</table>
Program generates coding table with average amino acid composition and stops set to mean:

<p>| | | | | |</p>
<table>
<thead>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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</tr>
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<td>16</td>
<td>C</td>
<td>tgt</td>
</tr>
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<td>15</td>
<td>C</td>
<td>tgg</td>
</tr>
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</tr>
<tr>
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<td>gtg</td>
<td>4</td>
<td>A</td>
<td>gcc</td>
</tr>
</tbody>
</table>

8.3.11.4 Positional base preferences

This method for finding protein coding regions is a variant of the codon usage method. Here, instead of measuring the closeness to an table of codon frequencies whose main discriminating power is due codon preferences, we look for similarity to the codon usage that would be expected from a protein sequence of average amino acid composition, but with no codon preference. The method is surprisingly effective: When tested against all the E. coli sequences in the EMBL sequence library it correctly identified the coding frame for 91% of window positions. (The E. coli sequences were chosen only for technical reasons: we have no reason to think the method would work less well on other organisms with roughly even base composition.) Staden R. (1990) Finding protein coding regions in genomic sequences. In Doolittle, R.R (ed), Methods in Enzymology, 183, Academic Press, San Diego, CA, 163-180.

The average amino composition used to derive the values in the codon table is that described by McCaldon and Argos McCaldon and Argos (1988), Proteins 4, 99-122.
Above is the result of applying this method to the C. elegans sequence analysed above with a codon preference table. Note that, as would be expected, the main difference is the that the range of observed scores is very much reduced.

8.3.11.5 Author test

This is an unpublished method for distinguishing between coding and noncoding segments of a DNA sequence. It is basically an extension of the Codon Usage method in which we compare the sequence to two tables of codon usage to see which of the two it is most like. One table should contain typical codon usage from a coding sequence and the other typical codon usage from a noncoding region. It is based on methods used to decide authorship of text - is the usage of words (codons) more like that of author A (coding) or that of author B (noncoding)?
The results for each reading frame are plotted in the graphics window with frame 1 in the top panel, frame 2 the middle and frame 3 in the bottom panel. Frame 1 is the frame of the first base in the active region. At each position along the sequence the program also plots a single dot for the reading frame with the highest score. These dots appear at the midpoints of the three panels and will form a continuous line if one reading frame is consistently the highest scoring. The figure shows a SPIN Sequence Plot containing the results of the author test method on a sequence from E. coli. Also visible are the cross hairs. Their x position is shown in sequence base numbers in the left hand box above the plot, and the y coordinate, expressed using the score values of the gene search, is shown in the right hand box. Each line in the window has its own colour and can be dragged and dropped to new locations to reorganise the plot. The cursor in the plot can be used to control the position of the cursor in the sequence display.
A typical pair of concatenated codon tables for use by the Author test

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F</td>
<td>G</td>
<td>H</td>
<td>I</td>
<td>J</td>
</tr>
<tr>
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<td>K</td>
<td>L</td>
<td>M</td>
<td>N</td>
<td>O</td>
</tr>
<tr>
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<td>P</td>
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<td>R</td>
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</tr>
</tbody>
</table>

The mathematical treatment of the data is very different from that of the codon usage method.

Given the two tables of codon usage the algorithm works out the optimal weighting to give each codon to obtain the best discrimination between coding and noncoding sequence. The user sets the expected error rate as a percentage and the algorithm will choose the corresponding window length to use for the analysis.
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The user should supply the name of a file containing two concatenated codon usage tables - the first being from coding sequence and the second from noncoding sequence. This double codon table can be calculated by spin using the Codon Usage function (see Section 8.3.4 [Calculate codon usage], page 309).

If the user gives the name of a file that contains only a single codon table the algorithm will assume that it is from coding sequence, and will generate a noncoding table that consists of the frequencies that would be expected if the sequence being analysed was random. The region to be analysed can also be set.

8.3.11.6 Uneven positional base preferences

This method is used to find regions of a sequence that code for a protein. It is based on the method of Fickett Fickett, J. (1982) Nucl. Acid Res. 10, and unlike the other methods currently in the package does not attempt to say either which strand or frame is likely to be coding, only which regions of the sequence.

The method looks for sections of the sequence in which the frequencies at which each of the four bases occupy the three positions in codons is nonrandom. The level of nonrandomness is plotted on a scale that shows the probability that the sequence is coding. At each position along a sequence the calculation gives the same value for all six possible reading frames, so only one value is plotted. Seventy six percent of coding regions score above 0.78 and 76% of noncoding regions below 0.78. No known window in a coding region has a value below 0.4, but 14% of windows in noncoding sequences score below it. No known window in a noncoding region reaches a score of 1.34, but this score is reached by 16% of known coding regions. These statements are now very much out of date.

The method was first described in Staden R. (1984) Nucl. Acid Res. 12, 551-567. It looks through the sequence in one fixed phase and counts the number of times each base appears in each of the three codon positions: for each window position it counts A1,A2,A3 and C1,C2,C3 and G1,G2,G3 and T1,T2,T3 and calculates AMEAN=(A1+A2+A3)/3, and similarly CMEAN, GMEAN and TMEAN; it then calculates ADIF=abs(A1-AMEAN)+abs(A2-AMEAN)+abs(A3-AMEAN) and similarly CDIF, GDIF and TDIF to measure the differences between an even base usage for all positions in the codons and the observed usage. The routine then calculates and plots the sum ADIF+CDIF+GDIF+TDIF.

In the figure shown below it will be seen that much of the sequence being analysed appears to be coding, and this is indeed the case. Many of the troughs between peaks correspond to the ends of genes in this E. coli sequence (which was not a good choice to illustrate the method!). The horizontal line is at 76%. 76% of coding regions achieve values above this line and 76% of noncoding regions achieve scores below the line.
As can be seen in the dialogue below the user can set the window length in codons (although around 67 codons is generally suitable) and can restrict the search to a sub region of the sequence. Note that the window length must be odd.

8.3.11.7 Splice site search

This method is used to search for mRNA splice junctions using a weight matrix. The default weight matrix is still that derived from the paper of Mount S.M, (1982) Nucl. Acids Res. 10, 459-472, but we are about to create a whole new set which will be organism specific, and will include them in later releases and make them available via ftp.

The results are displayed in three colours, one colour for each reading frame. The donors are plotted upwards from the base of the panel and the acceptors are plotted downwards from the top of the panel. The donors and acceptors with the same colour are compatible; eg red donors are compatible with red acceptors. Of course it is the combination of reading frame and splice sites that really matters, so donors and acceptors drawn in different colours can be compatible if the reading frame changes. By default all the sites are drawn in the same plot but in the figure shown below they have been separated by reading frame using the programs ability to reorganise the positions of graphical results. This layout of the donors and acceptors is designed to fit with the gene search methods and stop codon plots. The results are plotted as Log-Odds.
The frequency table shown below is used as a weight matrix and AG and GT are obligatory at the appropriate positions.

Mount acceptors redone 16-4-91

<table>
<thead>
<tr>
<th></th>
<th>18</th>
<th>15</th>
<th>0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>-14</td>
<td>-13</td>
<td>-12</td>
<td>-11</td>
</tr>
<tr>
<td>N</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>T</td>
<td>85</td>
<td>67</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>57</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>A</td>
<td>71</td>
<td>71</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>G</td>
<td>17</td>
<td>24</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Mount donors redone 16-4-91

<table>
<thead>
<tr>
<th></th>
<th>12</th>
<th>4</th>
<th>0.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>-2</td>
<td>-1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>N</td>
<td>136</td>
<td>136</td>
<td>136</td>
<td>136</td>
</tr>
<tr>
<td>T</td>
<td>28</td>
<td>15</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>41</td>
<td>6</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>A</td>
<td>40</td>
<td>9</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>27</td>
<td>12</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

8.3.11.8 tRNA search

This method is used to find segments of a sequence that might code for tRNAs. It looks for potential cloverleaf forming structures and then for the presence of the expected conserved bases. It presents results graphically and draws out the cloverleafs.

The algorithm uses a large number of parameters including some loop lengths, scores for each of the four stems, and scores for the conserved bases, but we have not yet included an interface for setting these. We apologise for this and plan to add the interface in a future release. Using individual base pair scores of A-T = G-C = 2, G-T = 1, in its present form the algorithm is set to search for segments of sequence satisfying the following minimum scores:

- aminoacyl stem 12
- tu stem 9
- anticodon stem 8
- du stem 4
- minimum total stem score 36
• minimum number of conserved bases 16

• no introns

The algorithm was first described in Staden, R. (1980) A computer program to search for tRNA genes. Nucl. Acid Res 8, 817-825, but has been completely rewritten since then. The tRNAs that have been sequenced so far have two characteristics that can be used to locate their genes within long DNA sequences. Firstly they have a common secondary structure - the cloverleaf - and secondly, particular bases almost always appear at certain positions in the cloverleaf. The cloverleaf is composed of four base-paired stems and four loops. Three of the stems are of fixed length but the fourth, the dhu stem which usually has four base pairs, sometimes has only three. All of the loops can vary in size. The following relationships between the stems in the cloverleaf are assumed in the program: (a) there are no bases between one end of the aminoacyl stem and the adjoining tuc stem; (b) there are two bases between the aminoacyl stem and the dhu stem; (c) there is one base between the dhu stem and the anticodon stem; (d) there are at least three bases between the anticodon stem and the tuc stem. The program looks first for cloverleaf structure and then for conserved bases.

The output shows the position of the possible gene in the sequence by a vertical line the height of which shows the number of basepairs made in the stems. Typical graphical output:

The cloverleaf structure is also drawn in the text Output Window. Typical text output:
8.4 Spin Comparison Functions

Spin contains three functions for finding local segments of similarity between pairs of sequences (see Section 8.4.1 [Finding Similar Spans], page 337), (see Section 8.4.2 [Finding Matching Words], page 340) and (see Section 8.4.5 [Aligning Sequences Locally], page 347), and two for finding global similarity (see Section 8.4.3 [Finding the Best Diagonals], page 342) and (see Section 8.4.4 [Aligning Sequences Globally], page 343). All functions produce results which are plotted in a dot matrix display called a SPIN Sequence Comparison Plot. Obviously global similarity consists of many small matching segments and so the local similarity searches, when plotted, will also reveal any larger scale relationships.

8.4.1 Finding Similar Spans

This method was first described by McLachlan McLachlan, A.D. Tests for comparing related amino acid sequences J. Mol. Biol. 61, 409-424 (1971). It involves calculating a score for each position in the plot by summing points found when looking forwards and backwards along a diagonal line of a given length (window length). The algorithm does not simply look for identity but uses a score matrix that contains scores for every possible pair of character types. At each point that the score is above a minimum score, a match is saved. The matches are plotted as a single point in the SPIN Sequence Comparison Plot, corresponding to the centre of the matching span (see Section 8.6.3 [SPIN Sequence Comparison Plot], page 362) (Although see "Rescan matches, below).
The dialogue box (shown above) requests the horizontal and vertical sequences and their ranges (see Section 8.8.4 [Selecting a sequence], page 371), the window span length and the minimum score. Only results above this minimum score are plotted. The default value for the minimum score is one that would produce approximately 500 matches between two random sequences of the same composition as the two under investigation (see Section 8.5.1 [Probabilities and expected number of matches], page 350). This value of 500 can be changed using the "Configure default number of matches" option of the "Options" menu on the main menubar (see Section 8.5.3 [Changing the default number of matches], page 351). The upper and lower limits of the minimum score are similarly determined except that the expected number of matches for the upper limit is 0 and for the lower limit is "maximum number of matches". The "maximum number of matches" value can be altered if more matches are required to be plotted by using the "Configure maximum number of matches" option of the "Options" menu (see Section 8.5.2 [Changing the maximum number of matches], page 351).

Further operations available for find similar spans are:

**Information**

This command gives a brief description of the sequences used in the comparison, the input parameters used and the number of matches found.

horizontal EMBL: hsproperd  
vertical EMBL: mmproper  
window length 11 min match 9  
number of matches 1772

**Results**

A detailed listing of all the hits found is displayed in the Output Window.
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Positions 2 h 630 v and score 9

Percentage mismatch 18.2

H agcctatcaac
V agcctatgagc

630 640

Positions 7 h 369 v and score 9

Percentage mismatch 18.2

H atcaacccaga
V aggaacccaga

369 379

Tabulate Scores
This option lists scores, probabilities, and their expected and observed numbers of matches.

<table>
<thead>
<tr>
<th>score</th>
<th>probability</th>
<th>expected</th>
<th>observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1.73e-04</td>
<td>365</td>
<td>1772</td>
</tr>
<tr>
<td>10</td>
<td>1.17e-05</td>
<td>25</td>
<td>601</td>
</tr>
<tr>
<td>11</td>
<td>3.60e-07</td>
<td>1</td>
<td>149</td>
</tr>
</tbody>
</table>

Rescan matches
It is also possible to plot a dot for each residue with a score above a minimum value within each matching span using the "Rescan matches" command. This is only a temporary result and will be destroyed if the SPIN Sequence Comparison Plot is altered (see Section 8.5 [Controlling and Managing Results], page 350).

Configure This option allows the line width and colour of the matches to be altered. See Section 9.5 [Colour Selector], page 378. A colour browser is displayed from which the desired line width or colour can be configured. Pressing OK will update the SPIN Sequence Comparison Plot.

Display sequences Selecting this command invokes the SPIN Sequence Comparison Display (see Section 8.6.4 [Sequence comparison display], page 365). Moving the cursor in the sequence display will move the cursors of the same sequence in any SPIN Sequence Comparison Plot (see Section 8.6.3.1 [Cursors], page 364). To force the sequence display to show the nearest match, use the "nearest match" button in the sequence display plot. To force the sequences to maintain their current register activate the "Lock" button.

Hide This option removes the points from the SPIN Sequence Comparison Plot but retains the information in memory.

Reveal This option will redisplay previously hidden points in the SPIN Sequence Comparison Plot.

Remove This command removes all the information regarding this particular invocation of Find similar spans and access to this data lost.
8.4.2 Finding Matching Words

The find matching words routine finds runs of identical characters in the sequence. Its main value is speed, being hundreds of times faster than the find similar spans function. It is of course not very sensitive but is useful for long DNA sequences.

The dialogue allows the horizontal and vertical sequences and their ranges to be selected (see Section 8.8.4 [Selecting a sequence], page 371). The word length is the minimum number of consecutive matching characters. All runs of identical characters that are at least as long as the word length will produce a line on the SPIN Sequence Comparison Plot of length proportional to the actual word length (see Section 8.6.3 [SPIN Sequence Comparison Plot], page 362).

Further operations available for find matching words are:

**Information**
This command gives a brief description of the sequences used in the comparison, the input parameters used and the number of hits found.

```
horizontal EMBL: hsproperd
vertical EMBL: mmproper
word length 8
Number of matches 140
```

**Results**
A detailed listing of all the matching words is obtained in the Output Window. The horizontal (h) and vertical (v) positions of the beginning of the match are listed along with the length of the match and the match itself.
Positions 162 h 4 v and length 14
\[\text{ttcacccagtatga}\]
Positions 225 h 67 v and length 18
\[\text{gaagactgctgtctcaac}\]
Positions 509 h 118 v and length 8
\[\text{ctctgtca}\]
Positions 276 h 118 v and length 9
\[\text{ctctgtcag}\]
Positions 288 h 130 v and length 8
\[\text{tgcaggtc}\]
Positions 626 h 131 v and length 8
\[\text{gcaggtct}\]
Positions 1208 h 144 v and length 8
\[\text{atggtcag}\]

Tabulate scores
This option lists scores, probabilities, and their expected and observed numbers of matches.

<table>
<thead>
<tr>
<th>Score</th>
<th>Probability</th>
<th>Expected</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2.06e-05</td>
<td>43</td>
<td>140</td>
</tr>
<tr>
<td>9</td>
<td>5.35e-06</td>
<td>11</td>
<td>67</td>
</tr>
<tr>
<td>10</td>
<td>1.39e-06</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>11</td>
<td>3.60e-07</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>9.35e-08</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>13</td>
<td>2.43e-08</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>6.30e-09</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>1.63e-09</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>16</td>
<td>4.24e-10</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>17</td>
<td>1.10e-10</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>18</td>
<td>2.86e-11</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>19</td>
<td>7.42e-12</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>1.93e-12</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>21</td>
<td>5.00e-13</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>1.30e-13</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>23</td>
<td>3.37e-14</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>8.74e-15</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Configure
This option allows the line width and colour of the matches to be altered. See Section 9.5 [Colour Selector], page 378. A colour browser is displayed from which the desired line width or colour can be configured. Pressing OK will update the SPIN Sequence Comparison Plot.

Display sequences
Selecting this command invokes the sequence display (see Section 8.6.4 [Sequence comparison display], page 365). Moving the cursor in the sequence display will move the cursors of the same sequence in any SPIN Sequence Comparison Plot (see Section 8.6.3.1 [Cursors], page 364). To force the sequence display to show the nearest match, use the "nearest match" button in the sequence display plot.

Hide
This option removes the points from the SPIN Sequence Comparison Plot but retains the information in memory.
Reveal  This option will redisplay previously hidden points in the SPIN Sequence Comparison Plot.

Remove  This command removes all the information regarding this particular invocation of Find matching words, and access to this data is lost.

8.4.3 Finding the Best Diagonals

This option is among the fastest and can be useful for a quick comparison of two long DNA sequences. The algorithm is as follows. First it finds the positions of runs of identical characters ("words") of length word length, as for the find matching words algorithm. These words are accumulated in an imaginary SPIN Sequence Comparison Plot and the number of hits on each diagonal is summed to produce a histogram. The histogram is analysed to find its mean and standard deviation. The diagonals that lie above some cutoff score (defined in standard deviation units), are rescanned using the find similar spans algorithm. Any window lengths reaching the cutoff score produce a dot which is plotted in the usual way.

The dialogue box requests horizontal and vertical sequences and their ranges (see Section 8.8.4 [Selecting a sequence], page 371), the minimum number of identical characters in a run "word length", the minimum standard deviation, the window length and the minimum score.

The points are plotted to the SPIN Sequence Comparison Plot (see Section 8.6.3 [SPIN Sequence Comparison Plot], page 362).

Further operations available for find best diagonals are:

Information  This command gives a brief description of the sequences used in the comparison and the input parameters used.

    horizontal EMBL: hsproperd
    vertical EMBL: mmproper
    window length 11 minimum score 9 word length 8 minimum sd 3.000000

Results  A listing of all the matches is obtained in the Output Window. The horizontal (h) and vertical (v) positions of the beginning of the match are listed.
Configure This option allows the line width and colour of the matches to be altered. See Section 9.5 [Colour Selector], page 378. A colour browser is displayed from which the desired line width or colour can be configured. Pressing OK will update the SPIN Sequence Comparison Plot.

Display sequences
Selecting this command invokes the sequence display (see Section 8.6.4 [Sequence comparison display], page 365). Moving the cursor in the sequence display will move the cursors of the same sequence in any SPIN Sequence Comparison Plot (see Section 8.6.3.1 [Cursors], page 364). To force the sequence display to show the nearest match, use the "nearest match" button in the sequence display plot.

Hide This option removes the points from the SPIN Sequence Comparison Plot but retains the information in memory.

Reveal This option will redisplay previously hidden points in the SPIN Sequence Comparison Plot.

Remove This command removes all the information regarding this particular invocation of Find best diagonals, and access to this data is lost.

8.4.4 Aligning Sequences Globally

This function will produce an optimal global alignment of two segments of the sequence. The dynamic programming alignment algorithm is based on Huang,X On global sequence alignment. CABIOS 10 227-235 (1994). There is no length limit of the sequences but the sequences to be aligned should be of the same type i.e. both be DNA or both protein.
A dialogue box (shown above) requests the horizontal and vertical sequences and the ranges over which they are to be aligned (see Section 8.8.4 [Selecting a sequence], page 371) and the gap start penalty and the gap extension penalty. In addition, if the sequence is DNA, the "score for match" and "score for mis-match" must be provided. These values are used to generate a score matrix. For protein sequences, the score matrix can be changed from the "Options" menu (see Section 8.5.5 [Changing the score matrix], page 351).

The alignment is displayed in the Output Window along with the percentage mismatch (see below) and on the SPIN Sequence Comparison Plot as a line. The line represents the path of the alignment.

The following plot shows a global alignment of two Xenopus Laevis sequences. The vertical sequence (xlaactcag) is genomic DNA, and the horizontal sequence (xlaacacr) is the corresponding cDNA. The vertical sections of the plotted path correspond to introns in the genomic DNA, which are obviously absent from the cDNA.
Below we show a typical alignment (from a different pair of sequences) as produced in the Output Window.
Percentage mismatch 29.6

1  11  21  31  41  51
hsproperd gagcctataaccagataaagcgggaggtgcagggggcagtac

mmproper ************************************************************

-157 -147 -137 -127 -117 -107

61  71  81  91 101 111
hsproperd tcaacatgacaacagggagcggcaggcccctgccagttgctgccgccgctgcctgc

mmproper ************************************************************

-97 -87 -77 -67 -57 -47

121 131 141 151 161 171
hsproperd tgctcaccctgccaagcagcagctcagacccgctgctcaccagagtgaagaat

mmproper **************************************tgtttcacccagtatgaggagt

-37 -27 -17  -7  3  13

181 191 201 211 221 231
hsproperd cctccggcaagtgcagaagcctctgggggttgtgcgtgaaagactgtgtctca

mmproper cctctggcaggtgcaaaggcctacttggagagacatcagggtagaagactgctgtctca

The two aligned sequences are automatically saved in memory and can be accessed through the sequence manager. They are assigned default filenames which are based on the parent with the addition of "number" where "number" is a unique identifier (see the twelfth and thirteenth entries of the sequence manager picture (see Section 8.8.3 [Sequence manager], page 369).

Further operations available for align sequences are:

Information
This command gives a brief description of the sequences used in the comparison and the input parameters used.

horizontal PERSONAL: m13mlp18.seq from 1 to 7250
vertical PERSONAL: lawrist7.seq from 1 to 5261

Configure
This option allows the line width and colour of the matches to be altered. See Section 9.5 [Colour Selector], page 378. A colour browser is displayed from which the desired line width or colour can be configured. Pressing OK will update the SPIN Sequence Comparison Plot.

Display sequences
Selecting this command invokes the Sequence Comparison Display (see Section 8.6.4 [Sequence comparison display], page 365). Moving the cursor in the sequence display will move the cursors of the same sequence in any SPIN Sequence Comparison Plot (see Section 8.6.3.1 [Cursor], page 364). To force the sequence display to show the nearest match, use the "nearest match" button in the sequence display plot.
8.4.5 Aligning Sequences Locally

The local alignment routine is based around the program SIM by Huang and Miller which is an implementation of the Smith-Waterman algorithm Huang, X.Q. & Miller, W. A Time-Efficient, Linear-Space Local Similarity Algorithm. Advances in Applied Mathematics 12 337-357 (1991).

