BioEdit version 7.0.0

This is the current help file for **BioEdit version 5.0.6**.
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Tom Hall
Ibis Therapeutics, a division of Isis Pharmaceuticals, Inc.

This is likely to be the final release of BioEdit.
There may be some bugs.
This is a free program and comes with a complete (but simple) disclaimer:
Simple DISCLAIMER: This software is provided as is. There are no warranties. The author will not be held responsible for any problems. This software may be freely distributed, provided that the original full installation is distributed along with the on-line documentation and the license agreement, and that the distributer realizes that that are other freeware programs packaged in the installation written by other authors.

That aside, if you have any questions or problems, you may email Tom Hall at: tahall2@isisph.com.

This file was last updated on7/2/2004.

I would like to thank **Isis Pharmaceuticals, Inc** for generous support to the Brown lab (James W. Brown, NCSU) for additions to BioEdit version 5.0.0, and for employment.

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About BioEdit

Introduction

BioEdit version 7.0.0
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Tom Hall
Current version built 7/2/2004

BioEdit is a biological sequence editor that runs in Windows 95/98/NT/2000/XP and is intended to provide basic functions for protein and nucleic sequence editing, alignment, manipulation and analysis. BioEdit is not a powerful sequence analysis program, but offers many quick and easy functions for sequence editing, annotation and manipulation, as well as a few links to external sequence analysis programs. Sequence lengths and numbers are limited only by available system memory. Alignments >100 Mb have been edited on an average desktop with reasonable efficiency. The document interface was originally modeled after the very nice programs SeqApp and SeqPup by Don Gilbert. SeqApp (Macintosh) and SeqPup (cross-platform) are offered free of charge from Indiana University at:

ftp://iubio.bio.indiana.edu/molbio/seqpup/

An exceptional alignment program that is freely available for Windows 95/98/2000 is called GeneDoc. GeneDoc is very professional and has nice protein alignment annotation and analysis, shading and structural definition features not offered in BioEdit, as well as an internal phylogenetic tree view of alignments. GeneDoc can also be found on the World Wide Web:

http://www.psc.edu/biomed/genedoc/

BioEdit is a C++ program written in Borland's C++ Builder. I am a graduate student in Microbiology at North Carolina State University, and not a trained programmer. This was my introduction to the C++ language and is necessarily a side project (this is not part of my doctoral work). This program could be *much* smaller and more efficient. Nevertheless, BioEdit provides an easy means for sequence alignment, output, and some analyses.

BioEdit Features

The main goal of BioEdit is to provide a useful tool for biologists who do not want to have to know much about a program to utilize it. BioEdit is intuitive, menu-driven, and highly graphical and offers a graphical interface for users to run external analysis programs. The main functions are intended to be visible by simply playing with the menu options.

Version 7.0.0 offers the following features:

The main goal of BioEdit is to provide a useful tool for biologists who do not want to have to know much about a program to utilize it. BioEdit is intuitive, menu-driven, and highly graphical and offers a graphical interface for users to run external analysis programs. The main functions are intended to be visible by simply playing with the menu options.

Version 7.0.0 offers the following features:

- An easy, graphical interface for sequence manipulation and editing.
- Variable editing options, including ‘select and drag’ sliding and 'grab and drag' sliding of residues, variable selection options, mouse-click insert and delete of gaps, full column selecting, on-screen editing with cut, copy and paste, and auto-scrolling of edit window.
- Split the window vertically or horizontally to manipulate two regions of an alignment at the same time.
- Collapse multiple columns of an alignment to hide them on the screen.
- Anchor alignment columns to protect fixed regions in an alignment.
- Automatically and manually annotate sequences with features such as introns, exons, promoters, CDS, and all standard GenBank feature types. Automatically annotate other sequences in an alignment using one sequence as a template.
- Download sequences into an alignment document directly from GenBank.
- Group sequences into color-coded families and lock group members for synchronized hand-alignment.
- User-defined character-relevance (any characters can be set to be considered as relevant bases in nucleic acid or amino acid sequences for the purposes of similarity shading, sequence identity matrices, and conservation plot views.
- User-defined motif searching using standard Prosite nomenclature and utilizing IUPAC characters to allow searching in nucleic acid or amino acid sequences, as well as exact text searches including or ignoring gaps.
- Lines may be defined as DNA, RNA, nucleic acid, protein, undefined, comments, sequence mask (basically the same as comments) or RNA structure mask. Comments may be used to hold general notes or things such as secondary structure mask definitions, but do not contribute to conservation calculations.
- Configure accessory application interfaces to run external analysis programs through a graphical interface created by BioEdit. Automatically feed information to and retrieve files from external apps. External apps run in a separate thread to allow simultaneous use of BioEdit while running time-consuming processes. Output from an external program may be automatically opened by another program.
- Merge alignments through a common reference sequence.

- Append one alignment to the end of another
- Rudimentary phylogenetic tree viewer that supports node flipping and printing.
- Display, print and edit ABI trace files from ABI autosequencer model 377, 373, and 3700, as well as SCF files of version 2 and 3, such as the files output by Licor sequencers.
- RNA comparative analysis tools, including covariation, potential pairings, and mutual information analyses.
- 2-D matrix plotter for mutual information output with dynamic data viewing with the mouse pointer. (Also allows image copy/paste and bitmap save).
- Interactive 1-D plots of mutual information matrix rows and columns.
- Color RNA secondary structure by base-pairs based upon a structure definition mask.
- Save sequence annotation information in BioEdit or GenBank format
- Align protein-encoding nucleic acid sequences through amino acid translation. Slide residues in toggled hybrid protein-DNA translations by toggling translation of annotated CDS features.
- Search for conserved regions in an alignment (find good PCR targets or help define motifs)
- Search for user-defined motifs in nucleic acid or protein sequences or search exact text with wildcards and choice of including or ignoring gaps.
- Dynamic memory allocation. Alignment size, number and length of sequences are limited only by avalailable memory.
- BioEdit currently reads and writes GenBank, Fasta, NBRF/PIR, Phylip 3.2 and Phylip 4 formats and reads ClustalW and GCG formats.
- Import/Export filter for 10 additional formats (Using Don Gilbert's ReadSeq).
- Import/Append one file on to the end of another (regardless of file format).
- Read and write large alignment files quickly with the BioEdit Project file format.
- ClustalW multiple sequence alignment (interface internal, external program by Des Higgins et. al.) with auto-update of aligned protein full titles and GenBank field information, as well as nucleotide coding sequence when aligned from a protein view of nucleotide sequences.
- Block copying of residues or sequence titles to clipboard allowing for pasting of full alignments or parts of alignments into a word processor or spreadsheet.
- Paste over blocks of sequence or sequence titles.
- Basic sequence manipulations (copy/paste of sequences between documents, translation and degenerate encoding, RNA->DNA->RNA, reverse/complement, upper/lowercase).
- Multiple document interface (Maximum of 50 open alignment documents at a time, but no set limit on other open windows).
- Six-Frame translation of nucleic acid sequences into Fasta-format ORF lists. Tested by translating the *E. coli* genome (4.6 Mbases) into 10,125 sorted raw codon stretches of 100 or more amino acids and 39,880 unsorted raw codon stretches of 50 or more amino acids.
- Semi-automated plasmid/vector drawing and annotation with vectored graphics, automatic restriction site and positional marking, automated polylinker view, and user-controlled drawing objects
- Save plasmid files as editable vectored graphic files or as bitmaps, copy to other graphics applications, and print plasmids at printer's full resolution.
- Amino acid and nucleotide composition summaries and plots
- 'Revert to Saved' and 'undo'/'redo' functions (up to 30 undo levels allowed).
- Edit both amino acid and nucleic acid sequences.

- Easy point-and-click color table editing, with different tables for protein and nucleic acid sequences.
- Alignment-responsive shading based on information content of alignment positions.
- Basic rich-text editor.
- Internal restriction mapping utility with any or all-frames translation, multiple enzyme and output options, including enzyme suppliers, and circular DNA option. Annotate sequences with restriction sites, fragment sequences with exact monoisotopic mass calculation of all resulting fragment strands.
- Browse restriction enzymes by manufacturer, or choose enzymes by properties or from a list.
- Auto-linking to your favorite Web Browser (e.g., Netscape or Internet Explorer).
- World Wide Web Bookmarks.
- NCBI BLAST tools, including BLAST 3.0 Internet client and local BLAST with the ability to compile local databases from Fasta files
- Configurable formatted text print with dynamic print preview,
- Configurable formatted shaded graphical output with dynamic preview, identity and similarity shading, and ability to cut and paste directly to graphics/presentation program for generation of figures.
- Entropy (lack of information) plotting of alignments
- Hydrophobicity profiles of multiple proteins using several hydrophobicity scales, with variable window width and option to analyze degapped sequences or alignments.
- Retain data from GenBank files, including LOCUS, DEFINITION, ACCESSION, VERSION, PID/SID, SOURCE, DBSOURCE, FEATURES, KEYWORDS, REFERENCE, FEATURES and COMMENT.
- Add table-based taxonomy data, as well as the NCBI-defined semicolon-delimited phylogeny string. Automatically map Bacterial phylogenies to a columnized phylogeny table. Map other phylogenies to your own curated phylogeny table.
- A variety of search functions, including all GenBank fields and phylogeny table.
- A variety of title search functions including a flexible search and replace using wilcards.
- Several sort functions, including phylogeny-based sorting.
- Calculate exact monoisotopic masses for DNA and RNA molecules.
- Rudimentary FTICR mass-spec data viewer foir BRUKER FTICR acqus+fid data files.
- Calculate oligo Tms with oligo/target mismatches based on mismatch parameters from John SantaLucia’s lab.
- Automatically grab Pubmed references associated with sequences directly from the web (requires Internet Explorer as an ActiveX component).
- Multiple levels of undo (up to 30), with more complete coverage of undoable operations (all should theoretically be undoable, but there have been some oversights in previous versions).

General overview of program and program organization

BioEdit was originally written in Borland C++ Builder 3.0 (started in C++ Builder 1.0). At the time, this was Borland’s newest C++ product which combined Borland C++ 5 with the Visual Component Library (VCL) of Delphi, allowing for visual development of the user interface. The benefit of using a Rapid Application Development (RAD) environment such as this is that it allows for the easy creation of a very rich graphical interface. The drawback is that the code is not portable. BioEdit runs only in Windows 95, 98, NT, 2000 and XP.

Organization: BioEdit currently supports the simultaneous editing of up to 50 documents. A main control form contains menus to open documents, create new documents, set global options such as color tables, codon table, and analysis preferences, and a window manager. Originally, each document had its own complete set of menus for all manipulations confined to that document, however, this has been abandoned for a more traditional multiple document interface. BioEdit does not use excessive physical memory (unless big alignments are being edited), but it does appear to be a bit of a resource hog. An alignment document currently has no set limit on number of sequences or sequence length.

The program file (BioEdit.exe) is found in the main installation directory. There should also be the following subdirectories:

- apps (accessory applications and WWW bookmarks)

Currently, the following files should be in the apps folder (as shown in the file manager sorted by name):

accApp.ini (accApp.def when first installed
blast.txt
blastall.exe
blastcl3.exe
blastcli.exe
bookmark.txt
cap.doc
cap.EXE
clustalw.exe
clustalw.txt
DNADIST.DOC
dnadist.exe
DNAML.DOC
dnaml.exe
DNAMLK.DOC
DNAMLK.EXE
DNAPARS.DOC
DNAPARS.EXE
DOS4GW.EXE
fastDNAml.doc
fastdnaml.EXE
FITCH.DOC

Fitch.exe
formatdb.exe
KITSCH.DOC
KITSCH.EXE
NEIGHBOR.DOC
NEIGHBOR.EXE
ncbi_presets.ini
phylip.map
PROML.DOC
proml.exe
promlk.exe
PROTDIST.DOC
PROTDIST.EXE
PROTPARS.DOC
PROTPARS.EXE
readseq.exe
ReadSeq.txt

- database (default for local BLAST databases). (empty)

- help
  BioEdit.cnt
  BioEdit.GID (not installed -- will appear after the first time help is accessed)
  Bioedit.hlp

- tables
  Bacterial_phylogeny.tab
  BLOSUM62
  BLOSUMcoloring.tab
  chao_fasman.tab
  codon.tab
  codonDegeneracyColoring.tab
  color.tab
  dayhoff
  defcolor.tab
  enzyme.tab
  GC.VAL
  gencodes.tab
  gonnet
  IDENTIFY
  kyteDoolittle.tab
  KyteDoolittleHydrophobicityColoring.tab
  ManuelRuizColorTable.tab
  match
  PAM120
  Pam250

PAM250Coloring.tab
PAM40
PAM80
SEQCODE.VAL
taxGroups.tab
Viral_Phylogeny.tab

The installation folder will also contain the following files:

_deisreg.isr
_isreg32.dll
BioEdit.exe (main program)
DeIsL1.isu
TreeV32.zip (the TreeView installation distribution)
TreeView.txt (TreeView information)
license.txt (license agreement)
Readme.txt (this file)

It is important that none of the folder names nor file names are changed, as parts of BioEdit will not run correctly if these names are changed.

All versions before 7.0.0 had the file "BioEdit.ini" in the main Windows directory. Version 7.0.0 has moved this file to the BioEdit installation folder, as a few complaints have come in referring to error dialogs saying "Cannot write to BioEdit.ini". This file contains the initialization defaults and preferences for BioEdit. Although this file can be edited manually, there should be no need and manual editing of this file is not recommended.

For a list of currently supported features and known problems, see BioEdit Features and Known Problems / Limitations.

Known problems / Limitations

BioEdit is intended to be a general-purpose interface for several simple sequence manipulations, general alignment of sequences with an option for automated multiple alignment, optimal pairwise alignment, and an emphasis on making hand alignment easy. Several accessory functions have been added over time (plasmid drawing, restriction mapping, ABI and SCF viewing, RNA comparative analysis and graphical annotation among other features). However, sophisticated search functions, specialized analyses such as protein secondary or tertiary structure predictions, thermodynamic predictions of RNA structure, statistical analyses of alignment quality, and probabilistic or neural network modeling of sequence patterns, alignment and structure prediction are outside the scope of this program.

Although command-line accessory applications may be configured by the user, there are programmed links to ClustalW and local BLAST and BLAST client 3. These links are not guaranteed to work correctly if the Clustal program or BLAST programs are replaced with an upgrade. Although the local BLAST and Clustal programs provided in the BioEdit installations will continue to work, BLAST client 3 may not work correctly after the next time the NCBI decides to change its client and I am no longer supporting this program directly. The source code may be offered for download at a later date, but is somewhat disorganized, not well commented, and really constrained to Borland C++ Builder (which is the main reason I don't bother to post the source code).

Also, automated web links which feed a selected sequence to the web page (e.g. for BLAST, PSI-BLAST, PROSITE profile scan) work by keeping a local HTML template for the web page, the source for which BioEdit edits to include the selected sequence within the query text area. Because of the highly mutable nature of the World Wide Web, these may not function correctly for very long. If the server addresses change, or the HTML interface changes substantially, these will no longer work correctly. They can possibly be updated by placing the newer web page locally into the BioEdit/apps folder under the same name as the current ones, but whether they work correctly will depend upon whether necessary URL references in the web page are specified as absolute or relative paths, and whether they depend on calling local CGI or Java programs, and other such potential problems.

The interface to configure command-line analysis programs does its best to be as complete as possible without requiring a complicated general-purpose scripting language. Because of the static nature of this interface and its options, however, there will be programs that just cannot be run correctly through BioEdit, though most programs that accept a command line should be able to be configured. Many people may prefer to run a program from the command line for better control of the options, anyway. The accessory application configuration is mainly intended for labs that want to be able to set up an easy method for several people who grew up on easy GUI interfaces to be able to run routine analyses without having to navigate the files and command-line options manually.

BioEdit performs fairly well with reasonably-sized alignments. However, there is an imposed limit on both the number of alignment documents that can be opened at once, as well as the number of sequences that can be contained in a single alignment. Currently the limit on open alignment documents is 50, though this may run Windows out of resources. The limit on the number of sequences in an alignment is 20,000.

The sequence number limit is independent of the lengths of the sequences. The absolute size of an alignment matrix is limited only by available system memory. If a document runs the system completely into virtual memory, editing will become *very* slow. If alignments on the scale of several thousand rRNA genes, or sequence lists from entire genomes, for example, will be used, it is recommended to have at least 64 to 128 Mb on a Win95/98 or NT machine, and probably at least 128 Mb on a Win2000 machine.

The open document and sequence number limits are a result of poor original program design that is a little cumbersome to change at this time. When the core of BioEdit first evolved, I was still getting a handle on memory handling and pointer manipulations, and so a static array of pointers to keep track of open documents by memory address or index is allocated at program startup, and at the time of creation of a document, an array of pointers to hold sequences that can be accessed either by memory address or array index is set aside. If this part of the core is ever redesigned, there will be no restriction on sequence number nor document number.

Another potential drawback that becomes evident with very large documents is that all lists of sequences are treated as an alignment matrix and the entire matrix is kept in physical memory for every open document. Having three documents open that are each 8000 or so sequences of about 4000 bases long each, for example, will run memory just for the alignment matrices up to >96 Mb, which, on top of the OS and all other allocated memory, will run into virtual memory even on a machine with 128 Mb RAM, and performance will slow to a crawl. At this time, there is no monitoring of memory use, nor internal swap-file system to reduce physical memory usage of idle matrix space.

The undo option is limited to one level at this point and needs to be redesigned (this probably won't happen, though). One undo level requires the same amount of memory as the entire alignment, and was admittedly programmed for ease of programming rather than performance. Therefore, for an alignment matrix where N x M > 40,000,000 (N = number of sequences and M = length of the longest sequence), undo is automatically disabled.

One more limitation is that BioEdit is written in Borland C++ Builder and is 100% Windows-based. It is basically non-portable as it is. Since the majority of this program is its rich graphical interface, creating a similar program on UNIX or Mac would require the program be written almost from the ground up, with very little porting possible.

Contacting the Author

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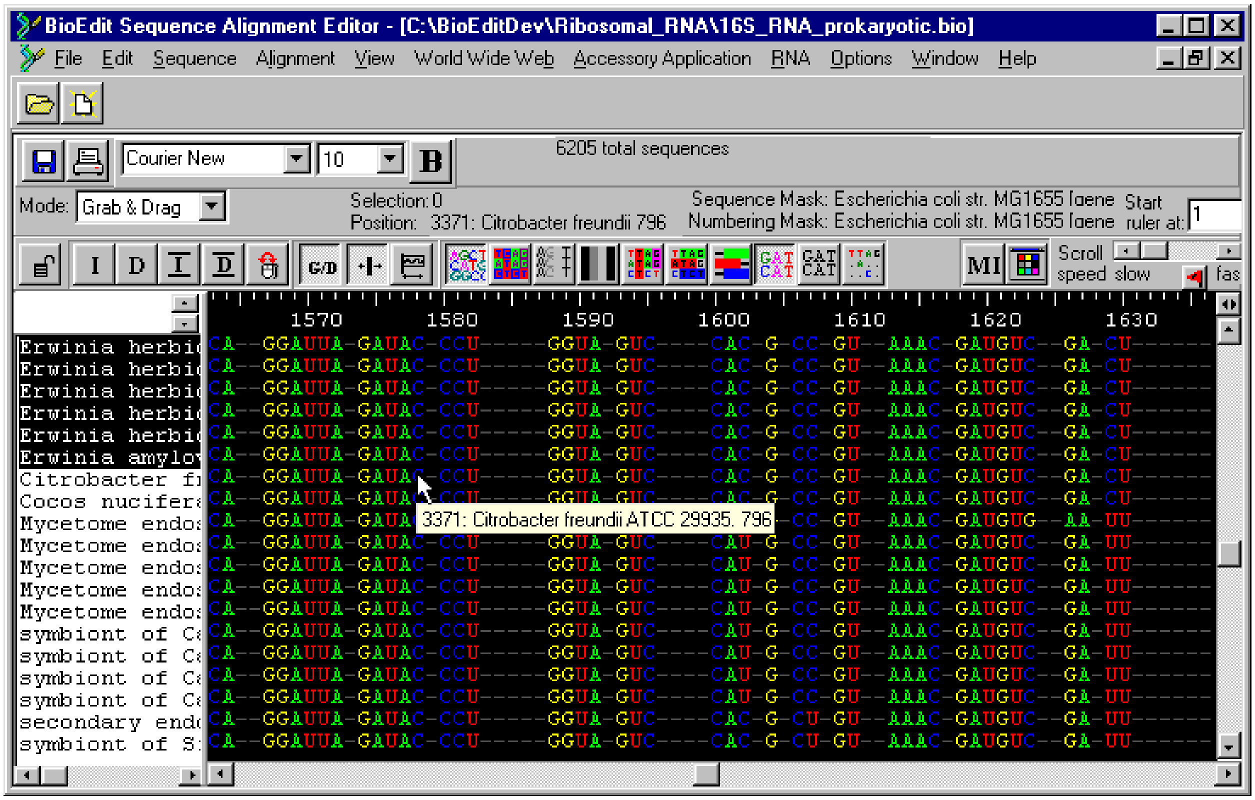
General Use of BioEdit

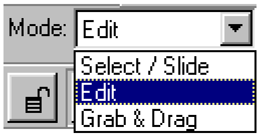
Sequence Editing / Manipulation

Manual alignment of sequences

Below is an image of the basic BioEdit alignment document window. Don't worry if you don't like the current view. The font, size, background color, residues colors, and title window width may all be changed. The yellow box to the lower right of the mouse arrow shows the absolute position in the current sequence. This also appears in the "Position" caption on the control bar, and the option to shut off the yellow boxes is found under View->show sequence position by mouse arrow.



The general manual alignment functions are:

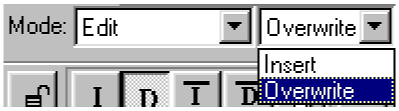


There are three basic modes available in the edit window: These options may also be found under Sequence->Edit Mode

Select / Slide mode: Select residues by boxing them with the mouse (left mouse button). Drag the selection back and forth with the mouse. The default is to "crunch" unlocked gaps in the direction you are sliding and open new unlocked gaps on the other side of the selection. To move the entire sequence downstream of the selection, regardless of gaps, hold down the shift key while dragging. You may also toggle the appropriate button on the buttons panel (see below) to change the default to moving the entire sequence downstream of the selection. With this option selected, use the shift key to "crunch" unlocked gaps when sliding.

Using the shift key while selecting will select all residues between the current selection and new selection. The CTRL key allows you to add only the new selection to the current selection (for instance, you may want to select residues in three sequences which are not right next to each other).

Edit mode: When in edit residues mode you may place the cursor anywhere in the document (except the titles) and type. You may move around between sequences with the arrow keys. There are two basic modes of editing, as in a word processor: insert and overwrite. When the editor is in "Edit" mode, a choice will be visible to the right of the edit mode drop-down:



Mode: Edit

Overwrite

When in the other two alignment modes, this choice will not be visible.

Grab & Drag mode: Choosing "Grab & Drag" from the "mode" list or toggling the "G/D" button (see below) allows you to grab and drag a single residue dynamically on the screen. Use the shift key to move the entire sequence downstream of the residue (or toggle the appropriate button on the buttons panel -- see below).

Grouping of sequences: Sequences may be grouped into groups (or "families"). The alignment for a group of sequences may be locked together, meaning that hand adjustments (insertion and/or deletion of gaps by sliding residues) will be automatically synchronized for a locked group. This ***only*** applies to sliding resides (Select / slide mode or Grab & Drag mode), not to single insertions and deletions of gaps with right mouse clicks. For information on grouping sequences and locking the alignment of groups of sequences, see grouping sequences.

Tool Bar / Speed buttons:

Lock and unlock all gaps in the entire alignment (shows all unlocked position). When an alignment is opened, this button is in the unlocked state, but gaps are present however they were saved. Changes are only made each time this button is pressed. To unlock all gaps in a current alignment, you must press this button twice to toggle it back to this state (the first press will lock all gaps).

Locked state of above button.

When down, allows you to insert single gaps by right-clicking the mouse.

Delete gaps by right-clicking the mouse.

Insert gaps in all sequences *except the one clicked on* with the right mouse button.

Delete gaps in all sequences *except the one clicked on* with the right mouse button. Sequences that do not have a gap at the selected position will be unchanged, but the gap will still be removed from any sequences that have one there.

Reverses the default functions of the left and right mouse buttons

Toggle "Grab & Drag" mode.

When this button is down, the default when sliding residues is to crunch or expand downstream gaps. Use the shift key while sliding to reverse this.

When this button is down, the default when sliding residues is to move the entire sequence downstream of the selection, rather than crunching or expanding gaps. Use the shift key while sliding to reverse this.

Normal view mode. When sequences are viewed in color, residues are colored according to the current color table. This option must be chosen to view sequences in monochrome. All other views override monochrome viewing.

Inverse color view mode. Background boxes are shaded according to the color table for each residue. Residue colors are the inverse of their normal colors.

"Strength of Alignment" -- Residues are shaded in grayscale according to the information content at each column position.

Residue backgrounds are shaded according to the information content at each column position.

Shade residues by identity and similarity in the document window. When this button is down, a drop-down list will appear on the control bar which controls the percent threshold for shading. The matrix file used for similarity shading of protein alignments can be specified from the Alignment->Similarity Matrix menu.

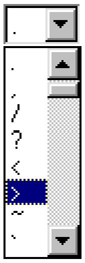
Draw features with sequences superimposed over them.

Draw features only. Do not show sequences.

View sequences in color, according to the current color table.

View sequences in monochrome, according to the currently selected sequence color. This mode only applies if the “normal view” button is also down.

Show identities to a reference sequence (default = top) with a character (default = '.').

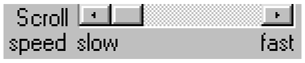


This drop-down allows for selection of the character to plot identities with, provided that the previous button is active (depressed).

Show or hide the mutual information examiner (for RNA analysis only).

Brings up the color table edit dialog.

Toggles “ignore anchor points” mode. When this is off (the button is not down), column anchors restrict the range of alignment. When this button is down, column anchors are ignored.



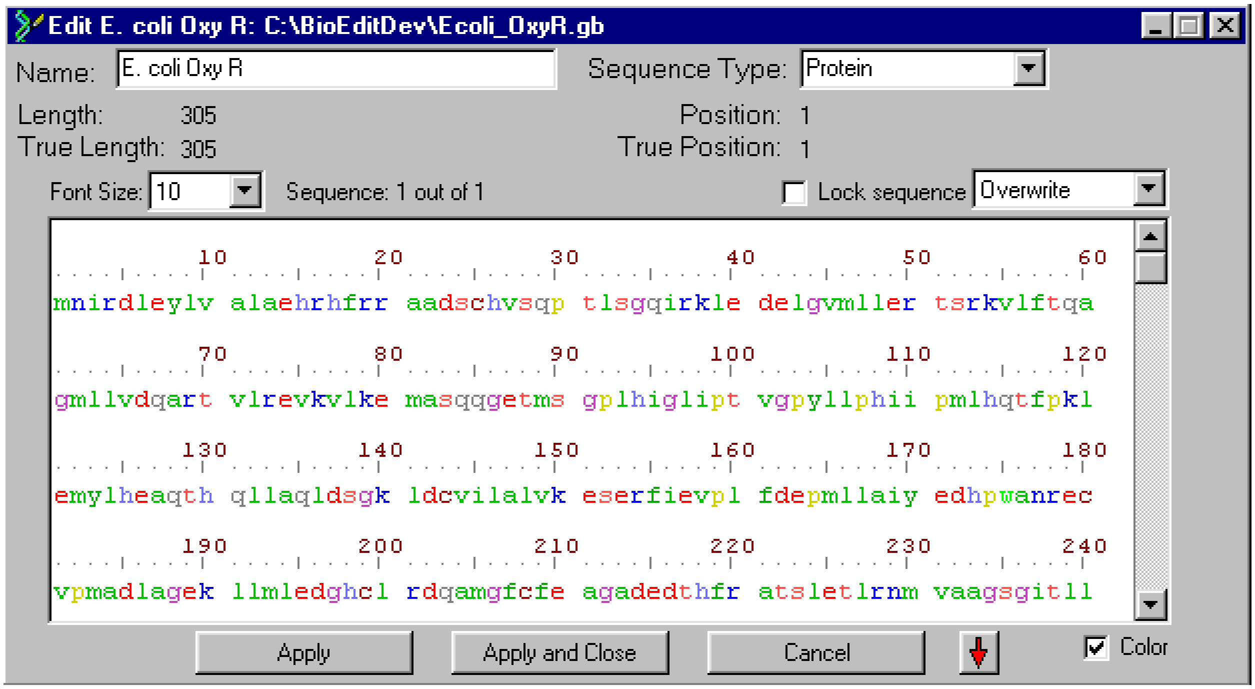
Scroll speed controller: controls the speed of the horizontal scroll bar (scrolling is in increments of residues).