SIM finds k best non-intersecting alignments between two sequences or within a single sequence using dynamic programming techniques. The alignments are reported in order of decreasing similarity score and share no aligned pairs. SIM requires space proportional to the sum of the input sequence lengths and the output alignment lengths, so it accommodates 100,000-base sequences on a workstation. Both sequences must be of the same type, ie both be DNA or both be protein.

A dialogue box (shown above) requests the horizontal and vertical sequences and the ranges over which they are to be aligned (see Section 8.8.4 [Selecting a sequence], page 371). Either a specified number of alignments can be requested or alternatively, all alignments above a certain score. If the sequence is DNA, the scores for a matching aligned pair, a transition and a transversion must be provided. These values are used to generate a score matrix. For protein sequences, the score matrix can be changed from the "Options" menu (see Section 8.5.5 [Changing the score matrix], page 351). Both DNA and protein sequences require the penalty for opening a gap and the penalty for gap extension.

The alignments are displayed in the Output Window along with the percentage mismatch (see below) and on the SPIN Sequence Comparison Plot as a series of lines, each line corresponding to a single alignment. The line represents the path of alignments.
The following two plots show local alignments of two Xenopus Laevis sequences. The vertical sequence (xlactcag) is genomic DNA, and the horizontal sequence (xlacacr) is the corresponding cDNA.

The first plot is of a local alignment using a higher than default penalty for each residue in the gap (1 as opposed to 0.2). It has also been specified that all alignments scoring more than 20 are to be shown. The result of this is seven aligned regions, represented by seven diagonal lines in the plot. These regions correspond to the exons that are present in both sequences, separated by the introns that are only present in the genomic sequence.

The second plot shows the result for the same two sequences when the default gap penalty is accepted and when only the highest scoring alignment is displayed. This best alignment covers five of the seven exons identified in the previous plot, with the lower gap penalty allowing it to span the introns that separate them.
Below is a typical alignment as written to the Output Window.

Percentage mismatch 35.7
438 448 458 468 478 488
h cagccctgtgaggaccagcagtgctgtcctgagatgggcggctggtctggctgggggcc
::: :::: :: :: :: :::: :::::
m caggcctgtgacacccagaagacctgccccacacatggggcctgggcatcctggggcccc
451 461 471 481 491 501

498 508 518
h tgggagccttgctctgtcacctgc
::: :: :::: :::::
m tggagcccccgctcaggatctgc
511 521 531

Further operations available for local alignments are:

Information
This command gives a brief description of the sequences used in the comparison and the input parameters used.
Configure
This option allows the line width and colour of the matches to be altered. See Section 9.5 [Colour Selector], page 378. A colour browser is displayed from which the desired line width or colour can be configured. Pressing OK will update the SPIN Sequence Comparison Plot.

Display sequences
Selecting this command invokes the Sequence Comparison Display (see Section 8.6.4 [Sequence comparison display], page 365). Moving the cursor in the sequence display will move the cursors of the same sequence in any SPIN Sequence Comparison Plot (see Section 8.6.3.1 [Cursor], page 364). To force the sequence display to show the nearest match, use the "nearest match" button in the sequence display plot.

Hide
This option removes the points from the SPIN Sequence Comparison Plot but retains the information in memory.

Reveal
This option will redisplay previously hidden points in the SPIN Sequence Comparison Plot.

Remove
This command removes all the information regarding this particular invocation of Local alignment, and access to this data is lost.

8.5 Controlling and Managing Results

Spin allows the parameters for each analytical option to be set in dialogues immediately prior to their execution, but there are other global parameters which can influence the results obtained, and they are described here.

This section also covers, in its description of the "Results Manager" how results can be manipulated after they have been obtained. As this implies, almost all searches conducted by spin produce results that are retained until the user explicitly deletes them, and these are termed "Permanent results". Any other results are termed "Temporary". At present, the only temporary results are those produced by a variant of the Similar Spans algorithm, (see Section 8.4.1 [Finding Similar Spans], page 337), in which the plot can be overlayed by marking every identical character in each matching span with separate dots. These extra dots are not stored as results and any changes to the SPIN Sequence Comparison Plot, for example plotting new data, will destroy them.

8.5.1 Probabilities and expected numbers of matches

To suggest reasonable ranges of cutoff scores for the Similar Spans (see Section 8.4.1 [Finding Similar Spans], page 337) and Matching Words (see Section 8.4.2 [Finding Matching Words], page 340) comparison functions, and later to help users assess the significance of the matches found between sequences, spin calculates their probabilities and the expected number of matches Staden R, Methods for calculating the probabilities of finding patterns in sequences. CABIOS 5 89-96 (1989). For both algorithms the probability depends on the composition of the two sequences, the cutoff score, and, for the matching spans algorithm, the score matrix. The probability is the chance
of finding the given score in infinitely long random sequences of the same composition as the pair being compared. The expected number of matches for any score is calculated by multiplying its probability value by the product of the lengths of the two sequences. Note that no correction is made for the case of comparing a sequence against itself.

The matches found for these two algorithms can be assessed by selecting the Tabulate Scores option, which will produce a list of observed and expected results as shown in the example below.

<table>
<thead>
<tr>
<th>score</th>
<th>probability</th>
<th>expected</th>
<th>observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1.73e-04</td>
<td>365</td>
<td>1772</td>
</tr>
<tr>
<td>10</td>
<td>1.17e-05</td>
<td>25</td>
<td>601</td>
</tr>
<tr>
<td>11</td>
<td>3.60e-07</td>
<td>1</td>
<td>149</td>
</tr>
</tbody>
</table>

In this case there are clearly many more matches at each score level than would be expected by chance.

8.5.2 Changing the maximum number of matches

The maximum number of matches is a guideline limit to the number of matches that a comparison function is allowed to produce. In conjunction with the probability calculations its value is used to determine the range of scores allowed for an option - for example, the lowest "minimum score" for the "find similar spans" function (see Section 8.4.1 [Finding Similar Spans], page 337). Altering the maximum number of matches value will in turn alter the range of scores available in a function. Note that this maximum only provides a guideline and each function will always attempt to calculate all matches. However, if the scores are set too low, and the sequences are long, very large numbers of matches matches may be produced and the functions may run slowly as the program gobbles up increasing amounts of memory to store them.

The maximum number of matches is altered using the Options menu.

8.5.3 Changing the default number of matches

The default number of matches is used to determine the default score in the function dialogue boxes. If the two sequences being analysed were scrambled (i.e. had the order of their bases or amino acids changed randomly) and then compared using the default score, they should be found to contain approximately the default number of matches. Hence this number provides users with a crude assessment of the significance of the matches found: if more than the default number are found, the sequences are more similar than is likely by chance.

The default number of matches is altered using the Options menu.

8.5.4 Hide duplicate matches

If the horizontal and vertical sequences are the same the comparison plots would be a mirror image about the main diagonal. In this case the default is that only the lower half of the plot is calculated. If the "Hide duplicate matches" checkbutton is not set the entire plot will be displayed. It is important to note this property of the algorithms: if only one half of the plot is displayed the main diagonal has been found to be identical!

8.5.5 Changing the score matrix

This option allows users to select their own score matrix for protein sequence comparison and is available from the "Options" menu which invokes a dialogue box. Enter the full filename of the matrix in the entry box. Clicking on the "browse" button will invoke a file browser. See Section 9.6 [File Browser], page 379.
The recommended format for the matrices is that used by blast. Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. Basic local alignment search tool. J. Mol. Biol. 215:403-10 (1990). Note that the NCBI make a whole range of protein score matrices available in this format and we include the one shown below in the package tables directory in a file named pam250.

```
# This matrix was produced by "pam" Version 1.0.6 [28-Jul-93]
# PAM 250 substitution matrix, scale = ln(2)/3 = 0.231049
# Expected score = -0.844, Entropy = 0.354 bits
# Lowest score = -8, Highest score = 17

A  2 -2  0  0 -2  0  0  1 -1 -1 -2 -1 -1 -3  1  1 -1 -6 -3  0  0  0  0 -8
R -2  6  0 -1 -4  1 -1 -3  2 -2 -3  3  0 -4  0  0 -1 -2 -4 -2 -1  0 -1 -8
N  0  0  2 -2 -4  1  1  0  2 -2 -3  1 -2 -3  0  1  0 -4 -2 -2  2  1  0 -8
D  0 -1  2  4 -5  2  3  1  1 -2 -4  0 -3 -6 -1  0  0 -7 -4 -2  3  3 -1 -8
C -2 -4 -4 -5 -5 -3 -3 -2 -6 -5 -5 -4 -3  0 -2 -8  0 -2 -4 -5 -3 -8
Q  0  1  1  2 -5  4  2 -1  3 -2 -2  1 -1 -5  0 -1 -1 -5 -4 -2  1  3 -1 -8
E  0 -1  1  3 -5  2  4  0  1 -2 -3  0 -2 -5 -1  0  0 -7 -4 -2  3  3 -1 -8
G  1 -3  0  1 -3 -1  0  5 -2 -3 -4 -2 -3 -5  0  1  0 -7 -5 -1  0  0 -1 -8
H -1  2  2  1 -3  3  1 -2  6 -2 -2  0 -2 -2  0 -1 -1 -3  0 -2  1  2 -1 -8
I -1 -2 -2 -2 -2 -2 -2 -3 -2  5  5 -2 -2  2  1 -2 -1  0 -5 -1  4 -2 -2 -1 -8
L -2 -3 -3 -4 -6 -2 -3 -4 -2  2  6 -3  4  2 -3 -3 -2 -2 -1  2 -3 -3 -1 -8
K -1  3  1  0 -5  1  0 -2  0 -2 -3  5  0 -5 -1  0  0 -3 -4 -2  1  0 -1 -8
M -1  0 -2 -3 -5 -1 -2 -3 -2  2  4  0  6  0 -2 -2 -1 -4 -2  2 -2 -1 -8
F -3 -4 -3 -6 -4 -5 -5 -5 -2  1  2 -5  0  9 -5 -3 -3  0  7 -1 -4 -5 -2 -8
P  1  0  0 -1 -3  0 -1  0  0 -2 -3 -1 -2 -5  6  1  0 -6 -5 -1 -1  0 -1 -8
S  1  0  1  0  0 -1  0  1 -1 -1 -3  0 -2 -3  1  2  1 -2 -3 -1  0  0 -8
T  1 -1  0  0 -1  2  0  0 -1 -2 -1  0  0 -2 -1 -3  0  1  3 -5 -3  0  0 -1 -8
W -6  2 -4 -7 -8 -5 -7 -7 -3 -5 -2 -3 -4  0 -6 -2 -5 17  0 -6 -5 -6 -4 -8
Y -3 -4 -2 -4  0 -4 -4 -5  0 -1 -1 -4 -2  7  5 -3 -3  0  1 -2 -3 -4 -2 -8
V  0 -2 -2 -2 -2 -1 -2 -2 -2  2 -1 -1 -1  0 -6 -2  4 -2 -2 -1 -8
B  0 -1  2 -3 -4  1  3  0  1 -2 -3  1 -2 -4 -1  0  0 -5 -3 -2  3  2 -1 -8
Z  0  0  1  3 -5  3  3  0  2 -2 -3  0 -2 -5  0  0 -1 -6 -4 -2  3  1 -8
X  0 -1  0 -1 -3 -1 -1 -1 -1 -1 -1 -1 -1 -2 -1  0  0 -4 -2 -1 -1 -1 -1 -8
* -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 -8 1
```

But for historical reasons the default matrix used by the program is the one shown below.

```
C  22 10  8  7  8  7  6  5  5  5  5  5  7  6  5  5  8  4  8  6  10  2  10  10  10  10
S 10 12 11 11 11 11 10 10  9 10 10  9 10 10  8  9  7  9  7  8  10  10  10  10
T  8 11 13 10 11 10 10 10  9 10 10  9 10 10  9  8 10  7  7  5 10 10 10 10 10
P  7 11 10 16 11  9  9  9  9 10  9 10 10  9  8  8  8  7  9  5  5  4 10 10 10 10
A  8 11 11 12 11 10 10 10 10 10  9  8  9  9  8 10  6  7  4 10 10 10 10 10
G  7 11 10 11 12 12 11 10 10 10  9  8 10  8  7  8  7  6  9  5  5  3 10 10 10 10
N  6 11 10  9 10 10 12 12 11 12 11 12 10 11  8  8  7  8  6  8  6 10 10 10 10 10
D  5 10 10  9 10 11 12 14 13 12 13 12 11  9  10  7  8  6  8  4  6 10 10 10 10 10
E  5 10 10  9 10 11 13 14 12 12 13 12 11  9  10  8  8  7  8  5  6  3 10 10 10 10
```
8.5.6 Set protein alignment symbols

This option allows users to set their own sequence similarity levels and alignment symbols for protein sequence alignments. The dialogue (shown below) provides for setting a symbol for identical characters and for three levels of similarity with corresponding symbols.

8.6 The Spin User Interface

Spin has several displays. The first is a top level window from which all the main options are selected and which receives textual results. Most analytical functions which operate on single sequences add their graphical results to a "SPIN Sequence Plot" that is associated with the sequence being analysed. (An exception is the restriction enzyme search which produces its own separate window.) Most functions which compare pairs of sequences add their results to a "SPIN Sequence Comparison Plot". The SPIN Sequence Plot and the SPIN Sequence Comparison Plot each have associated sequence display windows: the Sequence Display and the Sequence Comparison Display. These allow the text of the sequences to be viewed and use cursors to show the corresponding positions in the graphical displays.

Spin is best operated using a three button mouse, but alternative keybindings are available. Full details of the user interface are described elsewhere (see [User Interface], page 373).

The main window (shown below) contains an Output Window for textual results, an Error window for error messages, and a series of menus arranged along the top (see Section 8.2.4 [Spin menus], page 306). The contents of the two text windows can be searched, edited and saved. Each set of results is preceded by a header containing the time and date when it was generated.
As can be seen the main menu bar contains File, View, Options, Sequences, Statistics, Translation, Comparison, Search and Emboss menus.

8.6.1 SPIN Sequence Plot

Graphical results are shown in separate windows. Most functions add their graphical results to a SPIN Sequence Plot that is associated with the sequence being analysed, but some functions such as the restriction enzyme search produce their own separate windows. Each set of graphical results has its own particular default drawing method, but individual results can be "dragged and dropped" by users, hence allowing plots to rearranged and superimposed. Plots can also be extracted from the main graphics window and dropped to create new independent windows. The graphical results can be zoomed and scrolled in both x and y directions. Zooming is achieved using the X and Y scale bars at the bottom of the plot. The individual plots can be scrolled in y using the scroll bars attached to their right hand edge. The sequence can be scrolled using the scroll bar at the base of the plot.
The figure shows a SPIN Sequence Plot containing the results of a gene search method based on codon usage, upon which is superimposed a search for start codons and stop codons. The vertical blue line represents the cursor. The x position of the cursor is shown in the left hand box above the plot. Each result in the window has its own colour. At the right hand side of each panel is a set of square boxes with the same colours as the lines drawn in the adjacent plot. These icon-like objects represent individual results and allow the user to operate on them. For example at the right of the middle panel is a pop-up menu containing the items: "Information", "List results", "Configure", "Hide" and "Remove". (see Section 8.7.1 [Result manager], page 365).

The square icons can also be used to move the corresponding results to new locations. These operations are explained below (see Section 8.6.1.4 [Drag and drop], page 357). The cursor in the plot can be used to control the position of the cursor in the sequence display.

The SPIN Sequence Plot contains three menus: "File", "View" and "Results". The "File" menu contains the "Exit" command which closes down the plot; the "View" menu contains the "Results manager" command (see Section 8.7.1 [Result manager], page 365); and the "Results" menu contains a list of the results, colour coded i.e. the text is written in the same colour as the plot.
For each result a series of commands can be accessed from a cascading menu. (see Section 8.7.1 [Result manager], page 365).

8.6.1.1 Cursors

Each sequence displayed in a SPIN Sequence Plot will have a corresponding cursor of a particular colour. The same sequence displayed in several SPIN Sequence Plots will have a cursor of the same colour. To move a cursor, click on it with the middle mouse button, or Alt left mouse button, held down and drag the mouse. The cursor will move all other cursors displayed that relate to that sequence, whether these be in different SPIN Sequence Plots or within the sequence display.
8.6.1.2 Crosshairs

Crosshairs can be turned on or off using the check button labelled "crosshairs". The x and y positions of the crosshairs are indicated in the two boxes to the right of the check box respectively. The x value is the base position in the sequence and the y value the score for the corresponding plot.

8.6.1.3 Zoom

The graphical results can be zoomed and scrolled in both x and y directions. Zooming is achieved using the X and Y scale bars at the bottom of the plot. The individual plots can be scrolled in y using the scroll bars attached to their right hand edge. The sequence can be scrolled using the scroll bar at the base of the plot.

8.6.1.4 Drag and drop

The square boxes at the right edge of the SPIN Sequence Plot panels have the same colours as the individual results in the display. These icons can be used to drag and drop the results to which they correspond. This is activated by pressing the middle mouse button, or Alt left mouse button, over the box and then moving the cursor over the SPIN Sequence Plot to the new location. As the cursor moves over each part of the plot rectangular boxes will appear to indicate the position that the dragged result will occupy if the mouse button is released. Results can be dropped on top of another plot (signified by a rectangle drawn over the centre of the plot), above another plot (signified by a rectangle drawn in the top third of the plot), or below another plot (signified by a rectangle drawn in the bottom third of the plot). The figure below shows three panels containing a protein gene prediction and three panels showing the positions of stop codons in each of the reading frames.
The figure below shows the same SPIN Sequence Plot but with the rectangle indicating where the user has dragged the frame 1 stop codon results.
The figure below shows the result of releasing the middle mouse button, or Alt left mouse button: the stop codon plot has moved to be superimposed on the gene prediction plot.

8.6.2 Sequence display

Each sequence shown in the "Sequence manager" list can have its own "Sequence display" window. The Sequence display provides a way of viewing and scrolling along the characters of the sequence. It can also show translations to protein and the positions of restriction enzyme cutting sites. Movement along the sequence is controlled by standard mouse and cursor commands and by the use of a subsequence/string search routine. The cursor can also be controlled by dragging the cursor in the programs' graphical displays.
When restriction enzyme cutting sites are shown in the Sequence display window the number of rows of text to be displayed at any position along the sequence varies with the density of the sites. This presents a tricky problem about how to position the lines of text relative to the top and bottom of the window. Should the height of the window grow and shrink vertically as the user scrolls? Should the window maintain a fixed height, in which case should the top or the bottom be clipped if the number of lines of text exceeds the window height? We have programmed it so that the nucleotide sequence remains at a fixed height to provide a constant reference and the user can select this height by use of a vertical scroll bar and by growing or shrinking the window. The scroll bar will allow vertical movement when the number of lines of text exceeds the current window size.

8.6.2.1 Search

The Sequence display contains a subsequence or string search function which moves the cursor to the position of the next match. As shown in the dialogue the user selects the direction and strand over which the search should be performed, the search algorithm, the minimum percentage match, and the subsequence/string for which to search. The search algorithm allows either NC-IUB codes Cornish-Bowden, A. (1985) Nucl. Acids Res. 13, 3021-3030 or a literal search. The literal search will search for exact matches eg inputting a search string of "n" will search for the letter "n". The NC-IUB codes option can use any of the NC-IUB symbols shown in the figure below. Once activated the Search dialogue will remain visible until the user clicks on the "Cancel" button. The cursor will move to the next matching position each time the user clicks on the "Search" button, or will "beep" if there is no such match.
NC-IUB SYMBOLS

A, C, G, T
R (A, G) 'puRine'
Y (T, C) 'pYrimidine'
W (A, T) 'Weak'
S (C, G) 'Strong'
M (A, C) 'aMino'
K (G, T) 'Keto'
H (A, T, C) 'not G'
B (G, C, T) 'not A'
V (G, A, C) 'not T'
D (G, A, T) 'not C'
N (G, A, C, T) 'aNy'

8.6.2.2 Save

Saving the contents of the sequence display to a file

The Sequence display contents can be dumped to a file by selecting the "Save" option from the menu. Whatever options are currently activated (i.e. which of restriction enzyme sites, translation, ruler and strands) will be written to disk. The user can define the region of the sequence for which to dump the results and the name of the file to use.

8.6.3 SPIN Sequence Comparison Plot

When a comparison function has been run on a pair of sequences a SPIN Sequence Comparison Plot will appear. The results from each comparison can be viewed in a "Sequence Comparison Display" in which the two sequences can be scrolled passed one another.

The SPIN Sequence Comparison Plot display shows the results of comparison algorithms. Each match is represented as either a single dot ("Find similar spans", "Find best diagonals") or a line ("Find matching words", "Align sequences", "Local alignment"). Sets of matches from a single invocation of a comparison command are termed "a result". Each result is plotted using a
single colour which can be configured via the results manager (see Section 8.7.1 [Result manager], page 365). The maximum dimensions of the SPIN Sequence Comparison Plot are indicated on the rulers at the bottom and left hand side. It is possible within spin to compare many different sequences. This means that there may be more than one horizontal or vertical sequence shown in the spin plot. All the points are scaled to the largest sequence in each direction. Plots can also be extracted from or added to each SPIN Sequence Comparison Plot.

The diagram above shows the results of a "find similar spans" search (olive) (see Section 8.4.1 [Finding Similar Spans], page 337), and a "find matching words" (red) (see Section 8.4.2 [Finding Matching Words], page 340), between human and mouse properdin (hsproperd and mmproper). At the right hand side is a set of square boxes with the same colours as the dots drawn in the adjacent plot. These icon-like objects represent individual results and allow the user to operate on them. For example, in the figure above, the user has clicked the right mouse button on the icon to raise a pop-up menu beneath the "matching words" result (see Section 8.7.1 [Result manager], page 365).

The square icons can also be used to move the corresponding results to new locations. These operations are explained below (see Section 8.6.3.4 [Drag and drop], page 364).

The SPIN Sequence Comparison Plot has 3 menus, "File", "View" and "Results".

The "File" menu contains the "Exit" command to quit the SPIN Sequence Comparison Plot. This shuts down the SPIN Sequence Comparison Plot display removes all the results displayed in that plot.
The "View" menu contains the "Results manager" command, see Section 8.7.1 [Result manager], page 365.

The "Results" menu provides a quick method of interfacing with the menu obtainable via the "Results manager" (see Section 8.7.1 [Result manager], page 365).