Add or remove a positional marker flag.

Add or remove a column anchoring point.

Editing in an Edit Box

To make major edits to a sequence, it may be convenient to edit it in a text window. To open an edit window for a sequence, either double-click on the sequence title, or select the sequence and choose “Edit Sequence” from the “Sequence” menu. For changes to take effect, the “Apply” or “Apply and Close” button must be pressed. Canceling will cause no change in the sequence. The following window will appear when a sequence is first opened for editing.



Edit E. coli Oxy R: C:\BioEditDev\Ecoli_OxyR.gb

Name: E. coli Oxy R

Sequence Type: Protein

Length: 305

True Length: 305

Position: 1

True Position: 1

Font Size: 10

Sequence: 1 out of 1

□ Lock sequence

Overwrite

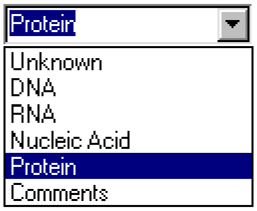
```
         10         20         30         40         50         60
mnirdleylv alaehrhfrr aadschvsqp tlsgqirkle delgvmller tsrkvlftqa

         70         80         90        100        110        120
gmllvdqart vlrevkvlke masqqgetms gplhiglipt vgpyllphii pmlhqtfpkl

        130        140        150        160        170        180
emylheaqth qllaqldsgk ldcvilalvk eserfievpl fdepmllaiy edhpwanrec

        190        200        210        220        230        240
vpmadlagek llmledghcl rdqamgfcfe agadedthfr atsletlrnm vaagsgitll
```

In the "Sequence Type" drop-down, the following options are available. If a sequence is "unknown", the protein color table is used for coloring, and it is treated like a protein sequence for the purposes of similarity shading.

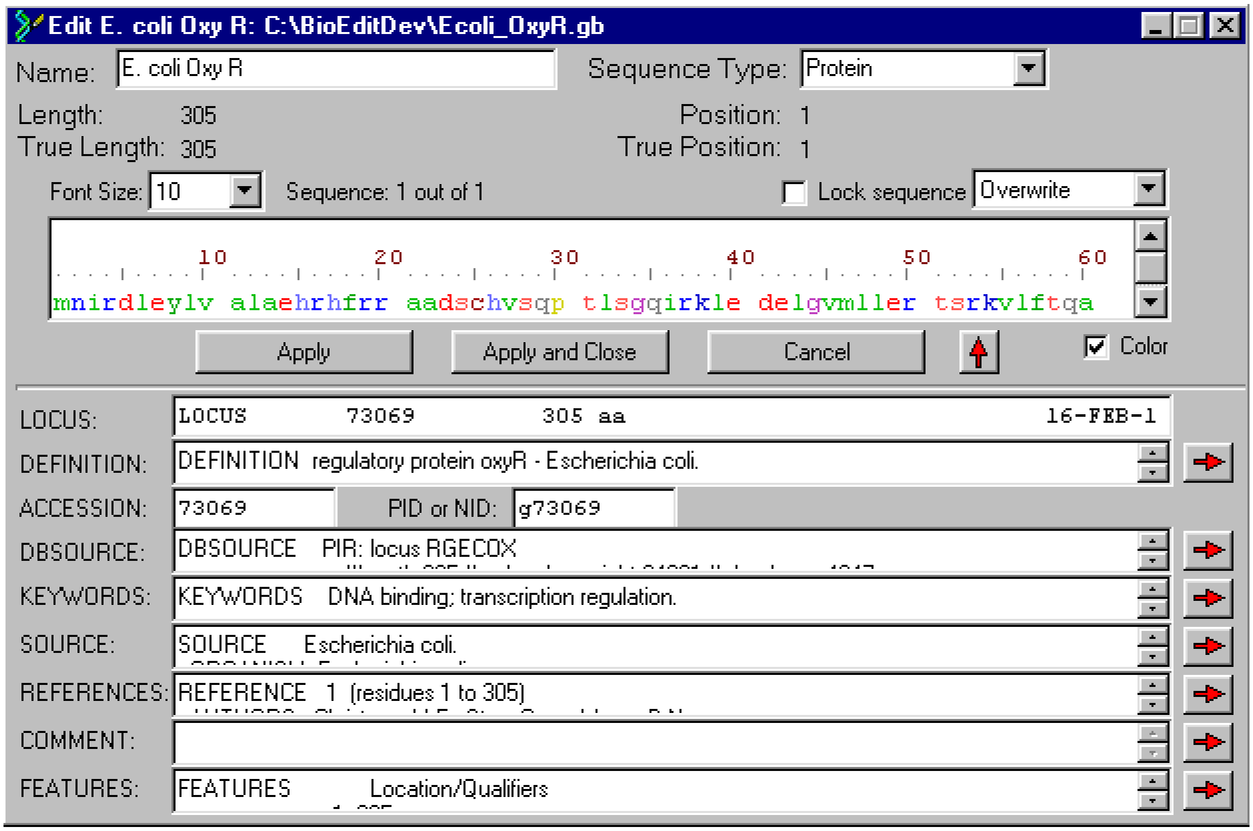


A "comment" may be reserved to hold information on the screen at any line in the alignment, but does not contribute to calculations of similarity and identity, and is not subject to the standard manipulations such as translation, complementing, automatic alignment, etc.

You may choose to lock any sequence with the "lock sequence" option within the single sequence editor. ☑ Lock sequence

When this option is applied, editing on screen and hand alignment by selecting/dragging or grab and drag will be disabled. Adding and deleting of gaps by right mouse clicks will still be enabled, however.

To expand the window to see associated GenBank information, press the ⬇ button
The window will expanded as follows:



Edit E. coli Oxy R: C:\BioEditDev\Ecoli_OxyR.gb

Name: E. coli Oxy R   Sequence Type: Protein

Length: 305   Position: 1
True Length: 305   True Position: 1

Font Size: 10   Sequence: 1 out of 1   ☐ Lock sequence   Overwrite

```
         10        20        30        40        50        60
mnirdleylv alaehrhfrr aadschvsqp tlsgqirkle delgvmller tsrkvlftqa
```

LOCUS: LOCUS 73069 305 aa 16-FEB-1

DEFINITION: DEFINITION regulatory protein oxyR - Escherichia coli.

ACCESSION: 73069   PID or NID: g73069

DBSOURCE: DBSOURCE PIR: locus RGECOX

KEYWORDS: KEYWORDS DNA binding; transcription regulation.

SOURCE: SOURCE Escherichia coli.

REFERENCES: REFERENCE 1 (residues 1 to 305)

COMMENT:

FEATURES: FEATURES Location/Qualifiers

The ➡ button may be used to bring up the associated field in a larger edit window.

** Note: GenBank information will only be saved in GenBank or BioEdit format
***Note: GenBank information, including the "features" field, is internally independent of user-defined graphical annotations.

Windowshading

A document may be "Window shaded", that is, reduced to its title bar, by double-clicking on the title bar of the window. Double-clicking again will bring it back to its original size. It can also be minimized and maximized in the normal manner.

Adding a new sequence

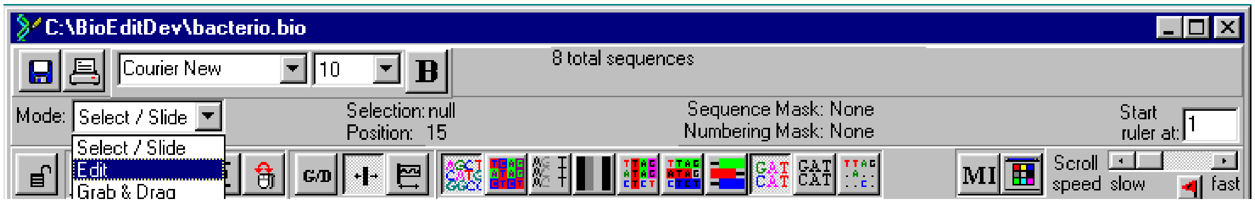
A new sequence may be added by:

1. Selecting the "New Sequence" option under the "Sequence" menu. The sequence may be typed, or copied as raw text, into the sequence window. Press "Apply" to add the sequence to the document.

2. Sequences may be copied and pasted from other BioEdit documents with the "Copy Sequence(s)" and "Paste Sequence(s) commands from the "Edit" menu. Also, current menu shortcuts may be used (defaults: Ctrl+F8 for copy and Ctrl+F9 for paste).

Editing on screen

Sequences may be edited on screen much like working in a word-processor. The "Mode" option of "Edit Residues" must be set first (BioEdit is installed with the "Slide Residues" mode as the default).



C:\BioEditDev\bacterio.bio

Courier New 10

8 total sequences

Mode: Select / Slide

Selection: null
Position: 15

Sequence Mask: None
Numbering Mask: None

Start ruler at: 1

When in edit mode, you may use the arrow keys to move around on the screen and type as in a text editor. There are two options for editing: Insert mode or overwrite mode, which each behave as the analogous functions in a common word-processor.

Selecting Sequences

Sequences are selected by clicking on their titles. Multiple sequences may be selected by drawing a box around them, or by shift-clicking to select everything between two selections. Use the Ctrl key with the mouse to de-select selected titles, or to add specific titles to the selection. Double-clicking on a title will open the single sequence editor. Clicking again on a previously selected title will put it into on-screen editing mode. You may then edit the title and either press <return> or click the sequence title panel anywhere off of the current title for the change to take effect.

Moving Sequences

To move a sequence (or sequences), select it (highlight its title by clicking it with the left mouse button) and drag it to where you would like it in the alignment.

Cut/Copy/Paste

Copy:

Text in edit window (sequence residues): Select the text with the mouse and choose “Copy” from the “Edit” menu. Unlike a word processor, you may copy discreet blocks of text without copying entire lines of text. A block of text copied this way may be pasted into any text edit-capable program.
If, and only if, there are no residues selected in the entire document, sequences whose titles are selected will be copied as BioEdit sequence structures to the BioEdit clipboard as well so that the entire sequence(s) may be pasted into a document by choosing Paste Sequence(s).

Entire sequences: Select the sequence title(s) with the mouse and choose “Copy Sequence(s)” from the “Edit” menu. Sequences whose titles are selected will also be copied to the Windows Clipboard in Fasta format. More than one selected sequence will be copied to the clipboard as a Fasta sequence list, and copied internally within BioEdit as a group of full BioEdit sequence structures that can be pasted into any BioEdit document.

Note: The BioEdit "clipboard" which contains all sequence-related data (GenBank information, graphical annotations) is internal to a single instance of BioEdit (they cannot be transferred between independent processes). To copy sequences between BioEdit alignment documents, make sure to have both documents open within the same instance of the program, as only Fasta-formatted sequences are copied to the general Windows clipboard.

Paste:

Text in edit window: To paste into a sequence within the main edit window, the interface must be in “Edit Residues” mode (see Editing On Screen). If a block of text is pasted into a sequence, only the first line (defined by a carriage return) will be pasted in. This is to avoid possible problems with pasting text into one sequence and inadvertently corrupting sequences below it. To paste segments of text into a block of an alignment, segments must be pasted into sequences one at a time. If the document is in “Slide Residues” or “Grab and Drag” mode, then Paste will behave the same as Paste Sequence(s) (see below).

Entire sequences: From the menu of the document to paste sequences into, choose “Paste Sequence(s)” from the “Edit” menu. The sequence(s) will be added to the end of the document. They may be then be moved to somewhere else within the alignment.

“Cut” and “Cut Sequence(s)”: Same as “Copy” and “Copy Sequences”, but deletes copied information from document. Residues are only deleted from the document if “Edit Residues” mode is active, however. Also, when Cut is used when no residues are selected in the document, sequences whose titles are selected are copied to the BioEdit Clipboard as sequence structures and to the Windows Clipboard in Fasta format, but they are not deleted from the document. To properly cut sequences from a document, choose “Cut Sequence(s)”.

Minimizing an Alignment

When an alignment is manipulated and tweaked extensively by hand, and when sequences are periodically added to an existing alignment and aligned manually, gaps often result which are present throughout a column in every sequence. To remove gaps that don’t change the actual alignment, simply choose “Minimize Alignment” from the “Alignment” menu.

Basic Manipulations / Sequence Menu

There are a few simple sequence manipulations which can be done automatically with BioEdit with a single menu option. These options are found in the “Sequence” menu.

Masking in BioEdit is at this point a little weak, and is provided mainly for use with the RNA comparative analysis functions. For an explanation of how BioEdit uses masks, see Masks.

Lock and unlock gaps: A locked gap will not be compressed when residues within a sequence are slid. To lock gaps, select the gaps to be locked and choose “Lock Gaps”. To lock all gaps in a sequence, select the sequence title, then choose “Lock Gaps”. To lock all of the gaps for an alignment, toggle lock/unlock button to the locked state: 🔒

Unlocking gaps is just the reverse of locking them. To unlock all gaps in an alignment, toggle the locked/unlocked button to the unlocked state: 🔓

The “Degap” option will remove all selected *unlocked* gaps. It will also remove and all unlocked gaps from sequences whose titles are selected.