8.6.3.1 Cursors

Each sequence displayed in a SPIN Sequence Comparison Plot will have a corresponding cursor of a particular colour. In the picture above, the sequence on the horizontal axis has a vertical blue cursor whereas the sequence on the vertical axis has a horizontal olive green cursor. The same sequence displayed in several SPIN Sequence Comparison Plots will have a cursor of the same colour unless the sequence has been plotted on a different axis. The x and y positions of the cursors are indicated in the two boxes to the right of the crosshair check box respectively. To move a cursor, click on it with the middle mouse button, or Alt left mouse button, held down and drag the mouse. The cursor will move all other cursors displayed that relate to that sequence, whether they are in different SPIN Sequence Comparison Plots or within the sequence display.

8.6.3.2 Crosshairs

Crosshairs can be turned on or off using the check button labelled "crosshairs". The x and y positions of the crosshairs are indicated in the two boxes to the right of the check box. The position of the crosshairs can be "frozen" at a particular position by pressing the control button and moving the mouse cursor outside the SPIN Sequence Comparison Plot window.

8.6.3.3 Zoom

Plots can be enlarged either by resizing the window or zooming. Zooming is achieved in two ways: either using the buttons at the base of the plot; or by holding down the control key and right mouse button and dragging out a rectangle around the region to be zoomed. Rectangles that are too small are ignored and a warning bell will sound. The Back button will restore the plot to the previous magnification. Zooming will increase the magnification of the plot so that the contents of the dragged out rectangle fill the display. The scrollbars allow the SPIN Sequence Comparison Plot to be scrolled in both directions.

It is not possible to zoom the results from Rescan matches (see Section 8.4.1 [Finding Similar Spans], page 337).

8.6.3.4 Drag and drop

The square boxes at the right edge of the SPIN Sequence Comparison Plot panels have the same colours as the individual results in the display. These icons can be used to drag and drop the results to which they correspond. This is activated by pressing the middle mouse button, or Alt left mouse button, over the box and then moving the cursor over the SPIN Sequence Comparison Plot to the new location or anywhere outside the SPIN Sequence Comparison Plot. As the cursor moves over each part of the plot rectangular boxes will appear to indicate the position that the dragged result will occupy if the mouse button is released. Results can be dropped on top of another plot (signified by a rectangle drawn over the centre of the plot), above another plot (signified by a rectangle drawn in the top third of the plot), or below another plot (signified by a rectangle drawn in the bottom third of the plot). Moving the mouse cursor outside the SPIN Sequence Comparison Plot and releasing the mouse button will create a new SPIN Sequence Comparison Plot containing that result.
8.6.4 Sequence Comparison Display

A sequence display is associated with a single set of results (see Section 8.7.1 [Result manager], page 365). To invoke a Sequence Comparison Display, bring up a pop up menu for the required result, either from the Results manager, the Results menu in the SPIN Sequence Comparison Plot, or the coloured square icon on the right of the spin plot. From the menu, select the "Display sequences" option.

```
<table>
<thead>
<tr>
<th>File</th>
<th>Nearest match</th>
<th>Nearest dot</th>
<th>Lock</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsproper.seq</td>
<td>0 570 580 590 600 610 620 630</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmproper.seq</td>
<td>agcagcagccctgttatgcagccagccttcaccgcccccagctgctagccagccagatagc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 570 580 590 600 610 620 630</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

The horizontal sequence is drawn above the vertical sequence. In the picture above, "hsproper" is the horizontal sequence and "mmproper" is the vertical sequence. The central panel indicates characters which are identical between the horizontal and vertical sequences. The buttons to the left of the sequences allow scrolling of the sequences either 1 character at a time (< or >) or a screen width (<< or >>). Pressing the Lock button "locks" the two sequences together and they can be scrolled as one. Movement of the sequences is also controlled by the scrollbars or by moving the corresponding cursor in the SPIN Sequence Comparison Plot (see Section 8.6.3 [SPIN Sequence Comparison Plot], page 362). The black cursors in the sequence display correspond to the position of the cursor in the SPIN Sequence Comparison Plot. The sequences can be made to "jump" to the nearest match in those results by pressing the "Nearest match" or "Nearest dot" buttons. Nearest match means the match whose x,y coordinate in sequence character positions is closest, whereas Nearest dot means the match which appears closest in screen coordinates. If the display edges were proportional to the sequence lengths the Nearest dot and Nearest match would be equivalent.

8.7 Controlling and Managing Results

8.7.1 Result manager

Many functions within spin produce "results" which are plotted to the SPIN Sequence Plot (see Section 8.6.1 [SPIN Sequence Plot], page 354) or the SPIN Sequence Comparison Plot (see Section 8.6.3 [SPIN Sequence Comparison Plot], page 362). The Result Manager provides a mechanism to interrogate and operate on these results.

```
K: Results manager
Thu 05:49:44 PM: Plot gene search (#1)
Thu 05:49:44 PM: Plot gene search (#2)
Thu 05:49:44 PM: Plot gene search (#3)
Thu 05:49:44 PM: Plot gene search (#4)
Thu 05:49:46 PM: Plot gene search (#5)
Thu 05:49:46 PM: Plot gene search (#6)
Thu 05:49:46 PM: Plot gene search (#7)
Thu 05:49:46 PM: Plot gene search (#8)
```

OK  Help
The Result Manager can be accessed via the "Results manager" command in the View menu on either the main menu or the menu bar of the SPIN Sequence Plot. Alternatively, the results can be accessed as a menu attached to the "Results" option on the SPIN Sequence Plot menu bar. In this case, the individual results are written in the same colour as the plots they refer to.

Each result is listed in the window containing the time the result was created, the name of the function which created the result and the result number. The number is simply a unique identifier to help distinguish between multiple results produced by the same function. The results are listed in time order, the oldest at the top.

Each item in the list is consuming memory on your computer. Running functions over and over again without removing the previous results will slow down your machine and it will, eventually, run out of memory. Removing items from the list solves this.
Pressing the right mouse button over an listed item will display a popup menu of operations to perform on this result.

**8.7.1.1 Information**

This option in the pop-up writes data about the parameters used to obtain the corresponding result.

**8.7.1.2 List**

This option in the pop-up writes all the numerical values for the result to the Output Window. This should be used sparingly as it requires a lot of memory.

**8.7.1.3 Configure**

This option allows the line width and colour of the matches to be altered (see Section 9.5 [Colour Selector], page 378). A colour browser is displayed from which the desired line width or colour can be configured. Pressing OK will update the SPIN Sequence Plot.

**8.7.1.4 Hide**

This option removes the points from the SPIN Sequence Plot but retains the information in memory.

**8.7.1.5 Reveal**

This option will redisplay previously hidden points in the SPIN Sequence Plot.

**8.7.1.6 Remove**

This command removes all the information regarding this particular result and access to this data is lost.

**8.8 Reading and Managing Sequences**

Spin manages sequences at two levels. First it provides for reading sequences into the program from disk files, and secondly it contains a range of facilities for deriving new sequences from them. For example it can internally produce protein sequences from DNA sequences, or produce the complement of a DNA sequence, rotate it about any position, or scramble it. Each of these types of internal operation produces a new sequence which can be analysed using the comparison functions, or which can be saved to disk. In the same way, performing a sequence alignment produces two new sequences which can be analysed or saved to disk.

The sections below deals first with reading sequences from disk, and then with what can be done to produce new sequences in memory. New sequences are obtained from disk using the Load sequences option in the File menu, and sequences are managed internally using the Sequences menu.

**8.8.1 Use of feature tables in spin**

At present spin can only read feature tables from EMBL style files and use them to perform translations to protein. Like many components of spin, for us this was an exercise in doing the hard part (ie parsing and using the table), but we still need to apply it to many other tasks. We also need to generalise it to read genbank files.
We also limited the types of record we accepted: only those with precisely defined endpoints. This means we do not store records which for example include <1..2000 or 1001.1005 but would store and use those with (1001..2000) or complement(join(2691..4571,4918..5163)).

8.8.2 Reading in sequences

This section describes how sequences are obtained from disk files.

Personal sequence files can be in plain text, "Staden", EMBL, Genbank, PIR, FASTA and GCG formats. If supported by the format, personal files can contain multiple entries preceded by entry names. As is explained below a browser is available for selecting entries from such files. The file format is worked out automatically.

New sequences are entered into spin using the "Load sequence" option in the File menu.

If a sequence is entered which has the same name as one already loaded, its name within the program is changed by the addition of '#number' where 'number' is a unique identifier. For example, if the sequence "hsproperd" has already been loaded and this sequence is loaded again, the name of this second sequence is changed to "hsproperd#0".

If only one sequence has been loaded, the comparison functions will compare this sequence against itself.

8.8.2.1 Simple search

This allows the selection of personal files. The second option button is an "Entry" / "Filename" selection menu and the entry box next to this should be completed with either an entryname or file_name accordingly. If a personal file is selected, the filename should be entered in the entrybox. The Browse buttons at the far right of the dialogue box either invoke a library browser or a file browser, see Section 9.6 [File Browser], page 379 depending on whether a sequence library or personal file has been selected. From the file browser multiple files can be entered by use of the Ctrl key and mouse. Library access is only available via EMBOSS.

8.8.2.2 Extracting a sequence from a personal archive file
This method invokes an archive browser. Enter the filename of the personal file in the entrybox. The Browse button to the right will invoke a file browser, see Section 9.6 [File Browser], page 379. If the file contains multiple entries, these will be displayed in the list box. It is necessary to press "Enter" after entering the filename in order for the entries to be displayed. Select the required entryname(s) and press OK. The selected entries should now have been loaded into spin.

8.8.3 Sequence manager

Spin allows more than two sequences to be available to the user. The sequence manager allows the user to perform operations on the sequences which have been loaded into spin. The same operations can also be invoked from the "Sequences" menu. The sequence manager is invoked from the "File" menu. This command invokes a list box showing all the sequences which have been read into spin together with their ranges, lengths and whether they are DNA (D) or Protein (P). The currently active horizontal and vertical sequences are marked with "H" and "V" respectively. In the picture below, these are "hsproperd" and "mmproper".

Clicking on the sequence name in the sequence manager with the right mouse button invokes a pop-up menu containing operations which may be performed on that sequence. The operations available depends on whether the sequence is DNA or protein.

These options are described in greater detail below.

8.8.3.1 Change the active sequence

To change the currently active horizontal or vertical sequence, use either the "Sequences" menu or select the sequence from the Sequence manager. Select "Horizontal" or "Vertical" from the menu. This sequence will now be the active "Horizontal" or "Vertical" sequence.

8.8.3.2 Set the range

If you are only interested in a particular region of a sequence, it is possible to specify the start and end positions of this region to create a new entry in the Sequence manager. The new sequence will have the same name as the parent, with the addition of a ",s" plus a unique number. The third entry in the picture above shows the range has been set from 100 to 1000, giving a total length of 901 bases for the sequence "hsproperd".
8.8.3.3 Copy Sequence

This option in the Sequences menu allows a sequence to be duplicated or copied. This simply creates a new entry in the Sequence Manager. The user can select which sequence and the segment start and end points to be copied.

8.8.3.4 Sequence type

This option allows the user to change the status of a sequence to become either circular or linear.

8.8.3.5 Complement sequence

This function will reverse and complement nucleic acid sequences. Select the "Complement" command from either the "Sequences" menu or the sequence manager pop-up menu. A new sequence will be added to the sequence manager list box with the same name as the parent but with ".c" appended to the end. The forth entry in the picture above is the complemented sequence of "hsproperd".

8.8.3.6 Interconvert t and u

This function interconverts T and U characters i.e. between DNA and RNA. A new sequence is added to the sequence manager list box with the same name as the parent but the addition to the end of ".r". The fifth entry is the picture above is the transcribed sequence of "hsproperd".

8.8.3.7 Translate sequence

This operation is only available for DNA sequences. Select the "Translate" command from either the "Sequences" menu or the sequence manager pop-up menu. It is possible to translate in any particular frame by selecting the appropriate check box. For each translation, a new sequence will be added to the sequence manager list box with the same name as the parent but with the addition to the end of either ".rf1", ".rf2" or ".rf3" to signify reading frames 1, 2 or 3 respectively.

The "all together" option will produce a single new sequence in the sequence manager, with the extension ".rf123", exemplified by the ninth entry in the picture above. Although at this point the sequence is still DNA, when it is used in a comparison function the program will translate it automatically into the three reading frames. The important point is that the results from the three reading frames will be superimposed in the plot, hence enabling frameshift errors to be spotted.

For example to compare a DNA sequence in all it’s reading frames with a protein:
1. Convert the DNA sequence using the "all together" command
2. Select this sequence as horizontal
3. Select the protein sequence as vertical
4. Invoke the relevant comparison function

8.8.3.8 Scramble sequence

This function produces a version of a given DNA or protein sequence in which the characters are randomly reordered. i.e. the new sequence has the same length and composition as the original but with the characters in a random order. The new sequence will be added to the sequence manager list box with the same name as the parent except with ".x" plus a unique number appended to the end. The tenth entry in the picture above is the scrambled version of "hsproperd". For long sequences, scrambling and then comparing should produce similar numbers of matches as are predicted by the probability calculations (see Section 8.5.1 [Probabilities and expected number of matches], page 350).
8.8.3.9 Rotate sequence

This function allows the user to specify a new origin for a sequence. A new sequence is added to the sequence manager list box with the same name as the parent except with ".0" plus a unique number appended to the end. The eleventh entry in the picture above is of a rotated version of "mmproper". This operation is not allowed for sub-sequences ie those created using "Set range".

8.8.3.10 Save sequence

To save a sequence to a file, select the "Save" option from either the "File" menu, the "Sequences" menu or the sequence manager pop-up menu. This command invokes a file name entry box. The browse button to the right of the dialogue box invokes a filebrowser. See Section 9.6 [File Browser], page 379. The sequence is written as either EMBL or FASTA format. If EMBL is selected and the sequence has an associated feature table, the feature table will also be written out (see Section 8.8.1 [Use of feature tables in spin], page 367).

8.8.3.11 Delete sequence

To delete a sequence, select the "Delete" option from either the "Sequences" menu or the sequence manager pop-up menu. This command will remove the sequence from the sequence manager and all plots and results that were produced from it.

8.8.4 Selecting a sequence

All the comparison functions request a horizontal and a vertical sequence and the ranges over which the function will operate. It is therefore possible to compare the same sequence over different ranges. The default sequences which appear when the dialogue box for the comparison function is brought up, are the current "active" sequences (see Section 8.8.3 [Sequence manager], page 369). To select a different sequence for this invocation of the function, press the Browse button to the right of the "Seq identifier" box. This will invoke a Sequence manager if one is not already displayed. Clicking with the left mouse button on the name of the required sequence in the sequence manager will update the function dialogue box with the sequence name and it’s currently defined range. To change the range, enter the new start and end positions. These positions will be remembered for future invocations of any of the comparison functions for this sequence.


9 User Interface

Introduction

This chapter describes the graphical user interface implemented in the programs gap4, pregap4, spin and trev.

9.1 Basic Interface Controls

The key components of any graphical interface are menus, buttons and text entry boxes. These are usually grouped together into 'panels' or 'dialogues'. For the sake of simplifying the rest of the documentation we describe the operation and control of the user interface here.

9.1.1 Buttons

There are three basic types of button: command buttons, radio buttons and check buttons. Buttons are used by moving the mouse pointer over the button (it will then be highlighted, as is "Choice 3" below) and pressing the left mouse button. The illustration below demonstrates each of the three types.

The buttons labelled "Command 1", "Command 2" and "Command 3" are command buttons. These perform a particular action, which is usually determined by the text within the button. Typically command buttons have a slightly raised look. A typical example is the "Clear" button visible on the main gap4 window. See the illustration in Section 9.3 [Output and Error Windows], page 376.

The buttons underneath, labelled "Choice 1", "Choice 2" and "Choice 3" are radio buttons. These have small diamonds to the left of each name. Only one of these boxes in each group of radio buttons (it is possible to have several distinct groups) may be set at any one time. In this example "Choice 1" has been selected. For example, selecting "Choice 3" will now clear the diamond next to "Choice 1" and fill the diamond next to "Choice 3".

The bottom row of buttons are check buttons. These each have small boxes to the left of each name. They act similarly to radio buttons except that more than one can be selected at any one time. Here we have "Check 2" and "Check 3" selected, with "Check 1" deselected. Pressing a check button will toggle it; so clicking the left mouse button on "Check 2" would deselect it and clear the neighbouring box. The "Scroll on output" button is an example of a check button. See the illustration in Section 9.3 [Output and Error Windows], page 376.

9.1.2 Menus

When many operations are available it is impractical to arrange them all in command buttons. For this reason we have menus. Typically we will use a "menubar" consisting of several menus arranged side by side.
The menubar is a series of menu buttons arranged side by side. In the above picture, the menus are "File" through to "Help". Selecting an item from a menu is done by pressing and holding the left mouse button whilst the cursor is above the menu button. The available menu choices will then be displayed. Whilst still pressing the mouse button, move down to the desired choice and then release the mouse button. Releasing the mouse button when the mouse cursor is not over a menu item will remove the menu without executing any options. Alternatively, it is possible to press and release the left mouse button whilst the cursor is above a menu button. The menu options will be revealed. Now move down and press and release the left button once more once on the selected item.
To see an overview of the menu contents press the left mouse button over a menu button and move the mouse cursor over the other menu buttons. As each menu button is highlighted the appropriate options for this menu will be shown.

Some menu items lead to further menus. These are called cascading menus. Treat these exactly as normal menus.

To tear off a menu pull down the menu using the left mouse button, select the dashed perforation line, and release the button. The menu will be redrawn with a title bar which can be used to move it to any position on the screen. Not all menus support tearing off.

### 9.1.3 Text Windows

A text window is simply an area of the screen set aside for displaying textual information. A typical example is the Output and Error windows seen on the main gap4 screen. See the illustration in Section 9.3 [Output and Error Windows], page 376.

The most basic use of text windows is to display data. If the data is large then there will usually be scrollbars on the right and bottom sides of the text display. If the data is of an editable nature (such as the comments in a tag in gap4) we may perform many editing operations on the text. The simplest commands follow.

- **Arrow keys**: Moves the editing cursor
- **Left mouse button**: Sets the editing cursor
- **Middle mouse button**: Panning - controls both scrollbars at once
- **Alt left mouse button**: Panning - controls both scrollbars at once
- **Delete**: Deletes the character to the left of the cursor
- **Most other keys**: Adds text to the window

In addition to the above, some more advanced features are available, mostly following the **Emacs** style of key bindings.

- **Delete**: Delete region (when highlighted), otherwise as above
- **Control D**: Delete character to the right of the cursor
- **Control N**: Down one line
- **Control P**: Up one line
- **Control B**: Move back on character
- **Control F**: Move forward on character
- **Control A**: Move to start of line
- **Control E**: Move to end of line
- **Meta b**: Move back one word
- **Meta f**: Move forward one word
- **Meta <**: Move to start
- **Meta >**: Move to end
- **Control Up**: Move up one paragraph
- **Control Down**: Move down one paragraph
- **Next**: Move done one page
- **Prev**: Move up one page
- **Control K**: Delete to end of line
- **Control T**: Transpose two characters
- **Drag left button**: Highlights a region (for cut and paste)
- **Control /**: Select all (for cut and paste)
- **Control \**: Deselect all (for cut and paste)
9.1.4 Text Entry Boxes

An entry box is basically a small, one line, text window. All of the same editing commands exist, although many are redundant for such a small window.

A typical entry box can be seen in the gap4 dialogue for opening new databases. Here the ringed region to the right of the "Enter new filename" text is the entry box. The current contents of this entry is "file". The vertical black line visible is the text entry point.

9.2 Standard Mouse Operations

The same mouse buttons are used for similar operations throughout the programs. A brief description of the mouse control is listed below. On UNIX three button mice are used, but on Windows or Linux two buttons are more common, and so the alternative of Alt-left-mouse button is used for the middle button.

**Left button Select**

In a dialogue this selects an item from a list of items. Within a graphical display (eg the template display) this "selects" an item. Selected items are shown in bold. Selecting an already selected item will deselect it.

**Drag left button Select region**

This operates only for the graphical displays. A rectangular box can be dragged out between where the left button was pressed (and held down) to the current mouse cursor position. Releasing the left button will then select all items contained entirely within the rectangle. Within the contig editor such selections are displayed by underlining the region instead.

**Drag middle button Move**

This currently operates only for the contig selector. The selected items (or the item under the mouse pointer if none are selected) are dragged until the middle button is released.

**Drag Alt left button Move**

This currently operates only for the contig selector. The selected items (or the item under the mouse pointer if none are selected) are dragged until the button is released.

**Right button Popup menu**

Within some displays this will pop up a menu displaying a list of commands that can be used on the selected item.

9.3 The Output and Error Windows

The main screen has three portions; the menubar, the output window and the error window. Of these, the output and error windows are identical except for the data that appears within them. Here we describe the general operation of the output window only, although the details apply to the error window too.
The output window consists of a text window with a set of labels and buttons above it. At the top left is the window name followed by a colon. After the colon the name of the current output file will be shown in blue italic letters. All new output appearing in this window will also be sent to this file. Initially no output file is specified and this label is blank (as can be seen in the error window). Using the "Redirect" menu located at the top right of the window a new file can be opened, or an existing one closed (in which case output is no longer sent to the specified file). The output and error windows may both have redirection files.

The "Search" button invokes a dialogue box requesting a string to search and whether to search forwards or backwards from the current position of the cursor. The search is case insensitive. Hitting the the OK button finds the next match. The bell is sounded if no more matches can be found.

The "Scroll on output" check button toggles whether the window should automatically scroll when new output appears to ensure that it is visible. The default (as seen in the illustration) state is to scroll. The "Clear" button removes all output from the window.

Each command, when run, adds a title to the output window. This contains the current time together with the command name. Output for this command then appears beneath the header. In the illustration the output from three commands is visible. Of these the "edit contig" command produces no output, but still has a header.

Pressing the right button with the cursor above a piece of output (either in its header or the text beneath it) will pop up a menu of operations. This operation is not valid for the error window. The commands are:

**Show input parameters**

Inserts the input parameters of the command, if any, beneath the command header

**Remove**

Deletes this text from the output window

**Output to disk**

Sends this text to a specified file
Output to list

Sends this text to a specified list

The output operations allow the user to specify whether the header, input parameters or text for the command, in any combination, are sent to the output.

The text in the error window has a different format to the output window. Instead of large portions of text separated by headers, each item in the error window consists of a single line containing the date, the name of the function producing the error, and a brief description of the error. Many error messages will be displayed in their own dialogue boxes (eg not having write access to a file) and hence will not appear in the error window. Each time an error message is added the bell is rung.

9.4 Graphics Window

The graphical displays have several features in common. Commands are selected from buttons and menus ranged along the top of the window. Menus can be "torn off" and positioned anywhere on the screen. Zooming is allowed using the mouse. The "Zoom out" button undoes the previous zoom command. Crosshairs and cursors can be toggled on and off, and their coordinates in base positions appear in boxes in the top right hand corner of the displays. Items plotted in the graphical displays have text attached, and as the cursor passes over an item, it is highlighted and its text appears in an Information line at the bottom of the display.

9.4.1 Zooming

Plots can be enlarged either by resizing the window or zooming. In some plots zooming is achieved by holding down the control key and right mouse button and dragging out a rectangle. Rectangles that are too small are ignored and a warning bell will sound. The content of the window is magnified such that the contents of the zoom box fill the window. The Zoom out button will restore the plot to the previous magnification. In other plots, x and y scale boxes achieve similar effects.

9.5 Colour Selector

A common operation is to change the colour of a plot. For this we use the colour selector dialogue shown below. The three sliders control the red, green, and blue intensities to use in producing the desired colour. The shaded box at the bottom illustrates the current colour. In some displays this will also interactively update the colour in the associated plot simultaneously.
Pressing the "OK" button will quit the colour selector and update the appropriate colours in the plot. Pressing "Cancel" will quit the colour selector without making any changes to the plot. Note that some colour dialogues may also be combined with extra controls for adjusting other graphical styles, such as the line width.

Many programs have a "Colours" command in the Options menu. This displays two colour selectors; one for each of the foreground and background colours. This can be used to adjust the main colour scheme used for the program. Pressing "OK" selects this colour scheme and keeps it in use until the program exits. Pressing "OK Permanent" accepts this colour scheme, but also updates the ‘$HOME/.tk_utilsrc’ file. This means that the colour scheme will be used for all future program uses. To revert to the default colours, manually edit the ‘$HOME/.tk_utilsrc’ file.

9.6 File Browser

The file browser is a dialogue that allows the user to select files from any directory. It is typically used when choosing a file for a particular action, such as opening a database in gap or saving a trace file in trev. The precise details of the layout may change depending on this context. In some circumstances, such as loading sequences into spin several files may be selected (in which case the dialogue will be titled "Open multiple files"), in others only a single file can be selected. The illustration below shows the file browser as displayed when opening files from within trev. The ‘Formats’ and ‘Filter’ section here are used to select different file types. These dialogue components may not appear in all file browsers.
The ‘OK’, ‘Filter’ and ‘Cancel’ buttons perform their usual tasks; ‘OK’ accepts the file currently shown in the selection component, and ‘Cancel’ quits the dialogue.

9.6.1 Directories and Files

The main component of the file browser dialogue consists of two scroll lists placed side by side. The left list is labelled "Directory" and shows a list of other directories to choose from. The right list is the list of files in the currently displayed directory.

Double clicking with the left mouse button on a directory updates the file list. If a filter file browser component is visible then the current directory will be displayed as the start of the filter. The directory named ".." is the parent directory of the current directory.

Single clicking on a file name updates the Selection component. Double clicking on a file name chooses this file and removes the dialogue. That is, it is equivalent to single clicking on the file to update the selection followed by pressing the ‘OK’ button.

When the dialogue allows multiple files to be selected (then titled "Open multiple files") holding down the Ctrl key will retain items already chosen.

9.6.2 Filters

The top component of the file browser shown in the introduction contained a Filter component. This consists of a text entry window containing a string of the form directory_name/file_pattern.

Some dialogues may not have a Filter component. In these cases the file_pattern is taken to be ".". Hence all files will be listed. The directory_name is the directory that the current list of files are contained within. The file_pattern is used to specify which of the files within this directory should be listed in the file list. The pattern uses the same form as UNIX shell wild card matching. To summarise this see the following table of simple examples.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>*</td>
<td>Every filename</td>
</tr>
<tr>
<td>xb*</td>
<td>Every filename starting in &quot;xb&quot;</td>
</tr>
<tr>
<td>a*b</td>
<td>Every filename starting with &quot;a&quot; and ending with &quot;b&quot;</td>
</tr>
<tr>
<td>a?b</td>
<td>Every three letter filename with &quot;a&quot; as the first letter and &quot;b&quot; as the last letter.</td>
</tr>
<tr>
<td>*scf</td>
<td>Every filename ending in &quot;scf&quot;</td>
</tr>
<tr>
<td>*[sS]cf</td>
<td>Every filename ending with &quot;scf&quot; or &quot;Scf&quot;</td>
</tr>
</tbody>
</table>
Every filename ending with "scf" or "SCF".

Some file browsers also include a Formats component. This is used to select the input or output format of the selected file. Updating the format will typically also update the filter.

### 9.7 Font Selection

The Options menu of most programs contains a "Set fonts" command. This brings up a font selection dialogue consisting of some sample text, three option menus to select the font name, family and size, and some check buttons for font styles.

In the above picture, **button_font** is the currently selected font name. This option menu contains several of the following font types. The exact ones available depends on the program being used.

- **button_font**
  - Used for buttons, labels, checkbuttons and radiobuttons.

- **menu_font**
  - Used for menu buttons and their contents, including pull down menu contents.

- **text_font**
  - Used for textual displays, such as the main output windows. This should be chosen to be a fixed width font, such as **Courier**.

- **sheet_font**
  - Used for the scrolled text displays such as the contig editor in gap4 and the sequence displays in spin. This too needs to be chosen as a fixed width font.

- **title_font**
  - Used as for headings within text windows such as contig names in the gap4 suggest probes function.

- **menu_title_font**
  - Used in the title line of popup menus.

- **trace_font**
  - Used for the sequence and number displays in the trace displays for both gap4 and Trev.

Next to the font name is the font family selector. The contents of this menu will depend on the fonts available to your system. Some may be inappropriate, or not even in the correct language. Next to the family selector is the size menu. This contains a range of sizes in both pixel and point units. If a font of a particular size is not available, the nearest font or size will be automatically chosen. Specifying fonts to be a fixed number of points states that the font should have a specific physical size, regardless of monitor size or screen resolution. There are 72.27 points to the inch. Underneath these we have Bold, Italic, Overstrike and Underline check buttons.

Whilst choosing the font, the fonts used in the entire program automatically update to show you how things will look. Pressing "Cancel" will reset the fonts back to their original state. Pressing "OK" will keep these chosen fonts, until the program is exited. Pressing "OK Permanent" will keep these fonts, but will also add them to the user’s `$HOME/.tk_utilsrc` file. This file is processed when the programs start up, and so your font choice will be permanently chosen. To remove this font choice, manual editing of the `$HOME/.tk_utilsrc` file is required.
10 File Formats

This section introduces the various file formats used by the package, but first we describe some limitations on the names of files.

There are restrictions on the characters used in file names and the length of the file names.

Characters permitted in file names:

QWERTYUIOPASDFGHJKLZXCVBNMqwertyuiopasdfghjklzxcvbnm1234567890.

A reading name or experiment file name used in a sequence assembly project must not be longer than 16 characters.

These restrictions also apply to SCF files which means, in turn, also to the names given to samples obtained from sequencing instruments. For example do not give sample names such as 27/OCT/96/r.1 when using and ABI machine: the / symbols will be interpreted as directory name separators on UNIX!

Currently the formats used by the package include the following.

10.1 SCF

SCF format files are used to store data from DNA sequencing instruments. Each file contains the data for a single reading and includes: its trace sample points, its called sequence, the positions of the bases relative to the trace sample points, and numerical estimates of the accuracy of each base. Comments and "private data" can also be stored. The format is machine independent and the first version was described in Dear, S and Staden, R. "A standard file format for data from DNA sequencing instruments", DNA Sequence 3, 107-110, (1992).

Since then it has undergone several important changes. The first allowed for different sample point resolutions. The second, in response to the need to reduce file sizes for large projects, involved a major reorganisation of the ordering of the data items in the file and also in the way they are represented. Note that despite these changes we have retained the original data structures into which the data is read. Also this reorganisation in itself has not made the files smaller but it has produced files that are more effectively compressed using standard programs such as gzip. The io library included in the package contains routines that can read and write all the different versions of the format (including reading of compressed files). The header record was not affected by this change. This documentation covers both the format of scf files and the data structures that are used by the io library. Prior to version 3.00 these two things corresponded much more closely.

10.1.1 Header Record

The file begins with a 128 byte header record that describes the location and size of the chromatogram data in the file. Nothing is implied about the order in which the components (samples, sequence and comments) appear. The version field is a 4 byte character array representing the version and revision of the SCF format. The current value of this field is "3.00".

/*
 * Basic type definitions
 */
typedef unsigned int uint_4;
typedef signed int int_4;
typedef unsigned short uint_2;
typedef signed short int_2;
typedef unsigned char uint_1;
typedef signed char int_1;

/* Type definition for the Header structure */
#define SCF_MAGIC ((((((uint_4)'.'<<8)+(uint_4)'s'<<8) \\
+ (uint_4)'c'<<8)+(uint_4)'f'))

typedef struct {
    uint_4 magic_number; /* Number of elements in Samples matrix */
    uint_4 samples; /* Byte offset from start of file */
    uint_4 bases; /* Number of bases in Bases matrix */
    uint_4 bases_left_clip; /* OBSOLETE: No. bases in left clip (vector) */
    uint_4 bases_right_clip; /* OBSOLETE: No. bases in right clip (qual) */
    uint_4 bases_offset; /* Byte offset from start of file */
    uint_4 comments_size; /* Number of bytes in Comment section */
    uint_4 comments_offset; /* Byte offset from start of file */
    char version[4]; /* "version.revision", eg '3' '0' '0' */
    uint_4 sample_size; /* Size of samples in bytes 1=8bits, 2=16bits*/
    uint_4 code_set; /* code set used (but ignored)!*/
    uint_4 private_size; /* No. of bytes of Private data, 0 if none */
    uint_4 private_offset; /* Byte offset from start of file */
    uint_4 spare[18]; /* Unused */
} Header;

For versions of SCF files 2.0 or greater (Header.version is 'greater than' "2.00"), the version number, precision of data, the uncertainty code set are specified in the header. Otherwise, the precision is assumed to be 1 byte, and the code set to be the default code set. The following uncertainty code sets are recognised (but still ignored by our programs!).

0 {A,C,G,T,-} (default)
1 Staden
2 IUPAC (NC-IUB)
3 Pharmacia A.L.F. (NC-IUB)
4 {A,C,G,T,N} (ABI 373A)
5 IBI/Pustell
6 DNA*
7 DNASIS
8 IG/PC-Gene
9 MicroGenie

10.1.2 Sample Points.

The trace information is stored at byte offset Header.samples_offset from the start of the file. For each sample point there are values for each of the four bases. Header.sample_size holds the precision of the sample values. The precision must be one of "1" (unsigned byte) and "2" (unsigned short). The sample points need not be normalised to any particular value, though it is assumed that they represent positive values. This is, they are of unsigned type.

With the introduction of scf version 3.00, in an attempt to produce efficiently compressed files, the sample points are stored in A,C,G,T order; i.e. all the values for base A, followed by all
those for C, etc. In addition they are stored, not as their original magnitudes, but in terms of
the differences between successive values. The C language code used to transform the values for
precision 2 samples is shown below.