Note: '~' and '.' (tilde and period) represent *unlocked* gaps, and '-' (dash) represents a *locked* gap. These conventions are used throughout every window and function in BioEdit. A period is never produced by BioEdit to represent a gap character, but is treated as a type of gap for computability with programs that prefer this character. Also, some programs may use a period to represent alignment positions that are neither residues nor gaps, but simply fill alignment slots before the beginning or after the end of a sequence. BioEdit does not directly pay attention to this distinction. Positions before or after a sequence's range are treated as gaps and BioEdit assumes each alignment consists of truly homologous sequences (although BioEdit is also designed to allow the user to ignore the alignment focus of the program and use it simply to manipulate lists of sequences).

Sequence Menu (excluding the “mask” functions)

New Sequence: Create a new sequence. This opens up the single sequence editor

Edit Sequence: Opens the first selected sequence in the single sequence editor

Select Positions: Opens a dialog that allows selecting of specified positions in all selected sequences.

Open at cursor position: If the document is in edit mode, and the cursor is showing, this option will open the sequence with the cursor at the cursor’s current position in the single sequence editor.

Rename: Rename sequence titles according to a submenu option:

> Edit title: Change the title of a sequence on-screen.
> with LOCUS: Change all selected titles to the LOCUS field.
> with DEFINITION: Change all selected titles to the DEFINITION field.
> with ACCESSION: Change all selected titles to the ACCESSION field.
> with PID/NID: Change all selected titles to the PID or NID field.

Sort: Sort sequences according to the following criteria:

> By Title
> By Locus
> By Definition
> By Accession
> By PID or NID
> By Reference
> By Comment
> By residue frequency in a selected column
>
> When the latter option (by residue frequency) is chosen, a single column of residues must be selected, and the sort is performed by order of greatest frequency of residues defined as valid residues.

Pairwise alignment: Optimal alignment of two sequences

> Align two sequences (optimal GLOBAL alignment): Align two sequences optimally with a global alignment algorithm based upon the Smith and Waterman optimal alignment method.
>
> Align two sequence (allow ends to slide): Align two sequences optimally with a local alignment algorithm based upon the Gotoh modification of the Smith and Waterman optimal alignment method which does not constrain the ends of either sequence (either

sequence end is allowed to slide freely over the other sequence). This alignment tends to be very useful for quickly identifying overlapping regions of sequence reads in small sequences where an auto-contig assembly program is not required.

Calculate identity/similarity for two sequences: Calculates the identity and similarity (according to the current similarity matrix) for two sequences *as they are currently aligned* in the document (does not align them).

Similarity Matrix (for pairwise alignments and shading): These matrices apply to amino acid sequences only. BioEdit does not use any matrix scoring schemes for nucleic acids (only simple identity).

BLOSUM62: The default matrix used by BLAST. The BLOSUM matrices are generally good for database searches and assume moderately large evolutionary distances (smaller BLOSUM number = greater evolutionary distance -- only the BLOSUM62 matrix [intermediate] is supplied in BioEdit).

PAM40: Intended for very closely related sequences (40 PAM units = relatively small evolutionary distance -- in the PAM matrices, *large* PAM number = greater evolutionary distance).

PAM80

PAM120

PAM250: Intended for more distantly related sequences (larger PAM distance).

IDENTIFY: Simple match or mismatch matrix with a very large (-10000) penalty for mismatches

DAYHOFF: Actually a PAM250 matrix -- M.O. Dayhoff's original PAM250 matrix (each value rounded to the nearest integer).

MATCH: Simple match or mismatch matrix with a -1 penalty for mismatches and a +1 score for matches.

GONNET: A modified PAM250 matrix recommended by Gonnet (1992).

Features (Feature annotation functions):

Automatically annotate from GenBank Feature Fields: This option allows you to add features according to the pre-existing GenBank data already deposited for the sequence.

Edit Features: Add, modify or delete features in a sequence.

Annotate Selection: Add a feature that will span the currently selected positions in all sequences with a selection in them.

Annotate selected sequences using the first sequence as a template

Sequence groups (or families): Group and ungroup sequences and edit current groups.

Edit Mode: Sets the current editing mode. See Manual alignment of sequences.

Mask (covered above).

Toggle color: toggles coloring of single sequences. This is a left-over of an early version and is pretty useless.

Gaps:

Lock gaps, Unlock gaps and Degap: explained above.

Insert multiple gaps: insert a variable number of gaps at the currently selected position in the alignment window.

Manipulations: Simple manipulations that are independent of sequence type.

lowercase and UPPERCASE: As indicated -- sequences only, not titles.

Reverse: Reverses any sequence

Remove numbers: As indicated. This was added by request to ease the process of pasting partial sequences from GenBank formatted text files and web pages.

World Wide Web:

Automated links are provided to the following selected WWW search functions:
BLAST, PSI-BLAST and PHI-BLAST.
Prosite profile and pattern scans
nnPredict protein secondary structure prediction

Nucleic Acid:

Nucleotide Composition: Plots nucleotide composition and gives a summary including G+C and A+T percentages and molecular weight

Complement: The complement of a DNA or an RNA sequence. This option has no effect upon protein sequences, and characters other than the standard five bases (A, G, C, T and U) and purines/pyrimidines are not affected (the complement of a purine (“R”) is a pyrimidine (“Y”)).

Reverse complement: Behaves the same as complement, but also reverses the sequence.

DNA->RNA and RNA->DNA: These really do nothing but toggle “T”’s and “U”’s and change the sequence type.

Translate: Translate sequence in frame 1, 2 or 3, or translate the currently selected region of a sequence. Codons are separated by spaces. The nucleotide sequence is shown on top of the protein sequence. The translated sequence is specified by three-letter or one-letter amino acid codes, depending on the preferences. If a selected part of a sequence is translated sequence is translated, either the entire nucleic acid sequence or only the translated region may be displayed, depending on the current preferences. A summary table may be displayed below the translation which shows the number of times each codon appears in the sequence, as well as the frequency with which each codon codes for a particular amino acid according to the codon table provided.

Find Next ORF: Searches the currently selected sequences from the point of the last current selection for ORFs according to the parameters defined in the preferences.

Create plasmid from sequence: A DNA sequence may be converted directly into a plasmid/vector. A restriction map is automatically run on the sequence. For help on annotating a plasmid, see Plasmid drawing with BioEdit

Restriction Map: Run a restriction map on a DNA or RNA sequence.

Sorted and Unsorted six frame translations: Translate nucleic acid sequences in all six frames by specifying a start codon (ATG, “any”, or user-defined), and a minimum and maximum ORF size. Sorted translations are limited to a few thousand output ORFs. To get a raw translation of entire genome (or larger), use an unsorted translation (in an unsorted translation, the output data is printed directly to a file, and very little memory is required).

Protein:

Amino Acid composition: Gives a plot and summary of the amino acid composition of a protein, including the molecular weight.

Hydrophobicity profiles:

Mean hydrophobicity is calculated by the method of Kyte and Doolittle (1982) using a choice of hydrophobicity scales.

Hydrophobic moment is calculated according to the method of Eisenberg et. al., 1984). The algorithm of Eisenberg *et. al*. for finding transmembrane alpha helices is ***not*** applied here, rather the hydrophobic moment of a user defined segment of sequence is plotted for each residue (each residue represents the beginning of a user-defined segment);

Mean hydrophobic moment: For each residue, the mean hydrophobic moment for a window the same size as that used to calculate each hydrophobic moment is applied.

> Note: I do not have the expertise to make any claims about the predictive power of these profile plots. BioEdit makes no conclusions about hydrophobic and/or transmembrane segments of proteins, and interpretation of these plots is up to the judgment of the user.
>
> For a description of the method and meaning of these plots, and references to the hydrophobicity scales and to hydrophobicity analysis algorithms, see Hydrophobicity Profiles.

Translate or Reverse-Translate: Translation from DNA or RNA to protein is done according to the codon table specified in the BioEdit.ini file. The default is "codon.tab" found in the /tables directory. The default is the *E. coli* codon usage table produced by J. Michael Cherry (cherry@frodo.mgh.harvard.edu) with the GCG program CodonFrequency. Any codon table with this format may be used, but the codon table must be in this format to be recognized by BioEdit. To choose a different table, see Codon Tables. A protein sequence will be degenerately encoded (to DNA) based upon codon preference for each particular amino acid. Obviously, if a nucleic acid sequence is translated to protein and back, information will be lost.

Translate in Selected Frame (Permanent): This allows you to translate a nucleotide sequence as if the currently selected column (defined as the start of a selection if more than one column is selected) is frame +1. When applied to a protein sequence, it simply results in the same degenerate reverse translation as the above option.

Toggle Translation: Toggles nucleotide sequences between the nucleic acid and encoded protein sequences, allowing for alignment of the sequences in either view. See Toggling between nucleotide and protein views

Toggle Translation in selected frame: This option allows you to toggle the translated view (without losing any nucleotide information) as if the currently selected column (defined as the start of the selection if more than one column is selected) was in frame +1.
Dot Plot (pairwise comparison): Create a dot plot of two sequences compared to each other in a matrix.

Customizing the View

BioEdit currently supports the following view options:

- Background colors for sequence and title windows
- Default monochrome sequence and title colors
- Character fonts.
- Font size
- View sequences in bold-face type.
- View sequences in monochrome or color (editing is faster in monochrome).
- Normal color view (residues colored)

- Inverse (background colored)
- Strength of alignment: shading is based upon the information contained at each position -- information is calculated as follows:
    - DNA/RNA: information = $ln5+\Sigma(fbx[ln(fbx)])$
    - Protein: information = $ln21+\Sigma(fbx[ln(fbx)])$,

  where fbx represents the frequency of each residue b occuring at position x. 5 represents the number of possible residues for nucleic acid (4 nucleotides plus gaps). This is not quite right, and the usefulness decreases if a lot of alternative characters are used. 21 represents the number of possibilities for amino acids (including the gap). $ln5$ and $ln21$ are the maximum information for a nucleic acid position or a protein position, respectively and the term $-\Sigma(fbx[ln(fbx)])$ represents the entropy (a measure of variability) at the position.

  The above description was true of BioEdit versions before 5.0.0. In BioEdit version 5.0.0, only the user-defined valid residues contribute to the entropy calculation. In this case, gaps only contribute to the calculation of entropy if they are defined as valid residues (or place-holding characters, if you'd rather think of it that way, as it is obvious that a gap cannot be a residue).
- Strength of Alignment - Inverse: Same as Strength of alignment, but the background instead of the residue is shaded.
- Identity/Similarity shading: Residues are background-shaded with there color-table defined colors if their frequency in a column equals or exceeds a user-defined cutoff (the option to choose the cutoff goes in increments of 10% and is visible when this mode is active). Nucleotide alignments are shaded according to identity only, while protein alignments are shaded according to identity and similarity according to the currently selected amino acid similarity scoring matrix. Only characters defined as valid residues and only non-comment sequences contribute to the similarity and identity calculations.
- Sequences and Graphical Features: Draw graphical sequence annotations on the document screen with the sequences superimposed on top of them.
- Graphical Features: Draw graphical sequence annotations in cartoon mode and do not draw the residue characters. When this mode is active, there is a scale-factor slide bar toward the top of the window that enables a scaling factor between 1:1 and 1:32768, by orders of 2.
- Conservation plot: Residues are plotted as a user-defined character (default = a period) if they are identical to the residue in the same column as a user-defined standard (default = the top sequence). To change the standard (reference) sequence, right-click the sequence title with the mouse that you want to be the new standard for the conservation plot. Only characters set as valid residues are recognized for the identity plot.
- Show or hide the mutual information examiner (this is only useful for RNA comparative analysis).
- Show or Hide the translation toggling control. This is mutually exclusive with the mutual information examiner control, because of space limitations.
- Show sequence position by mouse arrow: when moving the mouse over sequences in a document window, the absolute position of the mouse (ignoring gaps) is reported on the control bar above the sequence view window. The position may also e reported (including the full length of the title) at the mouse arrow. This option turns this feature on or off.
- Split window vertically: A duplicate window is created which sits inside the document window and is synchronized with the current document. The window is placed such that the document appears to be split by a vertical window splitter (it's really just two synchronized documents, one with most of it's interface removed). The vertical scroll position of the two

windows stays in register, but horizontal scrolling in each is independent of the other. The window may be resized by grabbing the window splitter within the main document. The window may be returned to normal by choosing this menu option again.

- Split window horizontally: A synchronized window is created which is placed directly below the original window such that the border between the bottom of the original window and the top of the new window behaves like a window splitter. Remove this window by choosing the option again.
- Save options as default: When "Auto-update view options" is off, choosing this item will save the view options of the current document as the default for all newly created or newly opened documents.
- Auto-update view options: when this item is checked, all changes made to the document views and preferences are automatically saved as the default for new documents.
- Customize menu shortcuts: brings up a dialog that allows changing of menu shortcuts to any key combination.
- Hide control bar or Show control bar: The main control bar may be removed in order to fit more sequences on the screen in a simple frame window. If the control bar is hidden, then the "Show control bar" option is offered. If the control bar is hidden, sequence editing modes may be changed through the Sequence->Edit Mode submenus. View defaults may be changed via menus as well.

To change these settings, choose the appropriate option from the "View" menu of an open document. To make the current view from any particular document into the default view for all subsequently opened documents, choose "Save Options as Default".

These views may be selected either through the "View" menu or by pressing the appropriate speed button on in alignment window.

Color Table

One color table is used for all documents. This table is called “color.tab” and is found in the \tables directory of the BioEdit install directory (see Program Organization). Although the table may be edited by hand, it is much easier to use the “Color Table” option under the “Options” menu of the main application control form.

Editing the color table: To edit the color table, choose “Color Table” from the “options” menu of the main application control bar. There is a different color table for nucleotide and protein sequences. To change the color of a residue, double-click on the colored box above the residue to get a color dialog. To add or delete a residue, click the “+” or “-” button. In the window that appears, push the button for the residue to be added or deleted. When adding a residue, it comes in with black as the default color. The color must then be changed to the desired color.