```c
void delta_samples2 ( uint_2 samples[], int num_samples, int job) {
    /* If job == DELTA_IT:
        * change a series of sample points to a series of delta delta values:
        * ie change them in two steps:
        * first: delta = current_value - previous_value
        * then: delta_delta = delta - previous_delta
        * else
        * do the reverse
    */
    int i;
    uint_2 p_delta, p_sample;

    if ( DELTA_IT == job ) {
        p_delta = 0;
        for (i=0;i<num_samples;i++) {
            p_sample = samples[i];
            samples[i] = samples[i] - p_delta;
            p_delta = p_sample;
        }
        p_delta = 0;
        for (i=0;i<num_samples;i++) {
            p_sample = samples[i];
            samples[i] = samples[i] - p_delta;
            p_delta = p_sample;
        }
    }
    else {
        p_sample = 0;
        for (i=0;i<num_samples;i++) {
            samples[i] = samples[i] + p_sample;
            p_sample = samples[i];
        }
        p_sample = 0;
        for (i=0;i<num_samples;i++) {
            samples[i] = samples[i] + p_sample;
            p_sample = samples[i];
        }
    }
}
```

The io library data structure is as follows:

```c
/*
 * Type definition for the Sample data
 */
typedef struct {
    uint_1 sample_A;       /* Sample for A trace */
```
typedef struct {
    uint_2 sample_A;  /* Sample for A trace */
    uint_2 sample_C;  /* Sample for C trace */
    uint_2 sample_G;  /* Sample for G trace */
    uint_2 sample_T;  /* Sample for T trace */
} Samples2;

10.1.3 Sequence Information.

Information relating to the base interpretation of the trace is stored at byte offset Header.bases_offset from the start of the file. Stored for each base are: its character representation and a number (an index into the Samples data structure) indicating its position within the trace. The relative probabilities of each of the 4 bases occurring at the point where the base is called can be stored in prob_A, prob_C, prob_G and prob_T.

From version 3.00 these items are stored in the following order: all "peak indexes", i.e. the positions in the sample points to which the bases corresponds; all the accuracy estimates for base type A, all for C,G and T; the called bases; this is followed by 3 sets of empty int1 data items. These values are read into the following data structure by the routines in the io library.

    /*
    * Type definition for the sequence data
    */
    typedef struct {
        uint_4 peak_index;  /* Index into Samples matrix for base posn */
        uint_1 prob_A;  /* Probability of it being an A */
        uint_1 prob_C;  /* Probability of it being an C */
        uint_1 prob_G;  /* Probability of it being an G */
        uint_1 prob_T;  /* Probability of it being an T */
        char base;  /* Called base character */
        uint_1 spare[3];  /* Spare */
    } Base;

10.1.4 Comments.

Comments are stored at offset Header.comments_offset from the start of the file. Lines in this section are of the format:

    <Field-ID>=<Value>

<Field-ID> can be any string, though several have special meaning and their use is encouraged.

<table>
<thead>
<tr>
<th>ID</th>
<th>Field</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACH</td>
<td>Sequencing machine model</td>
<td>MACH=Pharmacia A.L.F.</td>
</tr>
<tr>
<td>TPSW</td>
<td>Trace processing software version</td>
<td>TPSW=A.L.F. Analysis Program, Version=1.67</td>
</tr>
<tr>
<td>BCSW</td>
<td>Base calling software version</td>
<td>BCSW=A.L.F. Analysis Program, Version=1.67</td>
</tr>
<tr>
<td>DATF</td>
<td>Data source format</td>
<td>DATF=AM_Version=2.0</td>
</tr>
<tr>
<td>DATN</td>
<td>Data source name</td>
<td>DATN=a10c.alf</td>
</tr>
</tbody>
</table>
CONV Format conversion software CONV=makeSCF v2.0

Other fields might include:

<table>
<thead>
<tr>
<th>Field</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>Example</td>
</tr>
<tr>
<td>OPER</td>
<td>OPER=sd</td>
</tr>
<tr>
<td>STRT</td>
<td>STRT=Aug 05 1991 12:25:01</td>
</tr>
<tr>
<td>STOP</td>
<td>STOP=Aug 05 1991 16:26:25</td>
</tr>
<tr>
<td>PROC</td>
<td>PROC=Aug 05 1991 18:50:13</td>
</tr>
<tr>
<td>EDIT</td>
<td>EDIT=Aug 05 1991 19:06:18</td>
</tr>
<tr>
<td>NAME</td>
<td>NAME=a21b1.s1</td>
</tr>
<tr>
<td>SIGN</td>
<td>SIGN=A=56,C=66,G=13,T=18</td>
</tr>
<tr>
<td>SPAC</td>
<td>SPAC=12.04</td>
</tr>
<tr>
<td>SCAL</td>
<td>SCAL=0.5</td>
</tr>
<tr>
<td>ACMQ</td>
<td>ACMQ=99,6</td>
</tr>
<tr>
<td>ASTP</td>
<td>ASTP=143,12</td>
</tr>
</tbody>
</table>

/*
 * Type definition for the comments
 */
typedef char Comments[]; /* Zero terminated list of
  \n separated entries */

10.1.5 Private data.

The private data section is provided to store any information required that is not supported by the SCF standard. If the field in the header is 0 then there is no private data section. We impose no restrictions upon the format of this section. However we feel it maybe a good idea to use the first four bytes as a magic number identifying the used format of the private data.

10.1.6 File structure.

From SCF version 3.0 onwards the in memory structures and the data on the disk are not in the same format. The overview of the data on disk for the different versions is summarised below.

**Versions 1 and 2**

(Note Samples1 can be replaced by Samples2 as appropriate.)

<table>
<thead>
<tr>
<th>Length in bytes</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>header</td>
</tr>
<tr>
<td>Number of samples * 4 * sample size</td>
<td>Samples1 or Samples2 structure</td>
</tr>
<tr>
<td>Number of bases * 12</td>
<td>Base structure</td>
</tr>
<tr>
<td>Comments size</td>
<td>Comments</td>
</tr>
<tr>
<td>Private data size</td>
<td>private data</td>
</tr>
</tbody>
</table>

**Version 3**

<table>
<thead>
<tr>
<th>Length in bytes</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>header</td>
</tr>
<tr>
<td>Number of samples * sample size</td>
<td>Samples for A trace</td>
</tr>
<tr>
<td>Field</td>
<td>Description</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Number of samples * sample size</td>
<td>Samples for C trace</td>
</tr>
<tr>
<td>Number of samples * sample size</td>
<td>Samples for G trace</td>
</tr>
<tr>
<td>Number of samples * sample size</td>
<td>Samples for T trace</td>
</tr>
<tr>
<td>Number of bases * 4</td>
<td>Offset into peak index for each base</td>
</tr>
<tr>
<td>Number of bases</td>
<td>Accuracy estimate bases being 'A'</td>
</tr>
<tr>
<td>Number of bases</td>
<td>Accuracy estimate bases being 'C'</td>
</tr>
<tr>
<td>Number of bases</td>
<td>Accuracy estimate bases being 'G'</td>
</tr>
<tr>
<td>Number of bases</td>
<td>Accuracy estimate bases being 'T'</td>
</tr>
<tr>
<td>Number of bases</td>
<td>The called bases</td>
</tr>
<tr>
<td>Number of bases * 3</td>
<td>Reserved for future use</td>
</tr>
<tr>
<td>Comments size</td>
<td>Comments</td>
</tr>
<tr>
<td>Private data size</td>
<td>Private data</td>
</tr>
</tbody>
</table>

### 10.1.7 Notes

#### 10.1.7.1 Byte ordering and integer representation.

"Forward byte and reverse bit" ordering will be used for all integer values. This is the same as used in the MC680x0 and SPARC processors, but the reverse of the byte ordering used on the Intel 80x86 processors.

```
+-------+-------+
| MSB   | LSB   |
+-------+-------+
```

To read integers on systems with any byte order use something like this:

```c
uint_2 read_uint_2(FILE *fp)
{
    unsigned char buf[sizeof(uint_2)];
    fread(buf, sizeof(buf), 1, fp);
    return (uint_2)
           (((uint_2)buf[1]) +
            ((uint_2)buf[0]<<8));
}
```

```c
uint_4 read_uint_4(FILE *fp)
{
    unsigned char buf[sizeof(uint_4)];
    fread(buf, sizeof(buf), 1, fp);
    return (uint_4)
           (((unsigned uint_4)buf[3]) +
            ((unsigned uint_4)buf[2]<<8) +
            ((unsigned uint_4)buf[1]<<16) +
            ((unsigned uint_4)buf[0]<<24));
}
```
10.1.7.2 Compression of SCF Files

The SCF format version 3.00 has been designed with file compression in mind. No new information is recorded when compared to the version 2.02 format, except the data is stored in a manner conducive to efficient compression.

Experimentation\(^1\) has shown that 16 bit SCF version 3.00 files can achieve a 9:1 compression ratio and 8 bit SCF files a 14.5:1 compression ratio. These figures are for SCF files without quality values compressed using the \textit{bzip} utility. \textit{gzip} tends to give between 20 to 40\% larger files than \textit{bzip}. Compressed SCF files containing accuracy values tend to be around 10\% larger than those without accuracy values.

Whilst compression is not a specific part of the SCF standard, the size of trace files and the compression ratios attainable suggests that it is wise to handle compressed files. The Staden Package utilities, such as gap4 and trev, automatically uncompress and compress SCF files as needed.

Note that at present, on the fly compression, as just described, is not implemented for the Windows version of the package.

10.2 ZTR

The ZTR format is used for storing analogue chromatogram data from DNA sequencing instruments.

10.2.1 Header

The header consists of an 8 byte magic number (see below), followed by a 1-byte major version number and 1-byte minor version number.

Changes in minor numbers should not cause problems for parsers. It indicates a change in chunk types (different contents), but the file format is the same.

The major number is reserved for any incompatible file format changes (which hopefully should be never).

\begin{verbatim}
/* The header */
typedef struct {
    unsigned char magic[8]; /* 0xae5a54520d0a1a0a (be) */
    unsigned char version_major; /* 1 */
    unsigned char version_minor; /* 2 */
} ztr_header_t;

/* The ZTR magic numbers */
#define ZTR_MAGIC \0256ZTR\r\n\032
#define ZTR_VERSION_MAJOR 1
#define ZTR_VERSION_MINOR 2
\end{verbatim}

So the total header will consist of:

\begin{verbatim}
<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+=</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
\end{verbatim}

\(^1\) Analysed using a data set of 100 ABI (and their SCF equivalent) files
Hex values | ae 5a 54 52 0d 0a 1a 0d | 01 02 |

+--+--+--+--+--+--+--+--+--+--+

10.2.2 Chunk Format

The basic structure of a ZTR file is (header,chunk*) - ie header followed by zero or more chunks. Each chunk consists of a type, some meta-data and some data, along with the lengths of both the meta-data and data.

Byte number 0 1 2 3 4 5 6 7 8 9

+--+--+--+--+--+--+--+--+--+--+--+

Hex values | type |meta-data length | meta-data |data length| data .. |

+--+--+--+--+--+--+--+--+--+--+--+

Ie in C:

typedef struct {
    uint4 type; /* chunk type (be) */
    uint4 mdlength; /* length of meta-data field (be) */
    char *mdata; /* meta data */
    uint4 dlength; /* length of data field (be) */
    char *data; /* a format byte and the data itself */
} ztr_chunk_t;

All 2 and 4-byte integer values are stored in big endian format.

The meta-data is uncompressed (and so it does not start with a format byte). The format of the meta-data is chunk specific, and many chunk types will have no meta-data. In this case the meta-data length field will be zero and this will be followed immediately by the data-length field.

The data length is the length in bytes of the entire 'data' block, including the format information held within it.

The first byte of the data consists of a format byte. The most basic format is zero - indicating that the data is "as is"; it's the real thing. Other formats exist in order to encode various filtering and compression techniques. The information encoded in the next bytes will depend on the format byte.

10.2.2.1 Data format 0 - Raw

Byte number 0 1 2 N

+--+--+--+--+--+

Hex values | 0| raw data |

+--+--+--+--+--+

Raw data has no compression or filtering. It just contains the unprocessed data. It consists of a one byte header (0) indicating raw format followed by N bytes of data.

10.2.2.2 Data format 1 - Run Length Encoding

Byte number 0 1 2 3 4 5 6 7 8 N

+--+--+--+--+--+--+--+--+--+--+--+

Hex values | 1| Uncompressed length | guard | run length encoded data |

+--+--+--+--+--+--+--+--+--+--+--+
Run length encoding replaces stretches of N identical bytes (with value V) with the guard byte G followed by N and V. All other byte values are stored as normal, except for occurrences of the guard byte, which is stored as G 0. For example with a guard value of 8:

Input data:
20 9 9 9 9 10 9 8 7

Output data:
1 (rle format)
0 0 0 10 (original length)
8 (guard)
20 8 5 9 10 9 8 0 7 (rle data)

10.2.2.3 Data format 2 - ZLIB

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>2</td>
<td>Uncompressed length</td>
<td>Zlib encoded data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
+-------------+-----------+-------------------+
+-------------+-----------+-------------------+

This uses the zlib code to compress a data stream. The ZLIB data may itself be encoded using a variety of methods (LZ77, Huffman), but zlib will automatically determine the format itself. Often using zlib mode Z_HUFFMAN_ONLY will provide best compression when combined with other filtering techniques.

10.2.2.4 Data format 64/0x40 - 8-bit delta

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>40</td>
<td>Delta level</td>
<td>data</td>
<td></td>
</tr>
</tbody>
</table>
+-------------+-----------+-------+
+-------------+-----------+-------+

This technique replaces successive bytes with their differences. The level indicates how many rounds of differencing to apply, which should be between 1 and 3. For determining the first difference we compare against zero. All differences are internally performed using unsigned values with automatic an wrap-around (taking the bottom 8-bits). Hence 2-1 is 1 and 1-2 is 255.

For example, with level set to 1:

Input data:
10 20 10 200 190 5

Output data:
1 (delta1 format)
1 (level)
10 10 246 190 246 71 (delta data)

For level set to 2:

Input data:
10 20 10 200 190 5

Output data:
1 (delta1 format)
2 (level)
10 0 236 200 56 81 (delta data)

10.2.2.5 Data format 65/0x41 - 16-bit delta

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>41</td>
<td>Delta level</td>
<td>data</td>
<td></td>
</tr>
</tbody>
</table>

This format is as data format 64 except that the input data is read in 2-byte values, so we take the difference between successive 16-bit numbers. For example "0x10 0x20 0x30 0x10" (4 8-bit numbers; 2 16-bit numbers) yields "0x10 0x20 0x1f 0xf0". All 16-bit input data is assumed to be aligned to the start of the buffer and is assumed to be in big-endian format.

10.2.2.6 Data format 66/0x42 - 32-bit delta

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>42</td>
<td>Delta level</td>
<td>0</td>
<td>0</td>
<td>data</td>
<td></td>
</tr>
</tbody>
</table>

This format is as data formats 64 and 65 except that the input data is read in 4-byte values, so we take the difference between successive 32-bit numbers.

Two padding bytes (2 and 3) should always be set to zero. Their purpose is to make sure that the compressed block is still aligned on a 4-byte boundary (hence making it easy to pass straight into the 32to8 filter).

10.2.2.7 Data format 67-69/0x43-0x45 - reserved

At present these are reserved for dynamic differencing where the 'level' field varies - applying the appropriate level for each section of data. Experimental at present...

10.2.2.8 Data format 70/0x46 - 16 to 8 bit conversion

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>46</td>
</tr>
</tbody>
</table>

This method assumes that the input data is a series of big endian 2-byte signed integer values. If the value is in the range of -127 to +127 inclusive then it is written as a single signed byte in the output stream, otherwise we write out -128 followed by the 2-byte value (in big endian format). This method works well following one of the delta techniques as most of the 16-bit values are typically then small enough to fit in one byte.

Example input data:

0 10 0 5 -1 -5 0 200 -4 -32 (bytes)
(As 16-bit big-endian values: 10 5 -5 200 -800)

Output data:

70 (16-to-8 format)
10 5 -5 -128 0 200 -128 -4 -32
10.2.2.9 Data format 71/0x47 - 32 to 8 bit conversion

Byte number 0

+---+-

Hex values |47| data |

+---+-

This format is similar to format 70, but we are reducing 32-bit numbers (big endian) to 8-bit numbers.

10.2.2.10 Data format 72/0x48 - "follow" predictor

Byte number 0 1 FF 100 101 N

+---+- - - - -+-

Hex values |48| follow bytes | data |

+---+- - - - -+-

For each symbol we compute the most frequent symbol following it. This is stored in the "follow bytes" block (256 bytes). The first character in the data block is stored as-is. Then for each subsequent character we store the difference between the predicted character value (obtained by using follow[previous character]) and the real value. This is a very crude, but fast, method of removing some residual non-randomness in the input data and so will reduce the data entropy. It is best to use this prior to entropy encoding (such as huffman encoding).

10.2.2.11 Data format 73/0x49 - floating point 16-bit chebyshev polynomial predictor

Version 1.1 only. Replaced by format 74 in Version 1.2.

WARNING: This method was experimental and has been replaced with an integer equivalent. The floating point method may give system specific results.

Byte number 0 1 2 N

+---+-+-

Hex values |49| 0| data |

+---+-+-

This method takes big-endian 16-bit data and attempts to curve-fit it using chebyshev polynomials. The exact method employed uses the 4 preceeding values to calculate chebyshev polynomials with 5 coefficients. Of these 5 coefficients only 4 are used to predict the next value. Then we store the difference between the predicted value and the real value. This procedure is repeated throughout each 16-bit value in the data. The first four 16-bit values are stored with a simple 1-level 16-bit delta function. Reversing the predictor follows the same procedure, except now adding the differences between stored value and predicted value to get the real value.

10.2.2.12 Data format 74/0x4A - integer based 16-bit chebyshev polynomial predictor

Version 1.2 onwards This replaces the floating point code in ZTR v1.1.

Byte number 0 1 2 N

+---+-+-

Hex values |4A| 0| data |

+---+-+-
This method takes big-endian 16-bit data and attempts to curve-fit it using Chebyshev polynomials. The exact method employed uses the 4 preceding values to calculate Chebyshev polynomials with 5 coefficients. Of these 5 coefficients only 4 are used to predict the next value. Then we store the difference between the predicted value and the real value. This procedure is repeated throughout each 16-bit value in the data. The first four 16-bit values are stored with a simple 1-level 16-bit delta function. Reversing the predictor follows the same procedure, except now adding the differences between stored value and predicted value to get the real value.

10.2.3 Chunk Types

As described above, each chunk has a type. The format of the data contained in the chunk data field (when written in format 0) is described below. Note that no chunks are mandatory. It is valid to have no chunks at all. However some chunk types may depend on the existence of others. This will be indicated below, where applicable.

Each chunk type is stored as a 4-byte value. Bit 5 of the first byte is used to indicate whether the chunk type is part of the public ZTR spec (bit 5 of first byte == 0) or is a private/custom type (bit 5 of first byte == 1). Bit 5 of the remaining 3 bytes is reserved - they must always be set to zero.

Practically speaking this means that public chunk types consist entirely of upper case letters (eg TEXT) whereas private chunk types start with a lowercase letter (eg tEXT). Note that in this example TEXT and tEXT are completely independent types and they may have no more relationship with each other than (for example) TEXT and BPOS types.

It is valid to have multiples of some chunks (eg text chunks), but not for others (such as base calls). The order of chunks does not matter unless explicitly specified.

A chunk may have meta-data associated with it. This is data about the data chunk. For example the data chunk could be a series of 16-bit trace samples, while the meta-data could be a label attached to that trace (to distinguish trace A from traces C, G and T). Meta-data is typically very small and so it is never need be compressed in any of the public chunk types (although meta-data is specific to each chunk type and so it would be valid to have private chunks with compressed meta-data if desirable).

The first byte of each chunk data when uncompressed must be zero, indicating raw format. If, having read the chunk data, this is not the case then the chunk needs decompressing or reverse filtering until the first byte is zero. There may be a few padding bytes between the format byte and the first element of real data in the chunk. This is to make file processing simpler when the chunk data consists of 16 or 32-bit words; the padding bytes ensure that the data is aligned to the appropriate word size. Any padding bytes required will be listed in the appropriate chunk definition below.

The following lists the chunk types available in 32-bit big-endian format. In all cases the data is presented in the uncompressed form, starting with the raw format byte and any appropriate padding.

10.2.3.1 SAMP

Meta-data:

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data:
This encodes a series of 16-bit trace samples. The first data byte is the format (raw); the second data byte is present for padding purposes only. After that comes a series of 16-bit big-endian values.

The meta-data for this chunk contains a 4-byte name associated with the trace. If a name is shorter than 4 bytes then it should be right padded with nul characters to 4 bytes. For sequencing traces the four lanes representing A, C, G and T signals have names "A\0\0\0\0", "C\0\0\0\0", "G\0\0\0\0" and "T\0\0\0\0".

At present other names are not reserved, but it is recommended that (for consistency with elsewhere) you label private trace arrays with names starting in a lowercase letter (specifically, bit 5 is 1).

For sequencing traces it is expected that there will be four SAMP chunks, although the order is not specified.

### 10.2.3.2 SMP4

**Meta-data:** none present

**Data:**

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0 1 2 3 4 5 6 7</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The first byte is 0 (raw format). Next is a single padding byte (also 0). Then follows a series of 2-byte big-endian trace samples for the "A" trace, followed by a series of 2-byte big-endian traces samples for the "C" trace, also followed by the "G" and "T" traces (in that order). The assumption is made that there is the same number of data points for all traces and hence the length of each trace is simply the number of data elements divided by four.

This chunk is mutually exclusive with the SAMP chunks. If both sets are defined then the last found in the file should be used. Experimentation has shown that this gives around 3% saving over 4 separate SAMP chunks, but it lacks in

### 10.2.3.3 BASE

**Meta-data:** none present

**Data:**

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0 1 2 3</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>0</td>
<td>base calls</td>
</tr>
</tbody>
</table>

The first byte is 0 (raw format). This is followed by the base calls in ASCII format (one base per byte). The base call case an encoding set should be IUPAC characters [1].

### 10.2.3.4 BPOS

**Meta-data:** none present
Data:

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>0</td>
<td>padding</td>
<td>data</td>
<td>-</td>
<td>data</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This chunk contains the mapping of base call (BASE) numbers to sample (SAMP) numbers; it defines the position of each base call in the trace data. The position here is defined as the numbering of the 16-bit positions held in the SAMP array, counting zero as the first value.

The format is 0 (raw format) followed by three padding bytes (all 0). Next follows a series of 4-byte big-endian numbers specifying the position of each base call as an index into the sample arrays (when considered as a 2-byte array with the format header stripped off).

Excluding the format and padding bytes, the number of 4-byte elements should be identical to the number of base calls. All sample numbers are counted from zero. No sample number in BPOS should be beyond the end of the SAMP arrays (although it should not be assumed that the SAMP chunks will be before this chunk). Note that the BPOS elements may not be totally in sorted order as the base calls may be shifted relative to one another due to compressions.

10.2.3.5 CNF4

Meta-data: none present

Data:

<table>
<thead>
<tr>
<th>Byte number</th>
<th>0</th>
<th>1</th>
<th>N</th>
<th>4N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex values</td>
<td>0</td>
<td>call confidence</td>
<td>A/C/G/T conf</td>
<td></td>
</tr>
</tbody>
</table>

(N == number of bases in BASE chunk)

The first byte of this chunk is 0 (raw format). This is then followed by a series confidence values for the called base. Next comes all the remaining confidence values for A, C, G and T excluding those that have already been written (ie the called base). So for a sequence AGT we would store confidences A1 G2 T3 C1 G1 T1 A2 C2 T2 A3 C3 G3.

The purpose of this is to group the (likely) highest confidence value (those for the called base) at the start of the chunk followed by the remaining values. Hence if phred confidence values are written in a CNF4 chunk the first quarter of chunk will consist of phred confidence values and the last three quarters will (assuming no ambiguous base calls) consist entirely of zeros.

For the purposes of storage the confidence value for a base call that is not A, C, G or T (in any case) is stored as if the base call was T.

The confidence values should be from the "-10 * log10 (1-probability)". These values are then converted to their nearest integral value. If a program wishes to store confidence values in a different range then this should be stored in a different chunk type.

If this chunk exists it must exist after a BASE chunk.

10.2.3.6 TEXT

Meta-data: none present

Data: 0
This contains a series of "identifier\0value\0" pairs.

The identifiers and values may be any length and may contain any data except the nul character. The nul character marks the end of the identifier or the end of the value. Multiple identifier-value pairs are allowable, with a double nul character marking the end of the list.

Identifiers starting with bit 5 clear (uppercase) are part of the public ZTR spec. Any public identifier not listed as part of this spec should be considered as reserved. Identifiers that have bit 6 set (lowercase) are for private use and no restriction is placed on these.

See below for the text identifier list.

10.2.3.7 CLIP

Meta-data: none present

Data:
Byte number 0 1 2 3 4 5 6 7 8
+-+-+-+-+-+-+-+-+-+-+-+-+-+-+-+-+
Hex values | 0| left clip | right clip|
+-+-+-+-+-+-+-+-+-+-+-+-+-+-+-+

This contains suggested quality clip points. These are stored as zero (raw data) followed by a 4-byte big endian value for the left clip point and a 4-byte big endian value for the right clip point. Clip points are defined in units of base calls, starting from 0. (Q: is that correct!?)

10.2.3.8 CR32

Meta-data: none present

Data:
Byte number 0 1 2 3 4
+-+-+-+-+-+-+-+-+
Hex values | 0| CRC-32 |
+-+-+-+-+-+-+-+

This chunk is always just 4 bytes of data containing a CRC-32 checksum, computed according to the widely used ANSI X3.66 standard. If present, the checksum will be a check of all of the data since the last CR32 chunk. This will include checking the header if this is the first CR32 chunk, and including the previous CRC32 chunk if it is not. Obviously the checksum will not include checks on this CR32 chunk.

10.2.3.9 COMM

Meta-data: none present

Data:
Byte number 0 1 N
+-+- - -+-
Hex values | 0| free text |
+-+- - -+-
This allows arbitrary textual data to be added. It does not require a identifier-value pairing or any null termination.

10.2.4 Text Identifiers

These are for use in the TEXT segments. None are required, but if any of these identifiers are present they must confirm to the description below. Much (currently all) of this list has been taken from the NCBI Trace Archive [2] documentation. It is duplicated here as the ZTR spec is not tied to the same revision schedules as the NCBI trace archive (although it is intended that any suitable updates to the trace archive should be mirrored in this ZTR spec).

The Trace Archive specifies a maximum length of values. The ZTR spec does not have length limitations, but for compatibility these sizes should still be observed.

The Trace Archive also states some identifiers are mandatory; these are marked by asterisks below. These identifiers are not mandatory in the ZTR spec (but clearly they need to exist if the data is to be submitted to the NCBI).

Finally, some fields are not appropriate for use in the ZTR spec, such as BASE_FILE (the name of a file containing the base calls). Such fields are included only for compatibility with the Trace Archive. It is not expected that use of ZTR would allow for the base calls to be read from an external file instead of the ZTR BASE chunk.

[ Quoted from TraceArchiveRFC v1.17 ]

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Size</th>
<th>Meaning</th>
<th>Example value(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRACE_NAME *</td>
<td>250</td>
<td>name of the trace as used at the center unique within the center but not among centers.</td>
<td>HBBBA1U2211</td>
</tr>
<tr>
<td>SUBMISSION_TYPE *</td>
<td>-</td>
<td>type of submission</td>
<td></td>
</tr>
<tr>
<td>CENTER_NAME *</td>
<td>100</td>
<td>name of center</td>
<td>BCM</td>
</tr>
<tr>
<td>CENTER_PROJECT</td>
<td>200</td>
<td>internal project name used within the center</td>
<td>HBBB</td>
</tr>
<tr>
<td>TRACE_FILE *</td>
<td>200</td>
<td>file name of the trace relative to the top of the volume.</td>
<td>./traces/TRACE001.scf</td>
</tr>
<tr>
<td>TRACE_FORMAT *</td>
<td>20</td>
<td>format of the tracefile</td>
<td></td>
</tr>
<tr>
<td>SOURCE_TYPE *</td>
<td>-</td>
<td>source of the read</td>
<td></td>
</tr>
<tr>
<td>INFO_FILE</td>
<td>200</td>
<td>file name of the info file</td>
<td></td>
</tr>
<tr>
<td>INFO_FILE_FORMAT</td>
<td>20</td>
<td>file name of the base calls</td>
<td></td>
</tr>
<tr>
<td>BASE_FILE</td>
<td>200</td>
<td>file name of the base calls</td>
<td></td>
</tr>
<tr>
<td>QUAL_FILE</td>
<td>200</td>
<td>file name of the base calls</td>
<td></td>
</tr>
<tr>
<td>TRACE_DIRECTION</td>
<td>-</td>
<td>direction of the read</td>
<td></td>
</tr>
<tr>
<td>TRACE_END</td>
<td>-</td>
<td>end of the template</td>
<td></td>
</tr>
<tr>
<td>Field</td>
<td>Length</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>PRIMER</td>
<td>200</td>
<td>primer sequence</td>
<td></td>
</tr>
<tr>
<td>PRIMER_CODE</td>
<td></td>
<td>which primer was used</td>
<td></td>
</tr>
<tr>
<td>STRATEGY</td>
<td>-</td>
<td>sequencing strategy</td>
<td></td>
</tr>
<tr>
<td>TRACE_TYPE_CODE</td>
<td>-</td>
<td>purpose of trace</td>
<td></td>
</tr>
<tr>
<td>PROGRAM_ID</td>
<td>100</td>
<td>creator of trace file</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>phred-0.990722.h program-version</td>
<td></td>
</tr>
<tr>
<td>TEMPLATE_ID</td>
<td>20</td>
<td>used for read pairing</td>
<td></td>
</tr>
<tr>
<td>CHEMISTRY_CODE</td>
<td>-</td>
<td>code of the chemistry</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(see below)</td>
<td></td>
</tr>
<tr>
<td>ITERATION</td>
<td>-</td>
<td>attempt/redo</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(int 1 to 255)</td>
<td></td>
</tr>
<tr>
<td>CLIP_QUALITY_LEFT</td>
<td></td>
<td>left clip of the read in bp due to quality</td>
<td></td>
</tr>
<tr>
<td>CLIP_QUALITY_RIGHT</td>
<td></td>
<td>right &quot; &quot; &quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>CLIP_VECTOR_LEFT</td>
<td></td>
<td>left clip of the read in bp due to vector</td>
<td></td>
</tr>
<tr>
<td>CLIP_VECTOR_RIGHT</td>
<td></td>
<td>right &quot; &quot; &quot; &quot;</td>
<td></td>
</tr>
<tr>
<td>SVVECTOR_CODE</td>
<td>40</td>
<td>sequencing vector used</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(in table)</td>
<td></td>
</tr>
<tr>
<td>SVVECTOR_ACCESSION</td>
<td>40</td>
<td>sequencing vector used</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(in table)</td>
<td></td>
</tr>
<tr>
<td>CVVECTOR_CODE</td>
<td>40</td>
<td>clone vector used</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(in table)</td>
<td></td>
</tr>
<tr>
<td>CVVECTOR_ACCESSION</td>
<td>40</td>
<td>clone vector used</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(in table)</td>
<td></td>
</tr>
<tr>
<td>INSERT_SIZE</td>
<td>-</td>
<td>expected size of insert</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>in base pairs (bp)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(int 1 to 2^32)</td>
<td></td>
</tr>
<tr>
<td>PLATE_ID</td>
<td>32</td>
<td>plate id at the center</td>
<td></td>
</tr>
<tr>
<td>WELL_ID</td>
<td></td>
<td>well</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-384</td>
<td></td>
</tr>
<tr>
<td>SPECIES_CODE</td>
<td>*</td>
<td>code for species</td>
<td></td>
</tr>
<tr>
<td>SUBSPECIES_ID</td>
<td>40</td>
<td>name of the subspecies</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Is this the same as strain</td>
<td></td>
</tr>
<tr>
<td>CHROMOSOME</td>
<td>8</td>
<td>name of the chromosome</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ChrX, Chr01, Chr09</td>
<td></td>
</tr>
<tr>
<td>LIBRARY_ID</td>
<td>30</td>
<td>the source library of the clone</td>
<td></td>
</tr>
<tr>
<td>CLONE_ID</td>
<td>30</td>
<td>clone id</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>RPCI11-1234</td>
<td></td>
</tr>
<tr>
<td>ACCESSION</td>
<td>30</td>
<td>NCBI accession number</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AC000001</td>
<td></td>
</tr>
<tr>
<td>PICK_GROUP_ID</td>
<td>30</td>
<td>an id to group traces picked</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>at the same time.</td>
<td></td>
</tr>
<tr>
<td>PREP_GROUP_ID</td>
<td>30</td>
<td>an id to group traces prepared</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>at the same time.</td>
<td></td>
</tr>
</tbody>
</table>
RUN_MACHINE_ID 30  id of sequencing machine
RUN_MACHINE_TYPE 30  type/model of machine
RUN_LANE 30  lane or capillary of the trace
RUN_DATE  -  date of run
RUN_GROUP_ID 30  an identifier to group traces
                 run on the same machine

[ End of quote from TraceArchiveRFC ]

More detailed information on the format of these values should be obtained from the Trace Archive RFC [2].

10.2.5 References

10.3 Experiment File

Experiment files contain gel readings plus information about them, and are used during the processing of the sequence. They are used to carry data between programs: they provide input to the programs and programs may in turn add to or modify them. When the experiment file for a reading reaches the assembly program it should be carrying all the data needed for its subsequent processing. The assembly program will copy what it needs into the assembly database. The file format is based on that of EMBL sequence entries and, if required, can be read as such by programs like spin.

10.3.1 Records