To edit the color table by hand, the following format must be observed:

- Each color table is denoted by a line containing the exact text “/amino acids/” or “/nucleotides/” (without the double quotes).
- The end of each table is denoted by (exactly) “/////” (without the double quotes);
- Each residue color is specified by two lines in the file:
    - Line 1: A 3-byte hexadecimal number (or its integer value). The three bytes represent the values for blue, green and red, respectively (backwards RGB).
    - Line 2: An unbroken list of all characters representing all characters which should have this color. If a character color is redefined elsewhere in the file, the last occurrence will be the valid one.

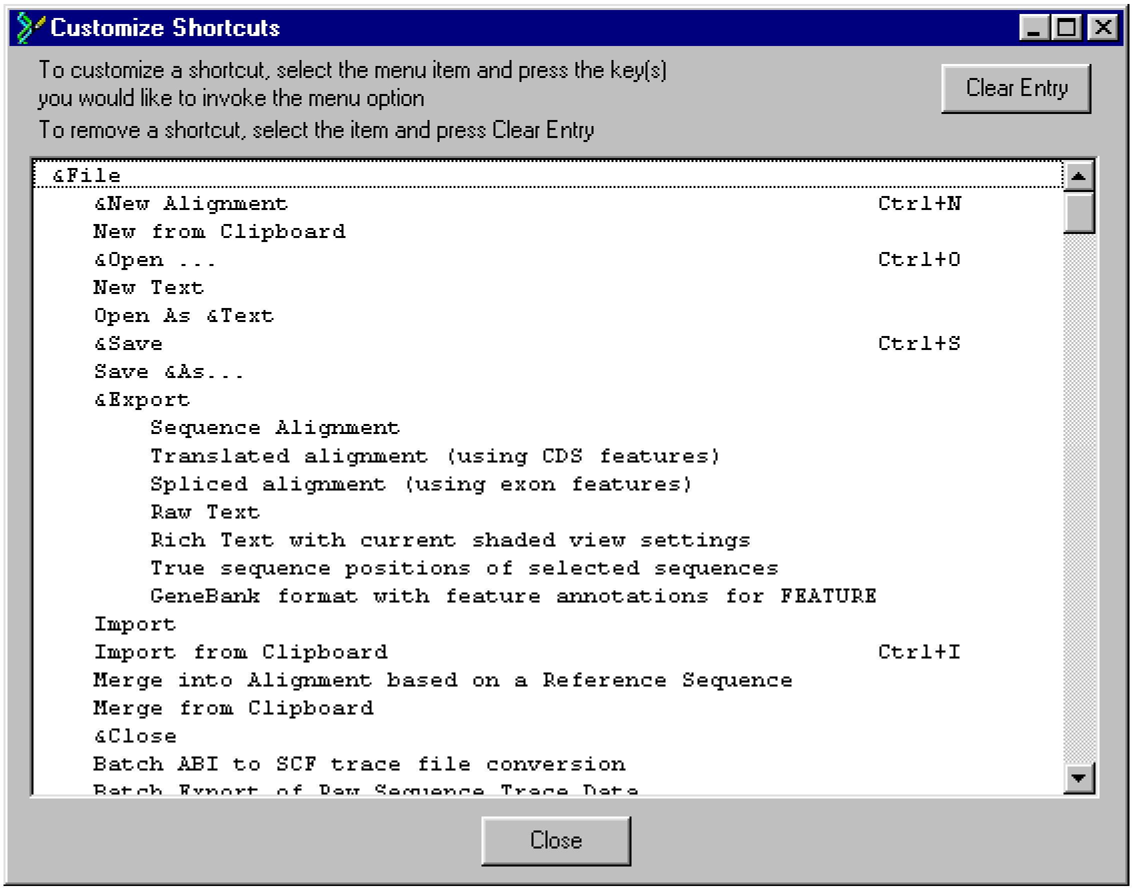
Note: Manual editing of the color table should not be necessary and is not recommended.

If the color table becomes corrupted, it may lead to program failure on startup or when the color table is edited. If this happens, you may delete the color table and create a new one, one residue at a time (you will get an error on startup and on choosing “Color Table”, but the program will create a new table when the “Save Table” button is pressed). This is tedious, so the /tables folder of BioEdit also comes with a file called “defcolor.tab”. If the color table becomes corrupted, you can make a copy of defcolor.tab and change the name of the copy to “color.tab”.

Customizing menu shortcuts

Preferred menu shortcuts may be created for any menu item or sub-menu item (but not to a third level). Shortcuts may only be customized for alignment document windows, however. For example, if Ctrl+Y was set to be a shortcut for "copy", when working in the text editor, Ctrl+C would still be the copy shortcut

To set shortcuts, choose View->Customize Menu Shortcuts. To set a shortcut, simply scroll to the menu item of interest, select it with the mouse, then press the particular key combination you would like to use to activate it. To completely remove a shortcut, highlight an item and press "Clear Entry"



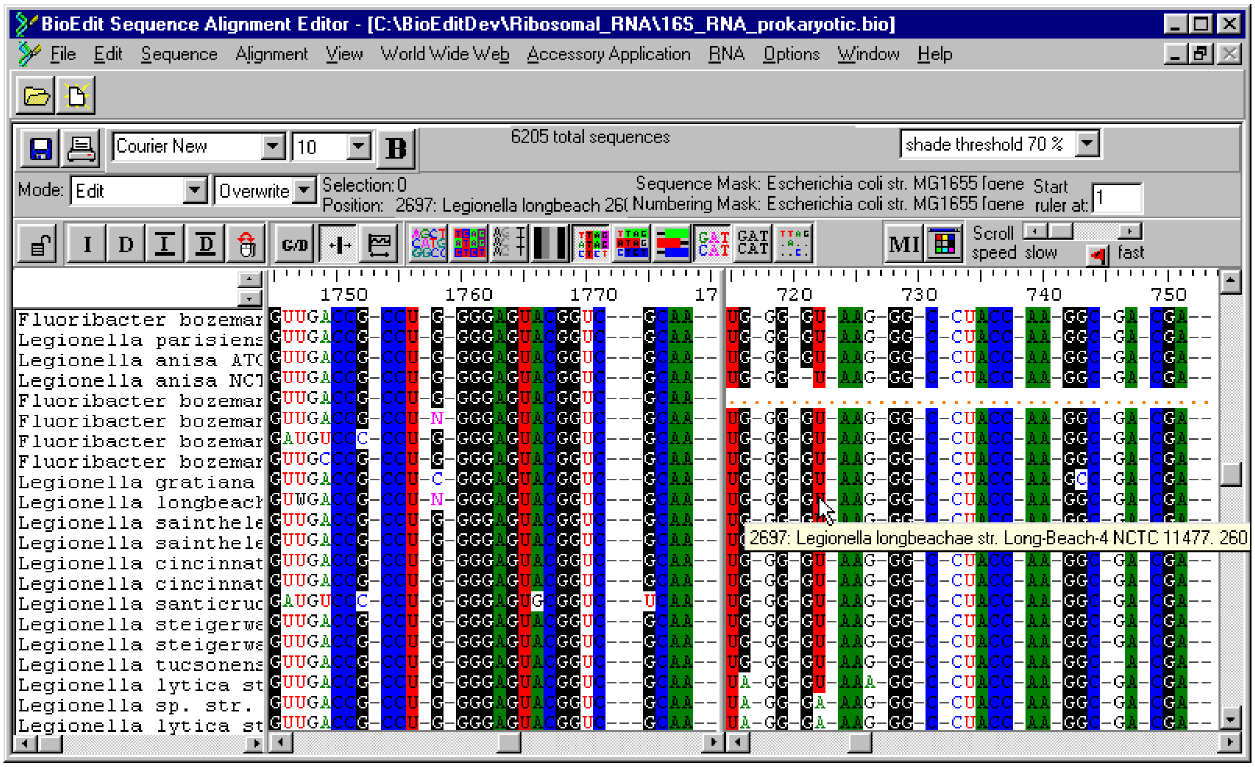
To customize a shortcut, select the menu item and press the key(s) you would like to invoke the menu option
To remove a shortcut, select the item and press Clear Entry

```
&File
   &New Alignment                                          Ctrl+N
   New from Clipboard
   &Open ...                                               Ctrl+O
   New Text
   Open As &Text
   &Save                                                   Ctrl+S
   Save &As...
   &Export
       Sequence Alignment
       Translated alignment (using CDS features)
       Spliced alignment (using exon features)
       Raw Text
       Rich Text with current shaded view settings
       True sequence positions of selected sequences
       GeneBank format with feature annotations for FEATURE
   Import
   Import from Clipboard                                   Ctrl+I
   Merge into Alignment based on a Reference Sequence
   Merge from Clipboard
   &Close
   Batch ABI to SCF trace file conversion
   Batch Export of Raw Sequence Trace Data
```

Splitting the window view

It may be convenient to edit two different parts of an alignment at one time. To allow for this, BioEdit offers two ways of splitting the document into two synchronized windows, one that splits the window vertically and the other which splits it horizontally.

To split the window vertically, choose View->Split Window Vertically. Shown below is a split view of part of the prokaryotic 16S rRNA alignment. The two sides share a vertical scroll bar, but scroll independently of each other in the horizontal direction. The window spit may be resized with the mouse.



BioEdit Sequence Alignment Editor - [C:\BioEditDev\Ribosomal_RNA\16S_RNA_prokaryotic.bio]

Courier New 10

6205 total sequences

shade threshold 70 %

Mode: Edit Overwrite

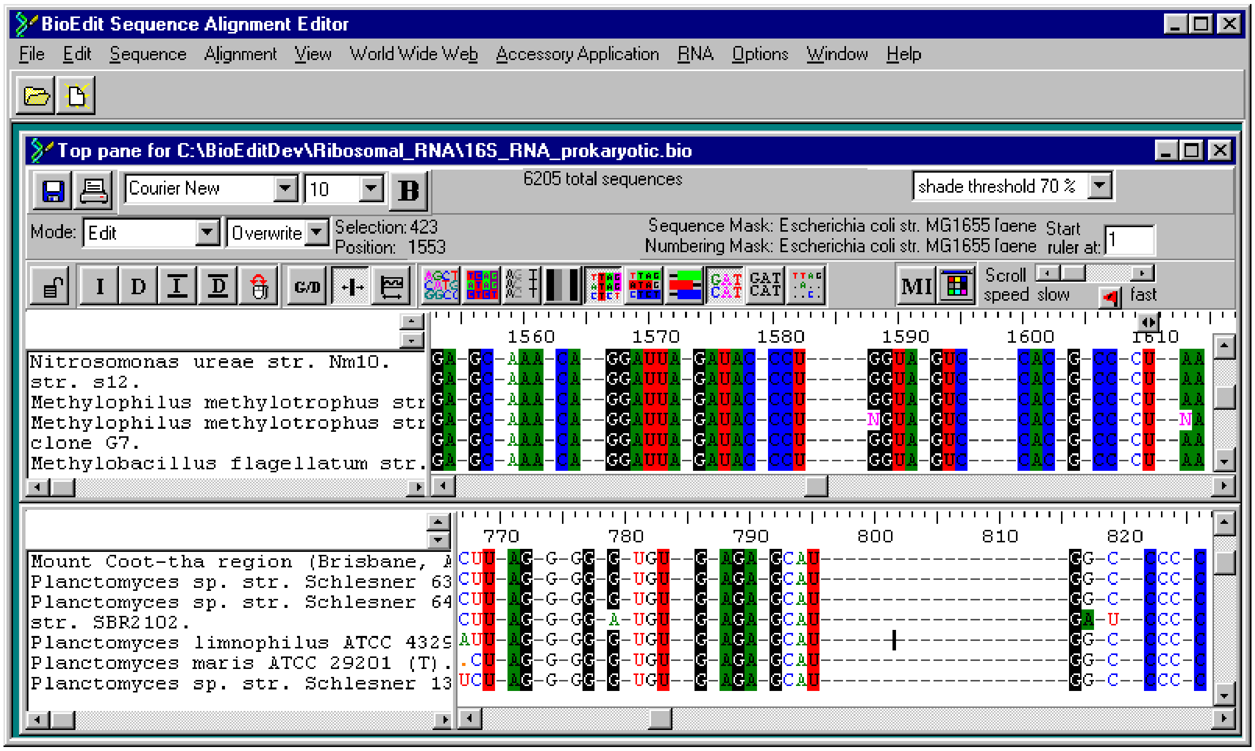
Selection: 0
Position: 2697: Legionella longbeach 26(

Sequence Mask: Escherichia coli str. MG1655 [gene
Numbering Mask: Escherichia coli str. MG1655 [gene

Start ruler at: 1

2697: Legionella longbeachae str. Long-Beach-4 NCTC 11477. 260

To split the window horizontally, choose View->Split Window Horizontally. Shown below is another split view of part of the prokaryotic 16S rRNA alignment. The two windows remain attached, but have independent vertical and horizontal scrolling


BioEdit Sequence Alignment Editor
File Edit Sequence Alignment View World Wide Web Accessory Application RNA Options Window Help
Scroll speed slow fast
MI

Top pane for C:\BioEditDev\Ribosomal_RNA\16S_RNA_prokaryotic.bio

6205 total sequences

Courier New 10

shade threshold 70 %

Mode: Edit Overwrite

Selection: 423
Position: 1553

Sequence Mask: Escherichia coli str. MG1655 [gene
Numbering Mask: Escherichia coli str. MG1655 [gene

Start ruler at: 1

```
Nitrosomonas ureae str. Nm10.
str. s12.
Methylophilus methylotrophus str
Methylophilus methylotrophus str
clone G7.
Methylobacillus flagellatum str.
```

```
Mount Coot-tha region (Brisbane, A
Planctomyces sp. str. Schlesner 63
Planctomyces sp. str. Schlesner 64
str. SBR2102.
Planctomyces limnophilus ATCC 4329
Planctomyces maris ATCC 29201 (T).
Planctomyces sp. str. Schlesner 13
```

Sorting Sequences

Sequences in an alignment document may be sorted by the following criteria:

- Title
- LOCUS
- DEFINITION
- REFERENCES
- COMMENT
- ACCESSION
- PID/NID
- residue frequency in a selected column