It is important to note that the assembly program gap4 (see Section 1.2 [Gap4 introduction], page 3) will not operate to its full effect if it is not given all the necessary data. For example gap4 contains many functions that can analyse the positions and relative orientations of readings from the same template in order to check the correctness of the assembly and determine the contig order. However if the records that name templates and their estimated lengths, and define the primers used to obtain readings from them are missing, none of these valuable analyses can be performed reliably. One way to ensure that all the necessary fields are present is to use the program pregap4 (see Section 3.2 [Pregap4 introduction], page 202).

In the descriptions below records containing * are those read into the database during normal assembly; those with ** are extra items required when entering pre-assembled data; those with *** are read from SCF files (after the experiment file has been read to obtain the SCF file name); (see Section 10.1 [SCF introduction], page 383) the record marked **** is an extra item required for Directed Assembly.

The order of records in the file is not important. They are listed here in alphabetical order with, where possible, reasons for the origin of their names. Several are redundant and no group is likely to make use of them all. Obviously others can be added in the future. Initially they might be of local use but if their use becomes wider they can be added to the standard set. Standard EMBL records such as FT are assumed to be included.

\begin{itemize}
  \item \textbf{AC} \hspace{1cm} ACcession number
  \item \textbf{AP} \hspace{1cm} Assembly Position ****
  \item \textbf{AQ} \hspace{1cm} AVerage Quality for bases 100..200
  \item \textbf{AV} \hspace{1cm} Accuracy values for externally assembled data **, ***
  \item \textbf{BC} \hspace{1cm} Base Calling software
  \item \textbf{CC} \hspace{1cm} Comment line
  \item \textbf{CF} \hspace{1cm} Cloning vector sequence File
  \item \textbf{CH} \hspace{1cm} Special CHemistry
  \item \textbf{CL} \hspace{1cm} Cloning vector Left end
  \item \textbf{CN} \hspace{1cm} Clone Name
  \item \textbf{CR} \hspace{1cm} Cloning vector Right end
  \item \textbf{CS} \hspace{1cm} Cloning vector Sequence present in sequence *
  \item \textbf{CV} \hspace{1cm} Cloning Vector type
  \item \textbf{DR} \hspace{1cm} Direction of Read
  \item \textbf{DT} \hspace{1cm} DaTe of experiment
\end{itemize}
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN</td>
<td>Entry Name</td>
</tr>
<tr>
<td>EX</td>
<td>Experimental notes</td>
</tr>
<tr>
<td>FM</td>
<td>sequencing vector Fragmentation Method</td>
</tr>
<tr>
<td>ID</td>
<td>IDentifier *</td>
</tr>
<tr>
<td>LE</td>
<td>was Library Entry, but now identifies a well in a micro titre dish</td>
</tr>
<tr>
<td>LI</td>
<td>was subclone LIbrary but now identifies a micro titre dish</td>
</tr>
<tr>
<td>LN</td>
<td>Local format trace file Name *</td>
</tr>
<tr>
<td>LT</td>
<td>Local format trace file Type *</td>
</tr>
<tr>
<td>MC</td>
<td>MaChine on which experiment ran</td>
</tr>
<tr>
<td>MN</td>
<td>Machine generated trace file Name</td>
</tr>
<tr>
<td>MT</td>
<td>Machine generated trace file Type</td>
</tr>
<tr>
<td>ON</td>
<td>Original base Numbers (positions) **</td>
</tr>
<tr>
<td>OP</td>
<td>OPerator</td>
</tr>
<tr>
<td>PC</td>
<td>Position in Contig **</td>
</tr>
<tr>
<td>PD</td>
<td>Primer data (the sequence of a primer)</td>
</tr>
<tr>
<td>PN</td>
<td>Primer Name</td>
</tr>
<tr>
<td>PR</td>
<td>PRimer type *</td>
</tr>
<tr>
<td>PS</td>
<td>Processing Status</td>
</tr>
<tr>
<td>QL</td>
<td>poor Quality sequence present at Left (5') end *</td>
</tr>
<tr>
<td>QR</td>
<td>poor Quality sequence present at Right (3') end *</td>
</tr>
<tr>
<td>RS</td>
<td>Reference Sequence for numbering and mutation detection</td>
</tr>
<tr>
<td>SC</td>
<td>Sequencing vector Cloning site</td>
</tr>
<tr>
<td>SE</td>
<td>SEnse (ie whether complemented) **</td>
</tr>
<tr>
<td>SF</td>
<td>Sequencing vector sequence File</td>
</tr>
<tr>
<td>SI</td>
<td>Sequencing vector Insertion length *</td>
</tr>
<tr>
<td>SL</td>
<td>Sequencing vector sequence present at Left (5') end *</td>
</tr>
<tr>
<td>SP</td>
<td>Sequencing vector Primer site (relative to cloning site)</td>
</tr>
<tr>
<td>SQ</td>
<td>SeQuence *</td>
</tr>
<tr>
<td>SR</td>
<td>Sequencing vector sequence present at Right (3') end *</td>
</tr>
<tr>
<td>SS</td>
<td>Screening Sequence</td>
</tr>
<tr>
<td>ST</td>
<td>STrands *</td>
</tr>
<tr>
<td>SV</td>
<td>Sequencing Vector type *</td>
</tr>
<tr>
<td>TG</td>
<td>Gel reading Tag *</td>
</tr>
<tr>
<td>TC</td>
<td>Contig Tag *</td>
</tr>
<tr>
<td>TN</td>
<td>Template Name *</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type trace</td>
</tr>
</tbody>
</table>
### 10.3.2 Explanation of Records

<table>
<thead>
<tr>
<th>Record</th>
<th>AC, ACcession line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>AC string</td>
</tr>
<tr>
<td>Explanation</td>
<td>A unique identifier for the reading.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>AP, Assembly Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>AP Name_of_anchor_reading sense offset tolerance</td>
</tr>
<tr>
<td>Explanation</td>
<td>For readings whose position has been mapped by an external program, these records tell the &quot;directed assembly&quot; algorithm where to assemble the data. Positions are defined as offsets from an &quot;anchor reading&quot; which is the name of any reading already in the database, an orientation (sense, + or -), and a tolerance. Readings are aligned at relative position offset + or - tolerance.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>AQ, Average Quality of the reading.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>AQ Numeric value in range 1 - 99.</td>
</tr>
<tr>
<td>Explanation</td>
<td>The average value of the &quot;numerical estimate of base calling accuracy&quot; as calculated by program eba. The value is useful for monitoring data quality and could also be used for deciding on an order of assembly - for example assemble the highest quality readings first.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>AV, Accuracy Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>AV q1 q2 q3 ... or a1,c1,g1,t1 a2,c2,g2,t2 ...</td>
</tr>
<tr>
<td>Explanation</td>
<td>The accuracy values lie in the range 1-99. Either 1 per base (eg 89 50 ... or 4 per base (eg 0,89,5,2 50,3,7,10). Bonfield,J.K and Staden,R. The application of numerical estimates of base calling accuracy to DNA sequencing projects. Nucleic Acids Res. 23 1406-1410, (1995).</td>
</tr>
</tbody>
</table>

| Record  | BC, Base Calling software |

<table>
<thead>
<tr>
<th>Record</th>
<th>CC, Comment line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>CC string</td>
</tr>
<tr>
<td>Explanation</td>
<td>Any comments can be added on any number of lines.</td>
</tr>
</tbody>
</table>
Record CF, Cloning vector sequence File
Format CF string
Explanation The name of the file containing the sequence of the cloning vector, to be used by vector_clip (see Chapter 5 [Screening Against Vector Sequences], page 267).

Record CH, Special CHemistry
Format CH number
Explanation Used to flag readings as having been sequenced using a "special chemistry". The number is a bit pattern with a bit for each chemistry type, thus allowing combinations of chemistries to be listed. Currently bit 0 is used to distinguish between dye-primer (0) and dye-terminator (1) chemistries. Bits 1 to 4 inclusive indicate the type of chemistry: unknown (0, 0000), ABI Rhodamine (1, 0001), ABI dRhodamine (2, 0010), BigDye (3, 0011), Energy Transfer (4, 0100) and LiCor (5, 0101). So for example a BigDye Terminator has bits 00111 set which is 7 in decimal.

Record CL, Cloning vector Left end
Format CL number
Explanation The base position in the sequence that contains the last base in the cloning vector. Currently gap4 only uses the CS line.

Record CN, Clone Name
Format CN string
Explanation The name of the segment of DNA that the reading has been derived from. Typically the name of a physical map clone.

Record CR, Cloning vector Right end
Format CR number
Explanation The base position in the sequence that contains the first base in the cloning vector. Currently gap4 only uses the CS line.

Record CS, Cloning vector Sequence present in sequence
Format CS range
**Explanation**
Regions of sequence found by vector clip (see Chapter 5 [Screening Against Vector Sequences], page 267) to be cloning vector. Used in assembly to exclude unwanted sequence.

**Record** CV, Cloning Vector type  
**Format** CV string  
**Explanation** The type of the cloning vector used.

**Record** DR, Direction of Read  
**Format** DR direction  
**Explanation** Whether forward or reverse primers were used. Allows mapping of forward and reverse reads off the same template. NOTE however that we do not encourage the use of this method as the terms direction, sense and strand can be confusing. Instead we encourage the use of the PRimer line.

**Record** DT, DaTe of experiment  
**Format** DT dd-mon-yyyy  
**Explanation** Any date information.

**Record** EN, Entry Name  
**Format** EN string  
**Explanation** The name given to the reading

**Record** EX, EXperimental notes  
**Format** EX string  
**Explanation** Another type of comment line for additional information.

**Record** FM, sequencing vector Fragmentation Method  
**Format** FM string  
**Explanation** Fragmentation method used to create sequencing library.
Record ID, IDentifier
Format ID string
Explanation This is the name given to the reading inside the assembly database and is equivalent to the ID line of an EMBL entry.

Record LE, Can be used to identify the location of materials
Format LE string
Explanation Originally a micro titre dish well number. Used in combination with LI.

Record LI, Can be used to identify the location of materials
Format LI string
Explanation Originally a micro titre dish identifier. Used in combination with LE.

Record LN, Local format trace file Name
Format LN string
Explanation The name of the local format trace file. This information is passed onto gap4, and allows for local formats to be used.

Record LT, Local format trace file Type
Format LT string
Explanation The type of the local trace file type (usually SCF).

Record MC, MaChine on which sequencing experiment was run
Format MC string
Explanation The lab’s name for the sequencing machine used to create the data. Used for logging the performance of individual machines.

Record MN, Machine generated trace file Name
Format MN string
**Explanation**
The name of the trace file generated by the sequencing machine MC.

**Record**

**MT**, Machine generated trace file Type  
**Format**

**MT string**  
**Explanation**
The type of machine generated trace file.

**Record**

**ON**, Original base Numbers (positions)  
**Format**

**ON (eg) 1..43 0 45..63 65..74 0 75..536**  
**Explanation**
The A..B notation means that values A to B inclusive, so this example reads that bases 1 to 43 are unchanged, there is a change at 44, etc.

**Record**

**OP**, Operator  
**Format**

**OP string**  
**Explanation**
Someone’s name, possibly the person who ran the sequencing machine. Useful, with expansion of the string field for monitoring the performance of individuals!

**Record**

**PC**, Position in Contig  
**Format**

**PC number**  
**Explanation**
For preassembled data, the position to put the left end of the reading.

**Record**

**PD**, Primer Data  
**Format**

**PD sequence**  
**Explanation**
The primer sequence.

**Record**

**PN**, Primer Name  
**Format**

**PN string**  
**Explanation**
Name of primer used, using local naming convention. Could be a universal primer.
Record PR, PRimer type
Format PR number
Explanation This record shows the direction of the reading and distinguishes between primers from
the ends of the insert and those that are internal. It is important for the analysis of
the relative orientations and positions of readings on templates. When the positions
of readings on templates are analysed (see Section 1.8.2 [Find read pairs], page 110)
primer types 1, 2, 3 and 4 are represented using the symbols F, R, f and r respectively.
0 Unknown
1 Forward from beginning of insert
2 Reverse from end of insert
3 Custom forward i.e. a forward primer other than type 1.
4 Custom reverse i.e. a reverse primer other than type 2.

Record PS, Processing Status
Format PS explanation
Explanation Indication of processing status.

Record QL, poor Quality sequence present at Left (5’) end
Format QL position
Explanation The sequence up to and including the base at the marked position are considered to
be of too poor quality to be used. It may overlap with other marked sequences - CS,
SL or SR. Used in assembly to exclude unwanted sequence.

Record QR, poor Quality sequence present at Right (3’) end
Format QR position
Explanation The sequence from and including the base at the marked position to the end is consid-
ered to be of too poor quality to be used. It may overlap with other marked sequences
- CS, SL or SR. Used in assembly to exclude unwanted sequence.

Record RS, Reference Sequence
Format RS string
Explanation The name of a sequence, usually in EMBL format, used to define the target sequence,
base numbering and feature table data for a project. Used to define the numbering
and changes produced by mutations in individual sequence readings (see Section 2.1
[Introduction to mutation detection], page 185).
<table>
<thead>
<tr>
<th>Record</th>
<th>SC, Sequencing vector Cloning site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>SC position</td>
</tr>
<tr>
<td>Explanation</td>
<td>The cloning site of the sequence vector. Used by vector_clip (see Chapter 5 [Screening Against Vector Sequences], page 267).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>SE, SEnse (ie whether complemented)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>SE number</td>
</tr>
<tr>
<td>Explanation</td>
<td>For preassembled data, the sense of the reading (0 for forward, 1 for reverse).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>SF, Sequencing vector sequence File</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>SF string</td>
</tr>
<tr>
<td>Explanation</td>
<td>The name of the file containing the sequence of the sequencing vector, to be used by vector_clip (see Chapter 5 [Screening Against Vector Sequences], page 267).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>SI, Sequencing vector Insertion length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>SI range</td>
</tr>
<tr>
<td>Explanation</td>
<td>Expected insertion length of sequence in sequencing vector. Useful for selecting templates for further experiments.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>SL, Sequencing vector sequence present at Left (5') end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>SL position</td>
</tr>
<tr>
<td>Explanation</td>
<td>The sequence up to and including the base at the marked position are considered to be sequencing vector. Written by vector_clip (see Chapter 5 [Screening Against Vector Sequences], page 267).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Record</th>
<th>SP, Sequencing vector Primer site (relative to cloning site)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Format</td>
<td>SP position</td>
</tr>
<tr>
<td>Explanation</td>
<td>Location of the primer using to sequence relative to cloning site. Used by vector_clip (see Chapter 5 [Screening Against Vector Sequences], page 267).</td>
</tr>
</tbody>
</table>
Record  SQ, SeQuence
Format   SQ \nsequence blocks...\n//\nExplanation
Complete sequence, as determined by the sequencing machine. The sequence is broken into blocks of 10 bases with 6 blocks per line separated by a space (see the example below).

Record  SR, Sequencing vector sequence present at Right (3') end
Format   SR position
Explanation
The sequence from and including the base at the marked position to the end are considered to be sequencing vector. Written by vector_clip (see Chapter 5 [Screening Against Vector Sequences], page 267).

Record  SS, Screening Sequence
Format   SS string
Explanation
Note that in earlier versions of this documentation this field was explained incorrectly. Due to this the field is not currently being used by any of our programs. The original meaning was to specify a sequence to screen against. Any number of SS lines could be present to denote any number of screening sequences. In the future we may change the meaning of this field to be a single SS line containing a file of filenames of screening sequences. If this causes problems for people then we will choose a new line type, so please inform us now. Also note that contrary to previous documentation, vector_clip does not use this field (it uses the SF field instead).

Record  ST, STrands
Format   ST number
Explanation
Denotes whether this is a single or double stranded template. This is useful for deducing suitable templates for later experiments.

Record  SV, Sequencing Vector type
Format   SV string
Explanation
Type of sequencing vector used. Can be used for choosing templates for custom primer experiments.

Record  TC, Tag to be placed on the Consensus.
Chapter 10: File Formats

Format
TC TYPE S position..length

Explanation
These lines instruct gap4 to place tags on the consensus. The format defines the tag type which is a 4 character identifier and should start at column position 5), its strand ("+", "," or ":" which means both strands), its start position followed by the position of its end. These two values are separated by ".". Following lines starting TG with space characters up to column 10 are written into the comment field of the tag. For example the next three lines define a tag of type comment that is to be on both strands over the range 100 to 110 and the comment field will contain "This comment contains several lines".

TC COMM = 100..110
TC This comment contains
TC several lines

Record
TG, Tag to be placed on the reading.

Format
TG TYPE S position..length

Explanation
These lines instruct gap4 to place tags on the reading. See TC for further information.

Record
TN, Template Name

Format
TN string

Explanation
The name of the template used in the experiment.

Record
WT, Wild Type trace file

Format
WT string

Explanation
The filename of the wild type trace file. Used for mutation studies.

10.3.3 Example

ID h4a01h6.s1
EN h4a01h6.s1
TN h4a01h6
EX lane 18, run time 10 hrs
MN Sample 18
MC A
MT ABI
LN h4a01h6.s1SCF
LT SCF
DT 08-Jan-1993
OP ak
TN h4a01h6
10.3.4 Unsupported Additions (From LaDeana Hillier)

Note the clash on AP which the io-lib uses for "Assembly Position" and PC which is used for "Position in Contig"

People to track:
TP Template Prep person
QP Sequencer Person, person who does sequencing reactions
LP Loader Person
AL Agar Loader person (when they run a gel to determine SI)
AP Agar reaction Person (person who does the reactions to prepare the template to be run on a gel)

Gel specific information
GN Gel Name
GL Gel Lane
GP Gel Pourer person
AG Agar Gel name (sizing gel)
AF Agar Fate, no insert, no bands, what else?

Name of library
LB Library name, probably not critical to assembly even though one CN may have more than one library. But it is important to the cDNA project although I could put it in CN, since the cDNA project wouldn’t have a CN otherwise.

Processing information
PC processing comment (a comment about PS)  
I think PS should just hold pass or fail and PC should hold additional information about why things passed.

Trace information gotten from the ABI machine (from info field in SCF file):
TS Trace Spacing
DP Dye Primer
HA signal strength A
HG signal strength G
HC signal strength C
HT signal strength T

(NOTE rs suggested these should go in a single record

PP Primer Position (position at which primer peak was detected in trace)

Stuff most likely specific to the cDNA project:
MP Map Position
TT Tissue Type of the library
EI dbEst Id
ER dbEst Remark
OE Other Est’s which are similar
NI NCBI ID
GB GenBank accession number
SD Submission Date (when est was submitted)
UD Update date (when it was last updated)
CI citation associated with this cDNA
10.4 Restriction Enzyme File

Restriction enzymes and their recognition sequences used by the package must be stored in the format described below. Updates of the files can be obtained from the REBASE restriction enzyme database of Dr R Roberts. Contact roberts@neb.com or macelis@neb.com to join the mailing list and state that you want the files sent in "staden" format.

Standard four-cutter, six-cutter and all-enzymes files are supplied with the package and users can create and use their own "personal" files. To create your own file of enzymes you may need to extract the information from the currently defined files. These are stored in the tables directory (folder) distributed with the package, and are named:

- RENZYM.4
- RENZYM.6
- RENZYM.ALL

We call the recognition sequences "strings". The format is as follows: each string or set of strings must be preceded by a name, each string must be preceded and terminated with a slash (/), and each set of strings by 2 slashes. For example AATII/GACG'TC// defines the name AATII, its recognition sequence GACGTC and its cut site with the ' symbol; ACCI/GT'MKAC// defines the name ACCI and its recognition sequence includes IUB symbols for incompletely defined symbols in nucleic acid sequences; BBVI/GCAGCN.../'NNNNNNNNNGCTGC// defines the name BBVI and this time two recognition sequences and cut sites are specified to enable the definition of the cut position relative to the recognition sequence. If no cut site is included the first base of the recognition sequence is displayed as being on the 3' side of the recognition sequence.

A section of a typical file follows:

AATII/GACG'TC//
ACCI/GT'MKAC//
AFLII/C'TTAAG//
AVAIII/G'GWCC//
AVRIII/C'CTAGG//
BANI/G'GYRCC//
BANII/GRGGY'C//
BBVI/GCAGCN.../'NNNNNNNNNGCTGC//
BCLI/T'GATCA//
BGLI/GCCN...'NGGC//
BGLII/A'GATCT//
BINI/GGATCN...'NNNNGATCC//
BSMI/GAATGCN...'/NG'CATTCC//
BSP1286/GDGCH'C//
10.5 Vector_primer File

The vector_primer files store the data for each vector/primer pair combination as a single record (line) and up to 100 records can be contained in a file. The items on each line must be separated by spaces or tabs (only the file name can contain spaces) and a newline character ends the record.

The items in a record are:

name seq_r seq_f file_name

name is an arbitrary record name. seq_r is the sequence between the reverse primer and the cloning site. seq_f is the sequence between the forward primer and the cloning site. file_name is the name of the file containing the complete vector sequence.

An example file containing two entries (for m13mp18, and a vector called f1) is shown below. "\" symbols have been used to denote wrapped lines and so it can be seen that the first record is shown on two lines and the next on 1.

m13mp18 attacgaattcgagctggtaccc ggggatctctagagctgacctgcaggcatgcaagcttggc \ 
/pubseq/tables/vectors/m13mp18.seq
f1 CCGGGAATTCGCGGCCGCGTCGACT CTAGACTCGAGTTATGCATGCA af_clones_vec

See Section 5.6 [Vector_Primer files], page 272 for information about creating new vector_primer file entries.
10.6 Vector Sequence Format

Sequences such as vectors or E. coli which are compared against readings using vector_clip (see [Vector_clip], page [undefined]) and screen_seq (see Chapter 6 [Screening for known possible contaminant sequences], page 279, usually via pregap4 (see Section 3.2 [Pregap4], page 202), must be stored as plain text. i.e. the files should contain only the sequence data (no header or title) on records (lines) of up to 60 characters. Each record should be terminated by a newline character. No other characters should appear in the file.
11 Man Pages
11.1 Convert_trace

NAME

convert_trace — Converts trace file formats

SYNOPSIS


DESCRIPTION

convert_trace converts between the various DNA sequence chromatogram formats, optionally performing trace processing actions too. It can read ABI (raw or processed), ALF, CTF, SCF and ZTR formats. It can write CTF, EXP, PLN, SCF and ZTR formats. (Note that EXP (Experiment File) and PLN formats are text sequences rather than a binary trace.)

There are two main modes of operation; either with a file of filenames specified using the -fofn filename option, or acting as a filter to process one single file. In this case the input and output file format may be specified as the last two options on the command line.

OPTIONS

-abi_data counts
  Only of use when processing ABI files. This indicates which ABI DATA channel numbers to use. For sequencing files this defaults to "9,10,11,12" which corresponds to the processed data. To read the raw data use "1,2,3,4".

-compress mode
  Specifies the name of a program to use to compress the trace data prior to writing. Due to limitations in the current implementation this option does not work when convert_trace is operating as a filter (and so requires use of the -fofn option). Valid values for mode are compress, bzip, bzip2, gzip, pack and szip. Note that for ZTR, ZTR2 and ZTR3 format files specifying compression modes will not reduce the file size as this format already contains internal compression algorithms. The ZTR1 format does not internally compress and so -compress will have an effect.

-failed fofn
  Produces a file listing the filenames which have failed to be converted. This only makes sense when also using -fofn.

-fofn file_of_filenames
  Processes several files instead of one, with the filenames to read from and written to being listed in file_of_filenames with one pair (input and output filenames) being listed per line, separated by spaces. If the filenames contain spaces then these may be "escaped" using backslashes. Similarly backslashes should be escaped using a double backslash. For example to convert "file a.scf" and "fileb.scf" to "file a.ztr" and "fileb.ztr" respectively we would use a file_of_filenames containing:

  file\ a.scf  file\ a.ztr
  fileb.scf   fileb.ztr
-in_format format
Specifies the format for the input data. Typically the input format is automatically
determined so this may not be required. format should be one of ABI, ALF, CTF,
EXP, PLN, SCF, ZTR, ZTR1, ZTR2 or ZTR3. The ZTR formats all conform to the
ZTR specification, but this indicates the compression level to be used.

-name id
When producing an Experiment File this specifies the value of the ID line. Without
this option default Experiment File ID line is the output filename, or if this is stdout
it is the input filename.

-normalise
Attempts to normalise the trace amplitudes to produce more even height peaks. This
may be useful to compensate for large spikes at either the start or end of the trace.

-out_format format
Specifies the output format for all files, whether read from a file of filenames or via a
filter. format should be one of ABI, ALF, CTF, EXP, PLN, SCF, ZTR, ZTR1, ZTR2
or ZTR3. The ZTR formats all conform to the ZTR specification, but this indicates
the compression level to be used.

-passed fofn
Produces a file listing the filenames which have been successfully converted. This only
makes sense when also using -fofn.

-scale range
Scales all trace amplitudes so that they fit within the range of 0 to range inclusive. Any
integer value of range may be used between 1 and 65535, but this option is designed
down-scaling traces in order to reduce file size.

-subtract_background
Attempts to remove background trace levels by analysing each trace channel indepen-
dently to determine the baseline. This option is mainly used when processing raw
data.

EXAMPLES
To convert several files to ZTR format using the same example file of filenames listed in the
-fofn option above:

convert_trace -out_format ZTR -fofn filename

To subtract the background from a raw ABI file and save this as an SCF file:

convert_trace -abi_data 1,2,3,4 -subtract_background ABI SCF < a.abi > a.scf

NOTES
If ABI files are manually edited before input to convert_trace then the internal formats of these
files may differ to the format expected by convert_trace.

SEE ALSO
See Section 10.1 [scf(4)], page 383. See Section 10.2 [ztr(4)], page 389. See Section 11.10
[makeSCF(1)], page 430.
11.2 Copy_db

NAME

copy_db — a garbage collecting gap4 database copier and merger

SYNOPSIS

```
copy_db [-v] from.vers ... to.vers
```

DESCRIPTION

Copy_db copies one or more gap4 databases to a new name by physically extracting the information from the first databases and writing it to the last database listed on the command line. This operation can be considered analogous to copying files into a directory. This is slower than a direct `cp` command, but has the advantage of merging several databases together and the resulting database will have been garbage collected. That is, any fragmentation in the original databases is removed (as much as is possible).

NOTE: Care should be taken when merging database. **No checks** are performed to make sure that the databases do not already contain the same readings. Thus attempting to copy the same database several times will cause problems later on. No merging of vector, clone or template information is performed either.

OPTIONS

```
-v   Enable verbose output. This gives a running summary of the current piece of information being copied.
```

EXAMPLES

To merge database X with database Y to give a new database Z use:

```
copy_db X.0 Y.0 Z.0
```

NOTES

To copy a database quickly without garbage collecting the UNIX `cp` command can be used as follows. This copies version F of database DB to version T of database XYZZY.

```
cp DB.F XYZZY.T; cp DB.F.aux XYZZY.T.aux
```

Care must be taken to check for the busy file (`DB.F.BUSY`) before making the copy. If the database is written to during the operation of the copy command then the new database may be corrupted.
11.3 Copy_{reads}

NAME

copy_{reads} — copies overlapping reads from a source database to a destination database

SYNOPSIS

Usage:


DESCRIPTION

During large scale sequencing projects where the genome is cloned into e.g. BACs prior to being subcloned into sequencing vectors it is generally the case that the ends of the DNA from one BAC will overlap that of two other BACs. Unless it is being used for quality control, it is a waste of time to sequence the overlapping regions twice, and so most labs transfer the relevant data between the adjacent gap4 databases. This is the function of copy_{reads} which copies readings from a "source" database to a "destination" database.

The consensus sequences for user selected contigs in each of the two databases are compared in both orientations. If an overlapping region is found, readings of sufficient quality are automatically assembled into the destination database. In the source database readings which have been added to the destination database will be tagged with a "LENT" tag and the equivalent readings in the destination database will be tagged with a "BORO" (borrowed) tag.

OPTIONS

-w\in Bring up a dialogue window

-source_trace_dir directory of source traces

The location of the traces of the source database can either be specified by giving the directory name or if this is not specified, determined from the rawdata note (see Section 1.20.8 [Trace File Location], page 179) held within the database. The program will add the location of the source traces into the rawdata note of the destination database. If the environment variable RAWDATA is set, this will be taken to be the location of the destination database traces and will also be added to the rawdata note of the destination database. If there are no traces for the source database, no rawdata note will be created.

-contigs_from file of contigs in source database

One or more contigs from the source database can be compared. These are selected either by providing a file containing a list of contig names (any reading name from within that contig, typically the first reading name). If no file is specified, all contigs will be compared.
-min_contig_len minimum contig length
Only contigs in the source database over a user defined length will be used. The default is 2000 bases.

-min_average_qual minimum average read quality
A minimum reading quality can be set so that only readings with an average quality over the specified amount will be entered into the destination database. The default is 30.0.

-contigs_to file of contigs in destination database
One or more contigs from the destination database can be compared. These are selected either by providing a file containing a list of contig names (any reading name from within that contig, typically the first reading name). If no file is specified, all contigs will be compared.

-mask masking mode
The consensus sequence is determined for each contig in both databases using either the standard consensus algorithm (none) or "Mask active tags" (mask). Masking the active tags means that all segments covered by tags that are "active" will not be used by the matching algorithms. A typical use of this mode is to avoid finding matches in segments covered by tags of type ALUS (ie segments thought to be Alu sequence) or REPT (ie segment that are known to be repeated elsewhere in the data (see Section 1.2.7.1 [Tag types], page 29). The default is none.

-tag_types list of tag types
A list of tag types to be used when the -mask option (above) is specified to be in "mask" mode.

-word_length word length
The consensus searching parameters are equivalent to those found in the find internal joins algorithm (see Section 1.8.3 [Find Internal Joins], page 114). The search algorithm first finds matching words of length Word length. Possible values are 4 or 8. The default is 8.

-min_overlap minimum overlap
The search algorithm only considers overlaps of length at least Minimum overlap. The default is 20.

-max_pmismatch maximum percentage mismatch
Only alignments better than Maximum percent mismatch will be reported. The default is 30.0.

-min_match minimum match
The algorithm considers in its initial phase only matching segments of length Minimum initial match length. However it does a dynamic programming alignment of all the chunks between the matching segments, and so produces an optimal alignment. The default is 15.

-band use banding algorithm
A banded dynamic algorithm can be selected, but as this only applies to the chunks between matching segments, which for good alignments will be very short and it should make little difference to the speed. Possible values are 0 (no) or 1 (yes). The default is 1.

-display_cons display consensus alignments
This allows the alignments between the consensus sequences to be displayed.

-align_max_mism maximum percent mismatch
If a match between two consensus sequences is found, the readings in that overlap are assembled into the destination database using the "directed assembly" function (see
Section 1.7.2 (Directed Assembly), page 99). Only readings for which the maximum percent mismatch is not exceeded, and which have an average reading quality higher than the specified minimum, will be entered into the database. The default value is 10.0.

-`display_seq` *display reading alignments*

This allows the alignments between the source database readings and the destination consensus to be displayed.

**EXAMPLE**

To copy readings from `source_db` to `destination_db` and display the consensus match

```
copy_reads -display_cons source_db destination_db
```
11.4 Eba

NAME

eba — Estimates Base Accuracy in an SCF or ZTR file

SYNOPSIS

eba [trace_file]

DESCRIPTION

Eba will calculate numerical estimates of base accuracy for each base in an SCF or ZTR file. The figures calculated should not be considered as reliable and better values can be obtained from phred or ATQA.

The method employed by eba to estimate the base accuracies performs the following calculation for each base. Calculate the area under the peaks for each base type. Divide the area under the called base by the largest area under the other three bases. From the 2002 release these values are normalised to the phred scale (this was achieved by comparing the original eba values and phred values for 4.6 million base calls of Sanger Centre data).

With no filename as an argument eba reads from standard input and writes to standard output. This enables eba to be used as a filter, or to estimate base accuracies for unwritable files. If a file is specified on the command line then the accuracy figures will be written to this file.

EXAMPLES

To write base accuracy figures to an SCF file named e04f10.s1SCF:

```
eba e04f10.s1SCF
```

To write base accuracy figures on the original eba scale to an SCF file named e04f10.s1SCF:

```
eba -old_scale e04f10.s1SCF
```

To write base accuracy figures to a ZTR file named e04f10.s1.ztr in another users directory, and to store the updated file in the current directory:

```
eba < ~user/e04f10.s1.ztr > e04f10.s1.ztr
```

SEE ALSO

See Section 10.1 [scf(4)], page 383.
11.5 Extract_seq

NAME

extract_seq — extracts sequence from a trace or experiment file.

SYNOPSIS

extract_seq [-r] [-abi|alf|scf|exp|pln] [-good_only] [-clip_cosmid] [-fasta_out] [-output output_name] [input_name] ...

DESCRIPTION

extract_seq extracts the sequence information from binary trace files, Experiment files, or from the old Staden format plain files. The input can be read either from files or from standard input, and the output can be written to either a file or standard output. Multiple input files can be specified. The output contains the sequences split onto lines of at most 60 characters each.

OPTIONS

-r       Directs reading of experiment file to attempt extraction of sequence from the referenced (LN and LT line types) trace file. Without this option, or when the trace file cannot be found, the sequence output is that listed in the Experiment File. This option has no effect for other input format types.

-abi, -alf, -scf, -exp, -pln
         Specify an input file format. This is not usually required as extract_seq will automatically determine the correct input file type. This option is supplied incase the automatic determination is incorrect (which is possible, but has never been observed).

-good_only
         When reading an experiment file or SCF file containing clip marks, output only the good sequence which is contained within the boundaries marked by the QL, QR, SL, SR, CL, CR and CS line types.

-clip_cosmid
         When the -good_only argument is specified this controls whether the cosmid sequence should be considered good data. Without this argument cosmid sequence is considered good.

-fasta_out
         Specifies that the output should be in fasta format

-output file
         The sequence will be written to file instead of standard output.

SEE ALSO

See Section 10.3 [ExperimentFile(4)], page 401. See Section 10.1 [scf(4)], page 383. Read(4)
11.6 Find_renz

NAME

find_renz — Identifies the position of a cut site within a sequence

SYNOPSIS

find_renz [-vp] enzyme filename ...

DESCRIPTION

find_renz may be used to determine the position that an enzyme cuts a sequence. It’s use as a command line utility is primarily designed for internal use within pregap4 and as a user utility for producing vector-primer files for use with vector_clip. As such it is dedicated to finding one and only one such cut site and considers no cuts sites or multiple cut sites to be an error.

Only one enzyme may be specified, which is given by the enzyme name (upper or lower case is not important). One or more filenames may be specified. If an enzyme does not cut a sequence the message "Enzyme not found in sequence" will be sent to stderr. If an enzyme cuts a sequence more than once the message "Found more than one match" will be sent to stderr. Otherwise output is produced to stdout. This means that wildcards may be used (find_renz -vp smai *.seq >> vpfile) with the output redirected without needing to consider whether the enzyme is suitable for all files matching the wildcard pattern.

OPTIONS

-vp Specifies that the output should be in a format suitable for saving to a vector-primer file (to use with vector_clip). Without this only the cut site position is listed.

SEE ALSO

See Section 11.16 [vector_clip(1)], page 444.
11.7 Get_comment

NAME

          get_comment — extract comments from trace files

SYNOPSIS

        get_comment [-c] [Field-ID ...]

DESCRIPTION

        The get_comment command extracts text fields from a variety of trace formats. Each comment is of the form Field-ID=comment, regardless of the file format. Field-ID is typically 4 character identifier.

        With no Field-ID arguments specified all comments are listed. Otherwise only those specified on the command line are listed.

OPTIONS

        -c          Suppresses the output of the Field-ID. Only the right hand side of the comment is displayed. The default action is the display the full comment in the form listed above.

SEE ALSO

        See Section 11.8 [get_scf_field(1)], page 428.
11.8 Get_scf_field

NAME

get_scf_field — extract comments from an SCF file

SYNOPSIS

get_scf_field [-cqs] filename [Field-ID ...]

DESCRIPTION

The get_scf_field command extracts comments from an SCF file. Each comment is of the form Field-ID=comment. Where Field-ID is a 4 character identifier.

With no Field-ID arguments specified all comments are listed. Otherwise only those specified on the command line are listed.

OPTIONS

-c Suppresses the output of the Field-ID. Only the right hand side of the comment is displayed. The default action is the display the full comment in the form listed above.

-q Query mode. Here no output is displayed, but it simply returns true or false depending on whether any of requested comments were found.

-s Silent mode. No error messages are produced, except for usage messages. It returns true or false for success or failure.

SEE ALSO

See Section 11.7 [get_comment(1)], page 427. See Section 10.1 [scf(4)], page 383.
11.9 Init_exp

NAME

init_exp — create and initialise an Experiment File

SYNOPSIS

init_exp [-abi|alf|scf|pln] [-output file] [-name entry_name] [-conf] file

DESCRIPTION

init_exp initiates an Experiment File for a binary trace file or a plain sequence file. The Experiment File created contains the ID, EN, LN, LT and SQ lines.

The experiment file is, by default, sent to standard output, unless an output file is specified using the -output option. The default entry name for the Experiment File is derived from the filenames used. If an output file has been specified, then this is taken as the EN field. Otherwise the input file name is used. The user can override the default by using the -name option.

OPTIONS

-abi, -alf, -scf, -pln

Specify an input file format. This is not usually required as init_exp will automatically determine the correct input file type. This option is supplied incase the automatic determination is incorrect (which is possible, but has never been observed).

-output file

The experiment file will be written to file instead of standard output. Additionally the value of the EN and ID fields, assuming -name has not been specified, will be file.

-name name

Sets the ID and EN fields to name, regardless of the output filename used.

-conf

Fills out the AV field with the quality values found in the SCF file.

NOTES

This program was formerly known as expGetSeq.

SEE ALSO

See Section 10.3 [ExperimentFile(4)], page 401. Read(4)
11.10 MakeSCF

NAME

makeSCF — Converts trace files to SCF files.

SYNOPSIS


DESCRIPTION

MakeSCF converts trace files to the SCF format. It can input ABI 373A, Pharmacia A.L.F., or previously created SCF files (although converting from SCF to SCF serves no useful purpose!).

OPTIONS

-8 Force conversion to 8 bit sample data. This shrinks the size of SCF files using 16 bit sample values, but at a loss of resolution. For trace display purposes this accuracy loss is acceptable.

-2 Force the output to be written in SCF version 2. By default the latest version (3) is used.

-3 Force the output to be written in SCF version 3. This is the default.

-s Silent mode. This prevents the output of the copyright message.

-abi, -alf, -scf, -any
Specify an input file format. A file format of "any" will force makeSCF to automatically determine the correct input file type.

-compress compression_mode
Requests the generated SCF file to be passed through a separate compression program before writing to disk. makeSCF does not contain any compression algorithms itself. It requires the appropriately named tool to be on the system and in the user’s PATH. Valid responses for compression_mode are (in order of best compression first) bzip, gzip, compress and pack. Note that bzip at present is only bzip version 1 and that bzip version 2 is incompatible.

-normalise
Performs some very simple trace normalisation. This subtracts the background signal (by defining the background signal to be the lowest of the four traces) and rescales the peak heights, averaging the height over a ‘window’ of 1000 trace sample points. This option may be useful for some unscaled ALF files.

-output file
Specifies the filename for the SCF file to be produced. If this is not specified the SCF file will be sent to standard output.
EXAMPLES

To convert an ABI 373A trace:

```bash
makeSCF -8 -abi trace.abi -output trace.scf
```

To convert an ALF archive to individual SCF files (Warning! this will most certainly fail if your clone names contain spaces):

```bash
alfsplit trace.alf | awk '/^Clone/ {print $3 "ALF"}r' > trace.files
cat trace.files.sh -c 'for i in `cat trace.files`;do makeSCF -alf $i -output $i.scf;done
```

NOTES

If ABI and A.L.F files are edited before input to makeSCF the contents of the resulting SCF files are unpredictable. To use Pharmacia A.L.F. files the `alfsplit` program should first be used. Then `makeSCF` should be run on each of the split files. See the example above.

SEE ALSO

See Section 10.1 [scf(4)], page 383. See Section 11.1 [convert_trace(1)], page 418. See Section 11.4 [eba(1)], page 424.
11.11 Make_weights

NAME

make_weights — makes weight matrices from sequence alignments

SYNOPSIS

make_weights [-v] [-m mark position] [-c minimum score] [-C maximum score]
[-w input weight matrix file name] [-o output weight matrix file name] [input aligned sequences file]

DESCRIPTION

make_weights is used to create weight matrix files from a file of aligned sequences. These weight matrices are for use with spin.

The simplest usage is to read in a file of aligned sequence motifs, and write out a weight matrix file created from their observed character frequencies at each position. The only command line input required is the name of the file of aligned sequence and the name for the output weight matrix file. In this mode, make_weights reads in the file of aligned motifs, counts the character frequencies at each position, calculates weights from these, and then applies the weights to all the input sequences, recording the score for each. By default the two cutoff scores written to the weight matrix file will be set to the minimum and maximum scores obtained from this process. In this mode nothing will be written to the output screen.

If no output file is supplied, none is written, but the scores for all the input sequences are written to the screen. In this way the user can decide whether to override the cutoff scores written to the weight matrix file. To set these values they can be supplied on the command line using the -c and -C options.

To see the range of scores for a set of aligned sequences and an existing weight matrix file, the -w option should be used. In this case the matrix file is read, applied to the set of aligned sequences, and the scores are listed on the screen.

The -m option is used to set the mark position and the -v option simply lists the current version number of the program.

The screen output produced by make_weights can be used as input to make_weights. An example is shown below.

Input to make_weights:

HSTGM1A    acagcggaccgtgtgaccat comments
HSARAF1G   aagtctaacagtatctatct
HSU01337   aagtctaacagtatctatct
HSA132695  gccgattgccgtatgtaaaa
HSCEL      ctctctgcaggtctcgggat

Output from make_weights:

HSTGM1A    acagcggaccgtgtgaccat            0 0.049247 comments
HSARAF1G   aagtctaacagtatctatct            1 0.010509
HSU01337   aagtctaacagtatctatct            2 0.010509
The output has added two extra columns between the sequences and the comments: a motif number and its score. This file could be passed through a sorting program to shift the lowest scoring motifs to the bottom of the file, and then the records with poor scores investigated, and perhaps removed. On UNIX the following creates a file as shown above, called don.s, and then sorts it on score to create the ordered file don.ss.

```
make_weights don.mw > don.s
sort -n -r +3 -o don.ss don.s
```

The weights are calculated in the following way.

The algorithm deals with the problem of zero counts by adding a small amount to every element. For alignments with few sequences the effect will be quite marked, but for large datasets it will be very small.

The score for unknown characters found in sequences is set to the mean for the column.

In calculating the log odds it is assumed that probability of each base type in a random sequence is 0.25.

Let the counts for each position (column) and character type in the alignment be stored in counts, and put the weights in matrix. Both are two dimensional arrays. Char_set_size is the character set size which is 4 for DNA.

```
for each column sum the counts to get the total
  set small to 1 if total = 0 otherwise 1/total
  set column total to total + small*char_set_size
for each character type
  set matrix to counts + small
  p = matrix/total
  p = log ( p / 0.25 )
  matrix = p
set unknown char (matrix) to mean for the column
end
```

Aligned sequences file format

```
name sequence comments
```

The file containing the aligned sequences should consist entirely of records containing data. Each record should contain a name, followed by the sequence, followed by arbitrary comments. Each record must be less that 2048 characters. At present make_weights is set to handle up to 10,000 records. Within a record fields (other than within the comments section) are separated by spaces. It is assumed that the sequences are aligned and do not contain leading spaces. For example, the last but 1 record below is not aligned in the file, but will be aligned after parsing.

```
AB002455 ctgacagaaggtgccagggt 1
AB002456 ccctggctgggtgagtatct 1
AB002456 tttgctccaggtagacactg 2
HSE27 atgtttgagggtgagggccc 1
AB002460 atccccaaaggtgccacagc 1 unusual
AB002461 cagggcccaggtaagggcgg 1
AB003312 aaatgcctcaaggtacagac 1
```

A weight matrix file (as shown below) consists of a single record title (here test matrix), a record containing the motif length (here 11), the "mark position" (here 5), and the minimum and
maximum scores (here 0.0 and 10.0). The "mark position" is an offset which is added to the position of any matches reported by the search routine in spin. The next two records are ignored by the programs. The first gives the matrix column positions, and the next the total counts in each column. The final records (4 for DNA weight matrices) give the counts for each character type at each position in the motif. These counts are converted into weights that are used during the searches. Any position in a sequence which scores at least as high as the minimum score (here 0.0) is reported as a match, and if the results are plotted they are scaled to fit the range defined by the minimum and maximum scores (here 0.0 and 10.0).

```
test matrix
11 5 0.0 10.0
P 0 1 2 3 4 5 6 7 8 9 10
n 8067 8067 8069 8067 8069 8069 8069 8069 8069 8069 8068
a 2572 4755 700 61 73 3759 5667 542 1236 2082 1624
c 3137 1109 301 33 89 260 671 518 1282 1821 2879 2098
g 1515 1146 6343 7897 103 3759 1060 6502 1821 2879 2098
t 845 1059 725 77 7803 288 670 506 3728 1303 1967
```

The maximum number of columns in a record is 20. Longer motifs will have weight matrix files with sufficient blocks of 20 columns. For example the one shown below has 22 positions and so a second block has been started.