To sort sequences, choose "Sequence->Sort-><sort type>

Graphical Feature Annotations

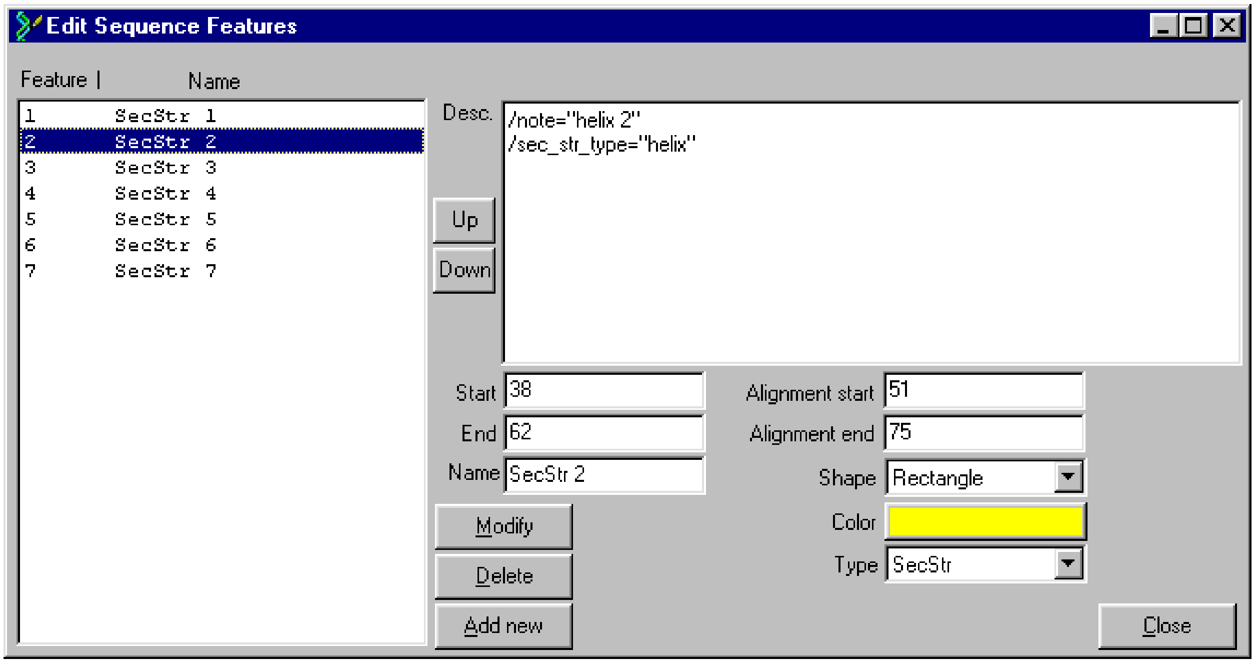
It is sometimes convenient to have information about certain elements of a sequence (e.g., exons, introns, helices, motifs, etc.) available for reference in a quick and easy way, without going to external sources such as a notebook, other files, or sources in the literature or on the WWW. For this reason, functions allowing for the graphical annotation of sequence features were added in version 5.0.0. Annotations may be done by hand, or automatically from existing GenBank format FEATURES data. Names and descriptions for features that span any position in a sequence are available right in the alignment window as ToolTips when the mouse arrow is moved over the sequence residues. The standard GenBank format feature types are used as a basis for internally keeping track of the sequence "type". If one is willing to adhere to GenBank standards when defining the "description" of each feature, the feature annotation functions of BioEdit can also provide a convenient way to annotate a sequence, or set of sequences, which can be exported in standard GenBank format using the user-defined graphical features to fill in the GenBank FEATURES field. This can be a useful starting point for a sequence submission using Sequin or BankIt.

When a feature is added to a sequence in BioEdit manually, the "true" positions are calculated and assumed to take precedent over the absolute positions in the alignment, and all future alignment adjustments are handled with these "true" positions in mind. For example, if 5 gaps are deleted within a feature, the end of a feature is drawn back by five in the alignment, but the absolute number of residues within the feature does not change. If 3 bases are deleted within the feature, the actual end position of the feature will be moved back by three. Likewise, when features are added automatically from GenBank information, the positions in the alignment which correspond to the true start and end of each feature are calculated and updated to reflect the correct aligned state of all elements. BioEdit allows control over the title of the feature, the color, the shape (rectangular, oval, diamond or arrow), the direction (only makes a difference for an arrow), the "type" (either "undefined" or any of the 67 standard GenBank feature types), and reserves space for a description (unlimited in length).

Adding, modifying and deleting sequence features manually

There are two ways to add or modify sequence features manually:

1. Highlight the sequence title and choose the menu option "Sequence->Features->Edit Features". There must be only one sequence title highlighted, or BioEdit will not know which sequence to edit the features from. The following dialog will appear (which of course would be empty if there were not yet any features added to the sequence:



Feature	Name
1	SecStr 1
2	SecStr 2
3	SecStr 3
4	SecStr 4
5	SecStr 5
6	SecStr 6
7	SecStr 7

Desc. /note="helix 2"
/sec_str_type="helix"

Start 38
End 62
Name SecStr 2
Alignment start 51
Alignment end 75
Shape Rectangle
Color 🟨
Type SecStr

To add a feature, fill in the "name" box with the title of the feature, add a description to the "Desc." box, and specify either the start and end positions in the sequence as if the sequence were a simple, unaligned sequence, or, if the sequence is in an alignment an it is easier to determine the alignment positions, you can specify those instead and BioEdit will figure out the true positions for you. If you fill them both in, BioEdit will ignore the alignment positions and recalculate them based on the true positions specified. Note: If you want a feature to reflect orientation, and the orientation is reverse, specify the start position as the higher number and the end position as the lower number. Next, choose a color by pressing the "Color" button (you will get a color picker dialog), choose the shape, and specify a type under "Type". After this, press "Add New" to add the feature to the sequence. Note that if two features overlap in position, the feature further down in the list will be drawn on top of the one further back in the list. To change the positions of features in the list, highlight one or more feature titles and press the "Up" or "Down" button.

To modify an existing feature, click the title of the feature on the left and do the same things as for adding a feature. Instead of pressing "Add New", just press "Modify" (Pressing "Add New" will effectively duplicate the feature). You may modify elements of multiple features at a time by selecting the titles of all of those features at once. The elements that are in common to all selected features will show up, while those that are not will not show up. If a change is made to any element, and "Modify" is pressed, the change will be applied to all selected features.

To delete one or more features, highlight the features and press "Delete".

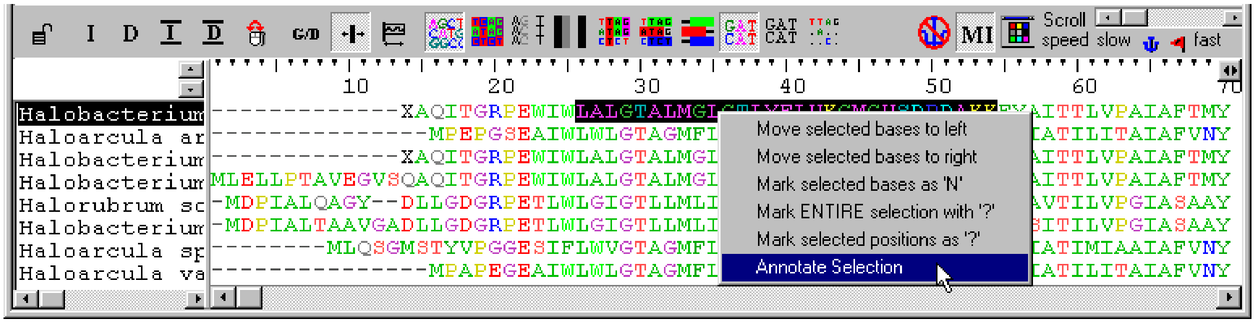
When finished adding or modifying features, press "Close" at the bottom right of the dialog.

2. Adding or Modifying a feature from the alignment window:

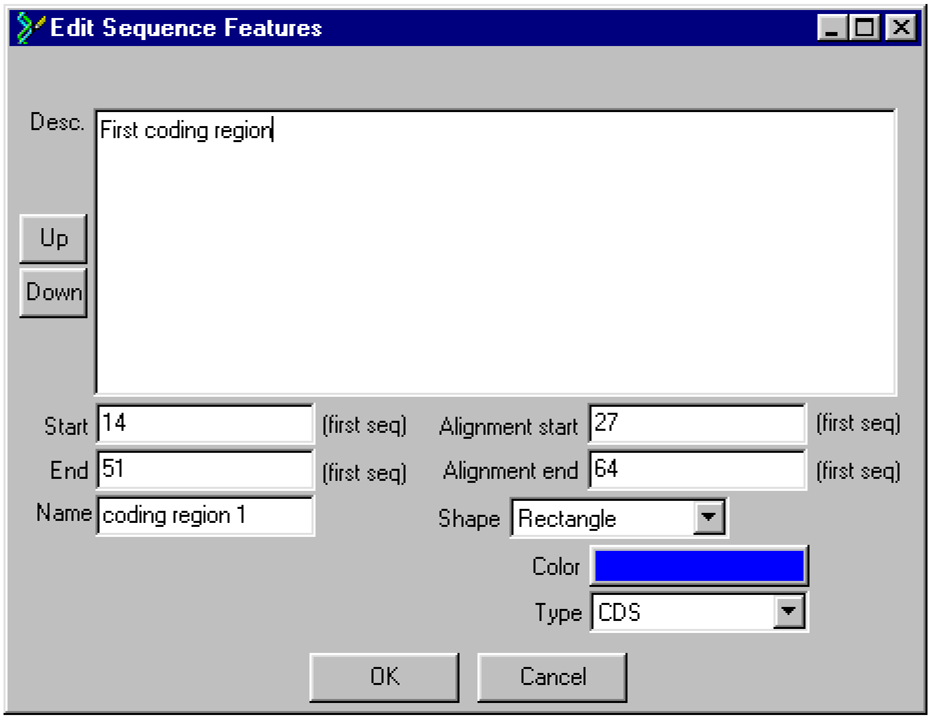
Adding or modifying a feature from right within the alignment window utilizes a right mouse-click context menu. For this menu to be available, all of the right mouse-click activated alignment features must be turned off. This means that the following four buttons must be in the up position (not depressed):

I D I D If any of these buttons is down, right-clicking in the alignment window will add or delete gaps, depending upon which button is down.

To add a new feature, highlight the desired span of the feature in the sequence within the alignment window. Next, right-click the mouse anywhere over the highlighted section and choose "Annotate Selection" in the context menu that comes up.



The following dialog will appear:



Edit Sequence Features

Desc. First coding region

Start 14 (first seq)
End 51 (first seq)
Name coding region 1

Alignment start 27 (first seq)
Alignment end 64 (first seq)
Shape Rectangle
Color 🟦
Type CDS

The rest of the options are the same as in adding a feature manually, but the feature positions are specified for you based upon the selection in the alignment window. You may annotate a block selection by selecting a block of residues that span multiple sequences and doing the same thing. In this case, the same feature will be applied to the same alignment positions in each sequence, and the "true" positions will be updated independently for each sequence according to the alignment positions.

To modify an existing annotation, right-click anywhere over the annotation, then choose "Update Annotation" (only visible if you right click over an annotation and *only* if there is only one annotation spanning that region) and you will get a dialog like the one above. Pressing OK will update that annotation, rather than adding a new one. If you simply right-click over the annotation, the positions in the dialog will reflect the current begin and end of the feature. If you make a new selection within the annotation before right-clicking, however, the positions in the dialog will reflect the selected positions in the alignment, assuming you want to easily alter the feature positions. You may also update the same positioned annotations in several sequences at a time this way by selecting a block, right-clicking and choosing "Update Annotation".

Annotating sequences automatically from existing GenBank FEATURES data

You may have BioEdit automatically add feature annotations from GenBank format FEATURES data, if it is present. To see if there is FEATURES data in a sequence, double-click on the sequence title, press the ⬇ button to expand the window, and look at the "FEATURES" box. If that box is filled in with data formatted similar to the following, then there is data formatted in the expected manner for auto-annotating:

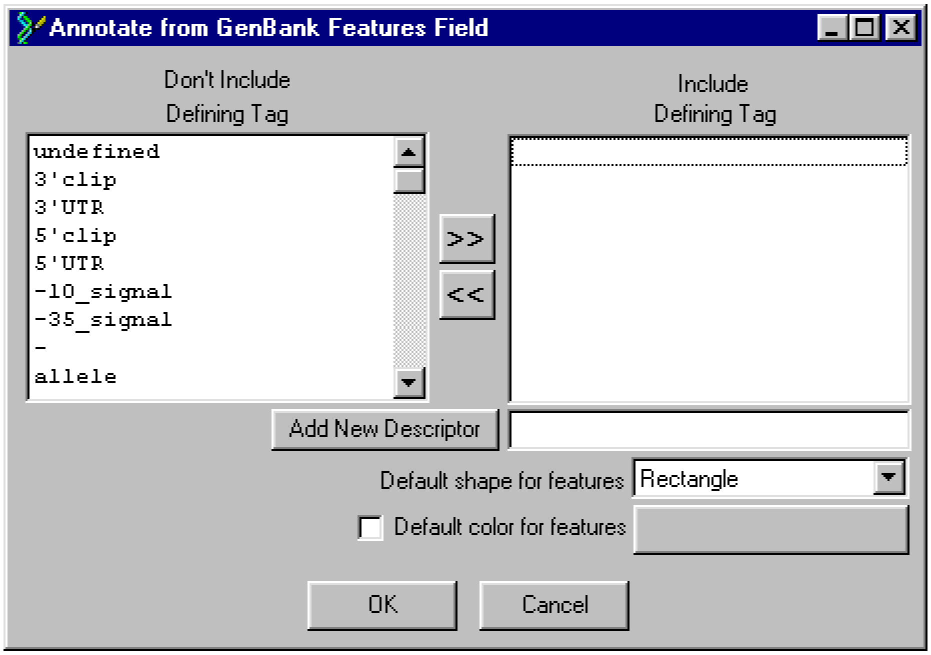
Example of formatting in the GenBank FEATURES field:

```
FEATURES             Location/Qualifiers
     source          1..247
                     /organism="Halobacterium salinarum"
                     /db_xref="taxon:2242"
     NonStdResidue   1
                     /non-std-residue="PCA NH3+"
     SecStr          10..31
                     /note="helix 1"
                     /sec_str_type="helix"

... etc.
```

For a complete list of the tags that BioEdit will look for, either look in the dialog while using the program, or see "GenBank Format".