```
title
22 0 0.0 3.0
P 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
n 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
a 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
C 1 1 1 1 1 1 1 1 1 1 0 0 0 1 1 1 1 1 1 1
G 0 0 0 0 0 0 0 0 1 4 4 3 0 0 0 0 0 0 0 0
T 1 1 1 1 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 1
P 20 21
n 4 4
a 2 2
c 1 1
g 0 0
t 1 1
```

**OPTIONS**

- `-v` Show the version number of the program.
- `-m` Set the mark position. When matches are found using the weight matrix offset m is added to the reported match position.
- `-c` Set the minimum score. When the weight matrix is used to search a new sequence all positions which reach this score are reported as a match.
- `-C` Set the maximum score. When the weight matrix is used to search a new sequence, matches are plotted using this value as the maximum.
- `-w` Apply an input weight matrix to the set of aligned motifs. Write the scores for each motif on the screen, but do not create a new weight matrix file.
- `-o` The file name for the weight matrix created.
EXAMPLE

make_weights
Usage: make_weights [options] input_file
Where options are:
    [-w input weights filename]       [-o output filename]
    [-c min score]                     [-C max score]
    [-m mark position]                [-v version]

SEE ALSO

See Section 8.3.10 [Motif search], page 321.
### 11.12 PolyA\_clip

**NAME**

polyA\_clip — Mark polyA and polyT heads and tails.

**SYNOPSIS**

```
polyA\_clip [-vt] [-t] [-x min\_length(0)] [p percent\_cutoff(95)] [w window\_length(50)] files...
```

**OPTIONS**

- `-v` Enable verbose output. This outputs information on which files are currently being clipped.
- `-t` Test mode. The SL and SR information is written to stdout instead of being appended to the Experiment file.
- `-x min\_length` Sequences which after clipping are shorter than `min\_length` are reported.
- `-w window\_length` The length of the window that is slid along the sequence to analyse the composition.
- `-p percentage` Windows containing this percentage of A or T bases are considered as polyA or polyT

**DESCRIPTION**

PolyA\_clip searches the 5' and 3' ends of sequence readings for the presence of polyA and polyT heads and tails. It marks them using the SL and SR experiment file records, and hence should be applied after quality clipping and sequence vector clipping. Any number of files can be processed in a single run. The algorithm is as follows. The user supplies `window\_length` and percentage. From MIN(QR,SR) slide the window left until percent\_A < percentage and percent\_T < percentage. Then from the right edge of the window look left until a C or G is found. Mark this base SR. Do the equivalent for the 5' end and mark SL.

**SEE ALSO**

See Section 10.3 [ExperimentFile(4)], page 401.

### 11.13 Qclip

**NAME**

qclip — an Experiment File sequence clipper
SYNOPSIS

Usage when confidence values are available (default mode):

```
qclip [-c] [-vt] [-m minimum_extent] [-M maximum_extent] [-w window_length]
[-q average_quality]
```

Usage when confidence values are not available or are to be ignored:

```
qclip [-c] [-vt] [-m minimum_extent] [-M maximum_extent] [-s start_offset] [-R r_length]
[-r r_unknown] [-L l_length] [-l l_unknown]
```

DESCRIPTION

Qclip is a simple program to decide how much of the 5' and 3' ends of a sequence, stored as an Experiment File, should be clipped off i.e. marked to be ignored during assembly.

The decision is made either by analysing the average confidence levels stored in the Experiment file (or an associated trace file), or by counting the numbers of unknown bases (eg - or N) found within windows slid left to right along the sequence.

Large numbers of files can be processed in a single run and each file argument is assumed to be a valid Experiment File. The sequence is read from the Experiment File SQ record and the trace is read using the LN and LT identifiers; clipping is performed and QL and QR identifiers are appended to the file.

For the default mode of clipping by confidence levels, the program firstly finds the region of highest average quality. A window is then slid from this point both rightwards and leftwards until the average quality over that window length (specified with the `-w` argument) drops below the average_quality argument. The exact position of the clip point within that window is determined by successively decreasing the window length.

When confidence values are not available, or when the `-n` argument is used, only the sequence base calls are analysed. In this case the right clip position is calculated by sliding a window of length `r_length` rightwards along the sequence, starting from base `start_offset`, and stopping when a window containing at least `r_unknown` unknown bases is found. The left clip position is calculated by sliding a window leftwards from base `start_offset`. The algorithm used is identical to the right clip position except that the `l_unknown` and `l_length` parameters are used.

The default arguments are "-c -m 0 -M 9999 -w 30 -q 10."

OPTIONS

- `v` Enable verbose output. This outputs information on which files are currently being clipped.
- `t` Test mode. The QL and QR information is written to stdout instead of being appended to the Experiment file.
- `c` Clip by confidence levels. This is the default mode of operation.
- `n` Clip by unknown base calls, even when confidence values are available.
- `m extent` If the clip algorithm returns a QL clip value of less than `extent`, use `extent` as the QL value.
- `M extent` If the clip algorithm returns a QR clip value of more than `extent`, use `extent` as the QR value.
-w
Only used for the confidence level clipping mode. The window length over which to compute the average confidence value.

-q
Only used for the confidence level clipping mode. The minimum average confidence in any given window for this window to be considered as good quality sequence.

-s offset
Only used for the unknown base clipping mode. Force the first window to start the calculations from position offset in the sequence. This can be useful to avoid poor data at the 5' end of a sequence.

-R length
Only used for the unknown base clipping mode. Set the length for the first rightwards window to length

-r unknown
Only used for the unknown base clipping mode. Stop sliding the first rightwards window when there are greater than or equal to unknown bases within the current window.

-L length
Only used for the unknown base clipping mode. Set the length for the second rightwards window to length. Setting this value to zero prevents the second window calculations from being performed.

-l unknown
Only used for the unknown base clipping mode. Stop sliding the second rightwards window when there are greater than or equal to unknown bases within the current window.

EXAMPLE
To clip a batch of sequences listed in the ‘fofn’ file with a minimum left clip value of 20 bases use:

qclip -m 20 ‘cat fofn’

SEE ALSO
See Section 10.3 [ExperimentFile(4)], page 401.
11.14 Screen_seq

NAME

screen_seq — filters out sequence readings containing contaminating DNA

SYNOPSIS

    screen_seq [-lcwmiSpft] [-l Length of minimum match (25)] [-m Maximum vector length (100000)] [-i Input file of reading file names] [-I Input file of single reading to screen] [-s Input file of sequence file names] [-S Input file of single sequence to screen against] [-p Passed output file of file names] [-f Failed output file of file names] [-t Test only mode]

DESCRIPTION

    screen_seq searches sequence readings to filter out those from extraneous DNA such as vector or bacterial sequences. We have separated this task from that of locating and marking the extents of sequencing vector and other cloning vectors. There we require precise identification of the junction between the vectors and the target DNA. The filtering process described here is designed to spot strong matches between readings and a panel of possible contaminating sequences, and it splits readings into passes and fails. Readings that fail have a PS line containing the word "contaminant" and a tag of type "CONT" added to their experiment file.

    Normal usage would be to compare a batch of readings in experiment file format against a batch of possible contaminant sequences stored in (at present) simple text files. Each batch is presented to the program as a file of file names, and the program will write out two new files of file names: one containing the names of the files that do not match any of the contaminant sequences (the passes), and the other those that do match (the fails). It is also possible to compare single readings and single contaminant files by giving their file names (i.e. it is not necessary to use a file of file names for single files).

    Given the frequent need to compare against the full E. coli genome the algorithm is designed to be fast. The user controls the speed and sensitivity by supplying a single parameter, "min_match". The program will find the longest exact match of at least min_match characters.

    The search is conducted only over the clipped portion of the readings. On our Alpha machine it takes about 1 second to compare both strands of a reading against the 4.7 million bases of E. coli.

OPTIONS

-1 Length of minimum match (25)
    The length of match required to initiate a closer search.

-m Maximum vector length (100000)
    The maximum length of the longest sequence to screen the readings against.

-i Input file of reading file names

-I Input file of single reading to screen

-s Input file of sequence file names to screen against

-S Input file of single sequence to screen against

-p Passed output file of file names

-f Failed output file of file names

-t Test only mode
-t Test only mode

In test mode no experiment files are changed and the results are written to stdout. When not in test mode a dot "." is written to stdout for each comparison, and an exclamation mark "!" for each error detected.

EXAMPLES

Usage: screen_seq [options and parameters]
Where options and parameters are:
- [-l minimum match (25)] [-m Max vector length (100000)]
- [-i readings to screen fofn] [-I reading to screen]
- [-s seqs to screen against fofn] [-S seq to screen against]
- [-t test only]
- [-p passed fofn] [-f failed fofn]

1. Screen the readings whose names are stored in fofn against a batch of possible contaminant sequences whose names are stored in vnames. Write the names of the readings that pass to file p and those that fail to file f. Increase the maximum sequence length to 5000,000 characters and require a minimum match of 20.

   screen_seq -i fofn -s vnames -p p -f f -l20 -m5000000

2. Screen the single reading stored in xpg33.g1 against a batch of possible contaminant sequences whose names are stored in vnames. If the reading does not match write its name to file p, otherwise to file f. Increase the maximum sequence length to 5000,000 characters and require a minimum match of 20.

   screen_seq -I xpg33.g1 -s vnames -p p -f f -l20 -m5000000

3. Screen the readings whose names are stored in fofn against a single possible contaminant sequence stored in ecoli.seq. Write the names of the readings that pass to file pass and those that fail to file fails. Increase the maximum sequence length to 5000,000 characters and require minimum match of 20.

   screen_seq -i fofn -S ecoli.seq -p pass -f fails -l20 -m5000000

NOTES

Limits

Screen_seq is currently set to be able to process a maximum of 10,000 readings and 5000 screening sequences in a single run. The maximum length of any screening sequence is 100,000 although this can be overridden by use of the -m parameter (set it to 5000000 for E. coli). At present the sequences to screen against must be stored in simple text files containing individual sequences, with no entry names, and <100 characters per line.

The following errors can be reported.

1. "Failed to open file of file names to screen against". Fatal failure to open the file of file names to screen against.
2. "Failed to open single file to screen against". Fatal failure to open the file to screen against.
3. "Failed to open file of file names to screen". Fatal failure to open the file of file names to screen.
4. "Failed to open single file to screen". Fatal failure to open the file to screen.
5. "Failed to open file of passed file names". Fatal failure to open the file of file names for readings that do not match.
6. "Failed to open file of failed file names". Fatal failure to open the file of file names for readings that match.
7. "Failed to open single file to screen". Fatal failure to open the file to screen.
8. "Error: could not open vector file". An individual sequence file could not be opened.
9. "Error: could not read vector file". An individual sequence file could not be read.
10. "Error: could not hash vector file". An individual sequence file could not be prepared for comparison.
11. "Error: could not open experiment file". The file does not exist or is unreadable.
12. "Error: no sequence in experiment file".
13. "Error: sequence too short". The reading is shorter than the minimum match length.
14. "Error: could not write to experiment file". The disk is full or the file is write protected.
15. "Error: hashing problem". An error occurred in the comparison algorithm. Please report to staden-package@mrc-lmb.cam.ac.uk

Inconsistencies in the selection of options, such as selecting -I and -i, should also cause the usage message (shown below) to appear, and the program to terminate.

PS record added to the experiment file for any reading that matches.

SEE ALSO

See Section 10.3 [Experiment File], page 401. See Chapter 5 [Screening Against Vector Sequences], page 267.
11.15 TraceDiff

NAME

tracediff — Compare two trace files for differences to detect mutations.

SYNOPSIS


DESCRIPTION

tracediff compares a pair of traces to look for mutations. It aligns the traces, and then subtracts one trace from the other to produce a "difference trace". This difference trace is analysed to distinguish between mutations and incorrect base calls. Bonfield,JK, Rada,C and Staden,R Automated detection of point mutations using fluorescent sequence trace subtraction. Nucl. Acids Res. 26, 3404-3409 (1998).

For an overview and more details about mutation detection see Section 2.1 [Search for Mutations], page 185.

To detect mutations, compute the mean and standard deviation of the difference trace, and then locate bases associated with a significant pair of peaks, one positive, the other negative. For example a base change from an A to T will cause a positive A trace difference and a negative T trace difference. If both the positive and negative differences are more than num_sd multiples of the standard deviation from the mean, then this is flagged as a potential mutation. Mutations are written to the experiment file as MUTA tags.

The experiment file contains records specifying the input trace, the reference trace and the strand direction. It also contains the clipping points for the input trace. A minimal experiment file for tracediff might look like this:

LN 27.17f.ztr PR 1 QL 10 QR 839 WT C:/my_dataset/09_5f

Where the LN record specifies the name of the input trace, the PR record specifies the strand direction 1=forward, 2=reverse, the QL and QR records specify the input trace left and right clip points respectively, and the WT record specifies the wildtype trace. You can also optionally specify clip points for the wildtype trace as WL and WR records. Pregap4 generates suitable experiment files automatically, so these would not normally be created manually.

OPTIONS

-a peak-alignment-deviation
The centres of each individual half-peak of a double peak above and below the baseline must align reasonably well for them to be considered to be a real mutation. The amount of half-peak alignment deviation allowable is specified in bases by this parameter, usually as a fraction of one base.

-c complement-reverse-strand-tags
After mutation detection and after readings have been assembled into a GAP4 database, GAP4 displays both forward and reverse readings in a single direction in the contig editor. This makes it much easier to compare sequences and traces in both directions simultaneously. When the corresponding traces are displayed, any reverse strand traces
are complemented automatically such that the bases are interchanged. In this case, the original mutation tag generated by tracediff will then be of the wrong sense, so if checked, this option complements the tag base labels to match the complemented trace displayed by GAP4.

\texttt{-d output-difference-traces}

After trace difference analysis, the generated traces are normally discarded and not written to disk. Checking this option lets you save the trace difference files to the same directory as the original traces. The .ZTR trace format is used for this purpose. The original filename is retained and a ".diff.ztr" suffix is appended.

\texttt{-f file-of-filenames}

Specifies the filename of a simple text file containing a list of experiment files to be processed by tracediff.

\texttt{-n analysis-window-length}

Analysis of the trace difference is done over a local region to counter the effects of non-stationarity in the trace signal. The analysis region is defined by a short window whose length is specified in bases. The window is asymmetric in that it’s located to the left of the base it’s positioned on. This avoids measurement problems when mutations are encountered. The window size is a tradeoff. If it’s too big, low level mutations may be missed. If it’s too small, there may be insufficient data to give unbiased measurements leading to many false positives.

\texttt{-q quiet-mode}

If specified, no information is output to stdout. The mutations will still be written to the experiment file as tags.

\texttt{-s analysis-sensitivity}

This threshold is used to determine when an above/below baseline double peak in the difference trace is considered to be a mutation. It is specified in standard deviations from the mean over the analysis window. The higher the value, the more stringent the test. This value is reduced dynamically by the algorithm in the presence of mutations since small mutations near larger ones can often be missed with a uniform sensitivity setting. It’s likely that some experimentation with this parameter will be required for optimal mutation detection in your data.

\texttt{-t noise-threshold}

This threshold is used to filter out low level noise during the analysis phase. It is specified as a percentage of the maximum peak-to-peak trace difference value. A high threshold will lead to fewer false positives but you run the additional risk of missing low level mutations.

\texttt{-w maximum-peak-width}

During analysis, the width of each peak is measured to avoid problems caused by gel artifacts. These often appear as broad peaks that overlap many bases. The maximum peak width is specified in bases. A lower value will lead to fewer false positives, but you run the additional risk of missing smeared mutations towards the end of a trace.
11.16 Vector\_clip

NAME

vector\_clip — finds and marks vector segments in sequence readings

SYNOPSIS

```
vector\_clip [-schr] [-w word\_length (4)] [-n num\_diags (7)] [-d diagonal\_score (0.35)] [-l minimum\_match (20/70\%)] [-m minimum\_5\_position] [-t] [-p passed\_fofn] [-f failed\_fofn] input\_fofn
```

DESCRIPTION

`vector\_clip` finds and marks vector segments in sequence readings stored in experiment file format. For sequencing vectors it can be used to find the 5’ primer and, for short inserts, the sequence to the 3’ side of the cloning site. It can also be used to find 3’ primer sequences. A further option can do a final check for any vector rearrangements that could be missed by the more specific searches around the cloning site. For cloning vectors it will search both orientations of the sequence and mark any segments found. The vector sequences must be stored as simple text files. For cloning vector searches the reading’s experiment file must contain the name of the cloning vector file. For sequencing vector searches, either the experiment file for each reading must contain the information about the vector sequence (the file name, cloning site and primer offset) or vector-primer files must be used. Vector-primer files contain sets of sequences from around cloning sites, and `vector\_clip` can use these to find the vector that matches each reading best. If the match is above the cutoff score the reading is clipped. Vector-primer files are the simplest method of providing `vector\_clip` with the data it needs for finding sequencing vectors. More information is available elsewhere (see Chapter 5 [Screening Against Vector Sequences], page 267).

The program processes batches of readings by the use of file of file names: one is used for input and two for output. The input file lists the names of all the readings to process, one name per line. One output file contains the names of all the readings that pass the screening and the other contains the names of those that fail.

OPTIONS

- `\(-s\)` Mark sequencing vector. Searches for 5’ primer, 3’ running into vector.
- `\(-c\)` Mark cloning vector. Searches both strands for cloning vector.
- `\(-h\)` Hgmp primer. Searches 3’ end for a primer.
- `\(-i \text{vector\_primer filename}\)` Mark transposon data.
- `\(-r\)` Vector rearrangements. Searches for sequencing vector rearrangements.
- `\(-t\)` Test only. Does not change the experiment files, displays hits.
- `\(-L \text{minimum percentage match 5’ end (60)}\)` sequencing vector searches and transposon search
- `\(-R \text{minimum percentage match 3’ end (80)}\)` sequencing vector searches and transposon search
-m minimum 5’ position
allows a minimum 5’ end cutoff to be set if a sufficiently good match is not found (i.e.
it is really a default 5’ cutoff position). If a value of -1 is used the program will set the
cutoff to be the distance between the primer and the cloning site.

-v vector-primer-pair filename
sequencing vector search using vector-primer-pair file

-V vector_primer length
the length of the sequence stored in the vector_primer file to use for the 5’ search

-w word_length (4)
cloning vector search hash length

-P probability
cloning vector search, (a score less likely than P is a match)

-n num_diags (7)
cloning vector search, old score based algorithm: number of diagonals to combine

-d diagonal score (0.35)
cloning vector search, old score based algorithm

-l minimum match (20)
sequencing vector rearrangements and transposon search minimum match length

-M maximum vector length (100000)
all algorithms, reset for vectors >100000 bases

-p passed fofn
file of file names for passed files

-f failed fofn
file of file names for failed files

input fofn ...
input file of file names

EXAMPLES

Usage: vector_clip [options] file_of_filenames
Where options are:

[-s mark sequencing vector]     [-c mark cloning vector]
[-h hgmp primer]                [-r vector rearrangements]
[-w word_length (4)]            [-n num_diags (7)]
[-d diagonal score (0.35)]      [-l minimum match (20)]
[-M maximum % 5’ match (60)]    [-R minimum % 3’ match (80)]
[-m default 5’ position]        [-t test only]
[-M Max vector length (100000)] [-P max Probability]
[-v vector_primer filename]     [-i vector_primer filename]
[-V vector_primer length]       [-f failed fofn]

Screen for sequencing vector using 5’ cutoff of 70%, a 3’ cutoff of 90% and default 5’ primer
position of 30. The batch of files to process are named in files.in, the names of the passed files are
written to files.pass and the names of those that fail to files.fail.

vector_clip -s -L70 -R90 -m30 -pfiles.pass -f files.fail files.in

Screen for sequencing vector using 5’ cutoff of 60%, a 3’ cutoff of 80% and default 5’ primer
position of 30. The batch of files to process are named in files.in, the names of the passed files are
written to files.pass and the names of those that fail to files.fail. This shows that the default search is for sequencing vector.

```
vector_clip -m30 -pfiles.pass -f files.fail files.in
```

Screen for sequencing vector using 5’ cutoff of 60%, a 3’ cutoff of 80% and a vector-primer-pair file called vector.primer_file. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -v vector_primer_file -pfiles.pass -f files.fail files.in
```

Screen transposon data using 5’ cutoff of 80%, a 3’ cutoff of 85%, a match length of 10 and a vector-primer-pair file called vector.primer_file. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -i vector_primer_file -L 80 -R 85 -l 10 -pfiles.pass \ 
-f files.fail files.in
```

Screen for cloning vector using the old algorithm with a word length of 4, summing 7 diagonals and diagonal cutoff score of 0.4. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -c -w4 -n7 -d0.4 -pfiles.pass -f files.fail files.in
```

Screen for cloning vector using the probability based algorithm with a word length of 4 and probability cutoff of 1.0e-13. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -c -P 1.0e-13 -pfiles.pass -f files.fail files.in
```

Screen for 3’ primer using a cutoff of 75%. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -h -R75 -pfiles.pass -f files.fail files.in
```

Screen for sequencing vector rearrangements using a cutoff of 20 bases. The batch of files to process are named in files.in, the names of the passed files are written to files.pass and the names of those that fail to files.fail.

```
vector_clip -r -l20 -pfiles.pass -f files.fail files.in
```

**NOTES**

The following error messages can be generated.

1. Error: could not open experiment file
2. Error: no sequence in experiment file
3. Error: sequence too short
4. Error: missing vector file name
5. Error: missing cloning site
6. Error: missing primer site
7. Error: could not open vector file
8. Error: could not write to experiment file
9. Error: could not read vector file
10. Error: missing primer sequence
11. Error: hashing problem
12. Error: alignment problem
13. Error: invalid cloning site
14. Warning: sequence now too short (no message)
15. Warning: sequence entirely cloning vector (no message)
16. Warning: possible vector rearrangement (no message)
17. Warning: error parsing vector_primer file
18. Warning: primer pair mismatch!
19. Aborting: more than X entries in vector_primer file

SL, SR, CL, CR, CS, PS, PR and SF records are written to the experiment files.

SEE ALSO

See Section 10.3 [Experiment File], page 401.

For notes on defining the cloning and primer sites, See Section 5.8 [Defining the Positions of Cloning and Primer Sites for Vector Clip], page 273.

See Section 10.1 [scf(4)], page 383.
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