To annotate a sequence automatically, highlight the titles of any sequences you want annotated (and that have GenBank FEATURES data), then choose "Sequence->Features->Automatically annotate from GenBank Feature Fields". You will get the following dialog:


Annotate from GenBank Features Field
Don't Include Defining Tag
Include Defining Tag
>>
<<
Add New Descriptor
OK
Cancel

undefined
3'clip
3'UTR
5'clip
5'UTR
-10_signal
-35_signal
-
allele

Default shape for features: Rectangle

☐ Default color for features

The available tags to search for are on the left. To add a tag or tags to the list of tags to look for, select whatever tags you would like to be included and press the ">>" button (you can move any back to the other side with the "<<" button). You can add your own tag to look for by typing it into the "Add New Descriptor" box and pressing "Add New Descriptor", but all of the standard GenBank tags should be available in the box on the left.

You may choose a default color to apply to all of the features, or you can let BioEdit automatically choose colors for you (they can be edited later). If BioEdit chooses the colors, all features of the same type will get the same color. If you choose a default color *all* features will be the same color, regardless of type. You may also choose a default shape. The default for all features is rectangular. The available shape options are: rectangle, oval, diamond and arrow. If arrow is chosen, then the start and end positions are important for determining orientation of a feature.

When BioEdit adds features, it searches through the FEATURES data looking for the specific tags specified in the above dialog. When it finds one (formatted in the proper place), a new feature is created. The title will be the feature type plus a number reflecting the present number of that feature in the list (e.g. "exon 1", "intron 1", "exon 2", etc.). The description will be all of the descriptive data that follows the tag in the file, including carriage returns to keep the formatting correct. For example, a CDS feature might have the following name and description:

Name:

```
CDS 2
```

Description:

```
/label=b0014
/gene="dnaK"
/product="DnaK protein (heat shock protein 70)"
/note="o638; 100 pct identical to DNAK_ECOLI SW: P04475"
/codon_start=1
/transl_table=11
/translation="MGKIIGIDLGTTNSCVAIMDGTTPRVLENAEGDRTTPSIIAYTQ
DGETLVGQPAKRQAVTNPQNTLFAIKRLIGRRFQDEEVQRDVSIMPFKIIAADNGDAW
VEVKGQKMAPPQISAEVLKKMKKTAEDYLGEPVTEAVITVPAYFNDAQRQATKDAGRI
AGLEVKRIINEPTAAALAYGLDKGTGNRTIAVYDLGGGTFDISIIEIDEVDGEKTFEV
LATNGDTHLGGEDFDSRLINYLVEEFKKDQGIDLRNDPLAMQRLKEAAEKAKIELSSA
QQTDVNLPYITADATGPKHMNIKVTRAKLESLVEDLVNRSIEPLKVALQDAGLSVSDI
DDVILVGGQTRMPMVQKKVAEFFGKEPRKDVNPDEAVAIGAAVQGGVLTGDVKDVLLL
DVTPLSLGIETMGGVMTTLIAKNTTIPTKHSQVFSTAEDNQSAVTIHVLQGERKRAAD
NKSLGQFNLDGINPAPRGMPQIEVTFDIDADGILHVSAKDKNSGKEQKITIKASSGLN
EDEIQKMVRDAEANAEADRKFEELVQTRNQGDHLLHSTRKQVEEAGDKLPADDKTAIE
SALTALETALKGEDKAAIEAKMQELAQVSQKLMEIAQQQHAQQQTAGADASANNAKDD
DVVDAEFEEVKDKK"
```

BioEdit will place the end toward the left (lower number) and the start toward the right (higher number) for features that are specified as "complement".

For features that are specified as multiple positions with a "join" command, a separate feature will be created for each individual start/end position set. In this case, the first feature will have the full description field. Subsequent features created by the join command will have the description "join #<number> to <feature type> <number>" (e.g., "join #4 to CDS 2").

Annotating other sequences based upon an annotated template

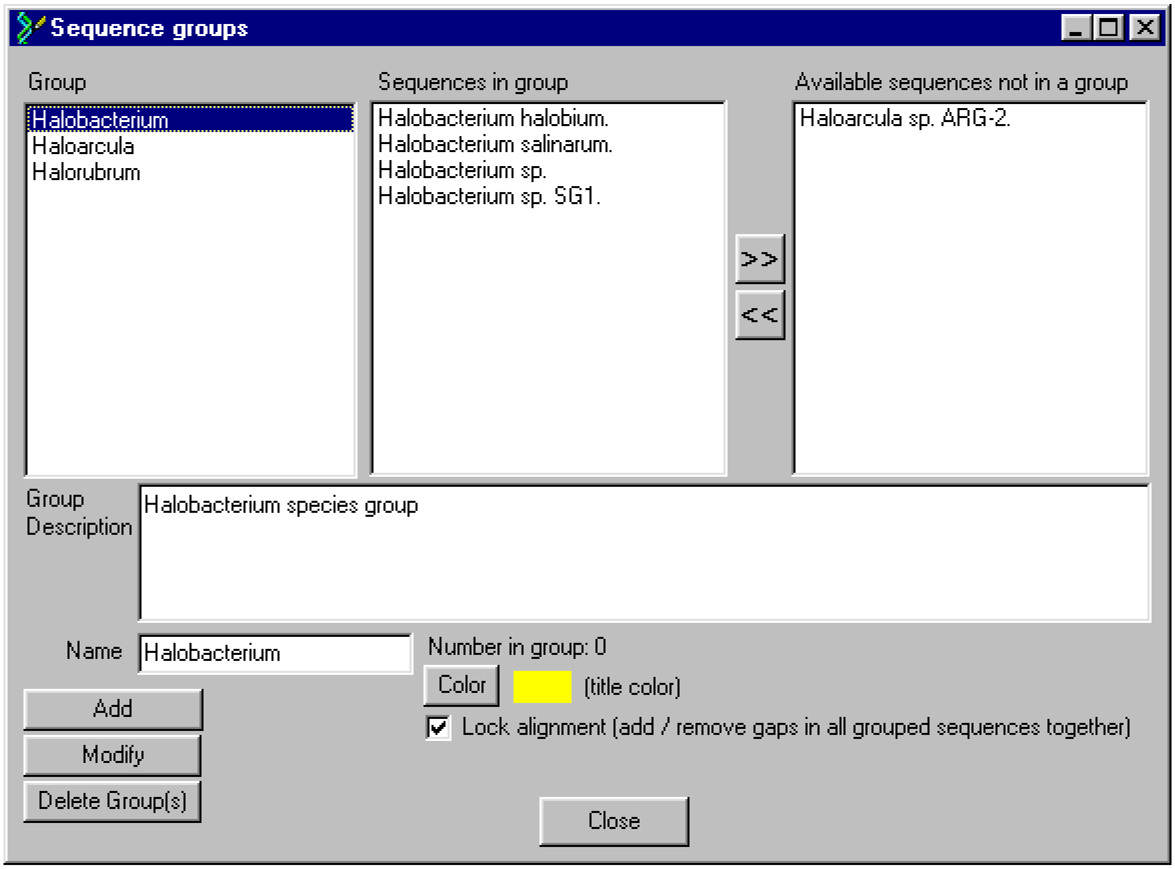
If you are dealing with an alignment of homologous sequences, chances are good that features you will be interested in that have to do with the function of the sequence will be lined up between sequences in a biologically relevant alignment. Therefore, for features such as RNA or protein helices, functional motifs, or introns, exons and CDS regions, etc. in many, if not most, aligned sequences it may be only necessary to annotate one sequence with the features of interest and then, once the sequences are properly aligned, annotate all of the others based upon the alignment positions of features in the annotated sequence. This way, even if the actual true positions and lengths of features differs between sequences, but their relative positions in a biologically relevant alignment line up vertically, then annotating correctly aligned sequences becomes much easier than having to annotate each sequence individually. The true positions for features created in this way are then calculated automatically for you by BioEdit.

To annotate sequences based using another annotated sequence as a template, first move the annotated sequence *to the top* of the alignment, select the titles of all sequences you want annotated, then choose "Sequence->Features->Annotate selected sequences using the first sequence as a template".

Grouping sequences into groups or families

Sequences may be grouped together to reflect their relationship by highlighting their titles with a group-specific color. Also, the alignment for grouped sequences may be locked together in order to synchronize alignment adjustments to pre-aligned, closely related sequences when making alignment adjustments based upon new data or added sequences.

To edit sequence groups, choose "Sequence->Sequence groups (or families)". You will get the following dialog:



Group
Halobacterium
Haloarcula
Halorubrum

Sequences in group
Halobacterium halobium.
Halobacterium salinarum.
Halobacterium sp.
Halobacterium sp. SG1.

Available sequences not in a group
Haloarcula sp. ARG-2.

Group Description: Halobacterium species group

Name: Halobacterium

Number in group: 0

🟨 (title color)

☑ Lock alignment (add / remove gaps in all grouped sequences together)

You may create groups by typing in the desired group name in the "Name" edit and pressing "Add". A new group will be created which does not have any sequences in it. To add sequences to a group, select the group title in the "Group" list and select the desired sequence titles from the far right list entitled "Available sequences not in a group" and press the "<<" button. You can remove sequence from a group by selecting them on the left and pressing the ">>" button. Each group has a description and a color. The title backgrounds in the alignment window will be colored according to the group color if the sequences belong to a group. You can remove groups by highlighting them in the "Group" list and pressing "Delete Group(s)".

Verbal confirmation of sequences

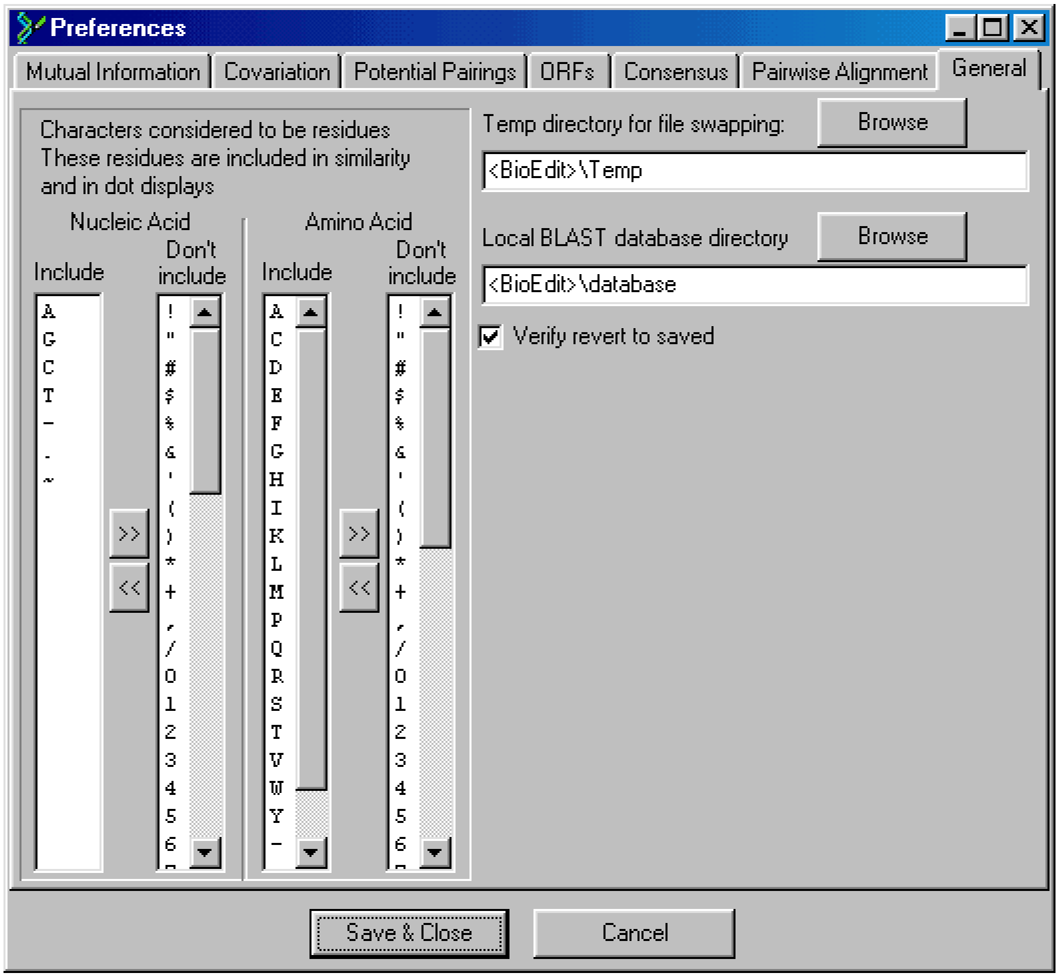
If you hand-type a small sequence into the single-sequence editor, for example a primer sequence to be stored in a file and ordered for synthesis, it is sometimes helpful to have someone read back the sequence for you as you verify on paper base by base as they read. If there is nobody available to read your sequence to you, BioEdit will slowly read a sequence back from within the single sequence editor, highlighting each base as it goes along (amino acid sequences may be read as well).

To read a sequence back from within the single sequence editor, choose "Edit->Read Sequence Back (Press escape to cancel)".

Note: This is only available from the single sequence editor. To open a sequence in the single sequence editor, double-click on its title, or highlight its title and choose "Sequence->Edit Sequence".

Valid residue characters vs non-residue characters

A researcher may wish to use characters in a sequence which are not defined in nature, that are ambiguous, or that simply hold a position, but are not known to be a residue or a gap. For this reason, there is an option to explicitly define which characters are considered to be valid for the purposes of calculations such as similarity shading and generation of an identity matrix. There are separate lists of valid residues for amino acid and nucleic acid sequences. To see or change the current settings for what is considered a "true" residue, choose "Options->Preferences->General. The following screen should appear:



Characters considered to be residues
These residues are included in similarity
and in dot displays

Nucleic Acid

Include: A G C T - . ~

Don't include: ! " # $ % & ' ( ) * + , / 0 1 2 3 4 5 6

Amino Acid

Include: A C D E F G H I K L M P Q R S T V W Y -

Don't include: ! " # $ % & ' ( ) * + , / 0 1 2 3 4 5 6

Temp directory for file swapping: <BioEdit>\Temp

Local BLAST database directory <BioEdit>\database

☑ Verify revert to saved

The default set of characters is AGUCT-~. for nucleic acids and ACDEFGHIKLMNPQRSTVWY-~. for amino acids. By default, gap characters are included, but may be removed by selecting them on the left and pressing the ">>" button to move them over to the left. Regardless of whether gap characters are included as valid residues for the purposes of shading calculations, '-', '~' and '.' characters are always treated as gaps internally. Also, although gaps may be included for the purposes of calculations (they may viewed as a mismatch on the basis that two homologous sequences differ at a position where one contains a base and the other has lost it [or the other is an insertion], gaps are still not shaded as identities, since in reality they are not true physical entities. Keep in mind that all characters are treated separately for the handling of valid residues vs non-residue characters, so if all gap characters are to be recognized (-, ~ and .), they *all* must be present in both the amino acid and nucleic acid lists of valid residues.

Locking a sequence to prevent accidental edits

A sequence may be locked to prevent the ability to slide residues in that sequence or insert or type over characters in the sequence either in the alignment window or the single sequence edit window. If that sequence is grouped, hand alignment (by sliding only, not right mouse click addition or deletion of gaps) of that sequence and *all other sequences in the group* is also blocked *if and only if* the sequence group has group alignment locked. To lock a sequence, open the sequence in the single sequence editor by either double-clicking on its title or by highlighting its title and choosing "Sequence->Edit Sequence". In the edit box, check the ☑ Lock sequence box, then press "Apply and Close".

Anchoring a column to protect aligned regions

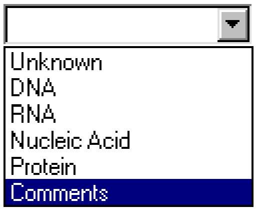
It is sometimes useful to be able to lock a column of an alignment without having to worry about accidentally pushing or pulling sequence over that position, although it may be off of the current viewing screen. BioEdit therefore allows the anchoring of as many columns as necessary to protect regions of an alignment that you don’t want to get messed up. To anchor a column, depress the add / remove column anchors button (⚓), then click the mouse over the column you want anchored. To anchor an entire region, add a column anchor to each side of the region you want protected. If you want to make sure that no alignment is possible in this region, simply add an anchor to each side, unselect all sequence titles, select all the residues within the region (highlight the region with the mouse by dragging the mouse on the ruler bar over the region, then choose “Sequence->Gaps->Lock Gaps” to lock all the gaps in that region. That region should be effectively locked until the anchors are removed (or the "“ignore anchors” button is pressed down).

To remove an anchor, depress the ⚓ button, then click over an existing anchor to remove it.

If you want to adjust the alignment in an anchored region, but don’t feel like resetting all of the anchors, you may depress the “ignore column anchors” button (🚫) and make your adjustments. Be sure to hit the “ignore column anchors” button again after the adjustments are finished to turn it off and make the anchors active again.

Comments

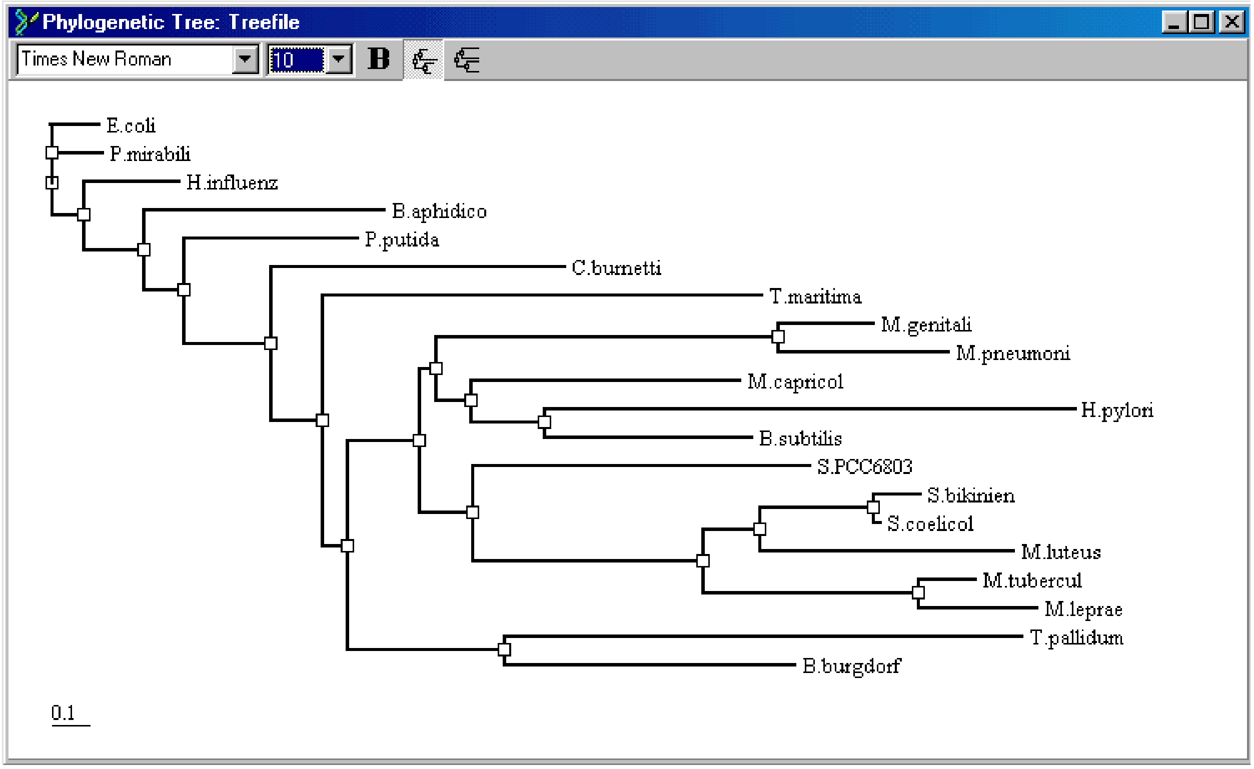
Any sequence may be made into a commment that simply takes up space in the alignment window but does not participate in shading or calculations and does not count as an actual sequence. Other than this, a comment is treated internally as just another sequence which is simply ignored in some situations and is italicized in the main alignment doc window. Any valid ASCII characters may be typed in a comment. To create a comment simply create a new sequence ("Sequence->New Sequence") and, in the single sequence editor, change its "Type" to "Comment":



Phylogenetic Tree Viewer

BioEdit version 5.0.6 contains a very rudimentary phylogenetic tree viewer that will open and view phylip-formatted tree files. Also, multiple trees may be linked directly to alignment files (up to 50 trees may be linked to one alignment), and phylogenetic tree information, along with the current node and branching pattern, is saved in the BioEdit file format. The tree viewer also allows flipping of nodes (in a way that does not alter the phylogeny), saving, printing, label-editing, and viewing the tree with or without distance information. Only a rectangular cladogram view is currently available, however. For alternative formatting options I recommend using TreeView, which is available on the WWW from Roderic Page at http:://taxonomy.zoology.gla.ac.uk/rod/rod.html. The installation for TreeView version 1.5.2 is distributed with BioEdit, and the TreeView.zip file can be found in the BioEdit installation folder.

To open a phylip tree in BioEdit, simply choose “File->Open” from anywhere in the program. BioEdit should automatically figure out that it’s a tree file and open it appropriately. A sample of how a tree might look in BioEdit is shown on the next page:



You may click the mouse on any node that has a small square (□) at its junction to flip the tree around that node. This will reverse the position of all downstream nodes and leaves (the final labels at the very end of each branch) while preserving the overall branch pattern and distances in the tree.

To edit a label, click the mouse on the label on the screen. The label will go into edit mode, and will become completely selected . You may then type the label you wish to rplace it with. When you are done, either select a different place on the tree window with the mouse, or press enter. To cancel the editing, press <Esc> (the escape key).

Note: Trees are sometimes written with more than two branches coming off of the same node. I've noticed that trees written by Phylip programs will sometimes have three branches coming directly off the first node. The BioEdit tree viewer allows more than one branch off of each node (up to 10, actually, just to be safe), but when a tree is opened directly in the BioEdit tree viewer from a file, if the tree has nodes with more than two branches, it is automatically converted to a completely binary tree by creating an extra node of distance 0 at each point where there is more than one branch point from a node. The tree topology does not change, and this allows one to orient all branch points relative to each other. Upon opening a tree, the tree is iterated through, moving each branch beyond 2 for any node to it's own, new node (with a distance of 0 from its parent), until there are no nodes with more than two branches. When a tree is imported into a BioEdit alignment, however, this conversion is not performed, and the tree is imported directly as it is written. It is viewed in the same viewer, but the original node organization is retained.

You may save the tree from the File menu (File->Save). The current version of BioEdit is limited to opening and saving phylip-formatted trees. In phylip format, the above tree looks something like this:

```
((P.mirabili: 0.13368,((B.aphidico: 0.6262,(((T.maritima:
1.14167,((((M.genitali: 0.24742,M.pneumoni: 0.43983): 0.88981,(M.capricol:
0.70024,(H.pylori: 1.37587,B.subtilis: 0.53651): 0.19415): 0.0886):
0.04525,(S.PCC6803: 0.87437,((M.tubercul: 0.14643,M.leprae: 0.30498):
0.56324,(M.luteus: 0.65897,(S.bikinien: 0.1209,S.coelicol: 0.01772):
0.29437): 0.14817): 0.59556): 0.14169): 0.18393,(T.pallidum:
1.3449,B.burgdorf: 0.75431): 0.40702): 0.06668): 0.13184,C.burnetti:
0.76309): 0.22955,P.putida: 0.45219): 0.10167): 0.15512,H.influenz: 0.24691):
0.08603): 0,E.coli: 0.12297);
```

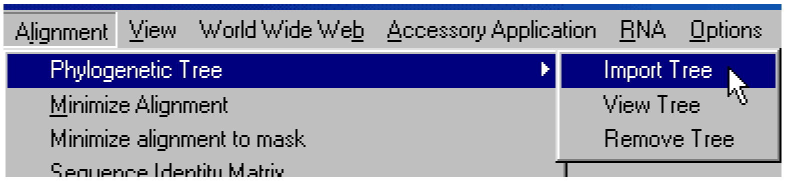
The BioEdit tree viewer supports only one tree at a time, and if a tree file is opened that has multiple trees in it, only the first tree will be loaded. However, when importing trees into an alignment file, all of the trees (up to 50 anyway) will be loaded into the alignment (as separate tree entries).

The tree viewer formats a tree to the current size of the viewing window, and does not now support multiple paging, zooming, or manual size specification, so it is only suitable for rather small trees. Also, printing is rather primitive, and simply scales to the size of the printer page. Right now, there is no copying to the clipboard. To produce an image of a tree, I recommend TreeView, which copies trees nicely to the clipboard as a Windows metafile.

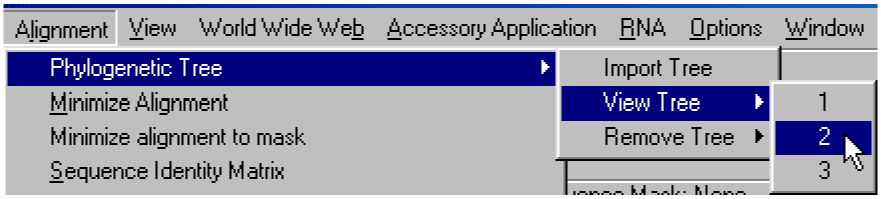
Importing Phylogenetic Trees into an alignment

It is sometimes convenient to have a phylogenetic tree handy showing the relationships between sequences in an alignment. For this reason, BioEdit 5.0.6 and above allows you to import one or more phylogenetic trees into an alignment file (as long as they are phylip-formatted), and to save those trees in a BioEdit-format alignment file. You may have up to 50 trees in one file. Normally, only one tree is probably desired, but one might have a set of equivalent trees generated by parsimony methods, or perhaps you want to have trees showing the relationships between sequences in subgroups of an alignment.

To import a tree into a BioEdit alignment, open the alignment (File->Open), then choose “Alignment->Phylogenetic Tree->Import Tree”. The menu will look something like this:



You will be prompted to specify the tree file to import. To view the imported tree, choose “Alignment->Phylogenetic Tree->View Tree-> (tree number)”. For example, if you have three trees associated with an alignment, the menu will look like this:



You may then save your file in BioEdit format and your associated trees will be saved with the file. Keep in mind that, if a file is not saved, a "Revert to Saved" operation will also remove any trees that were not saved with the file.

You may remove a tree with the "Alignment->Phylogenetic Tree->Remove Tree" option.

You may also open a tree in the tree viewer and choose to associate it with an open alignment file, if it is easier to see the tree to make sure it is the correct one. To do this, open the tree from the File->Open command from anywhere in the program, make sure you have your alignment file open, then, from the tree viewer, choose "File->Associate Tree With Alignment". You will get a dialog that lists all the currently open alignments, from which you can choose the appropriate alignment.

File formats

File formats read and written by BioEdit

BioEdit v5.0.0 reads and writes the following formats:

- BioEdit
- Genbank
- Fasta
- NBRF/PIR
- Phylip 3.2 / 2
- Phylip 4

- In addition, BioEdit version 4.7.0 and above will read ABI model 377 autosequencer files. The sequence is extracted and the trace is displayed on the screen and may also be printed in color. BioEdit version 4.7.7 and above allows editing the editable sequence. The current version also reads SCF trace files (versions 2 and 3), and ABI 373 and 3700 files.
- BioEdit 4.7.7 and above also read both ClustalW and GCG-formatted files, but it does not write them.

In addition to these formats, an external input/output filter (Don Gilbert's ReadSeq) is provided, allowing for the import and export of the following formats:

- IG/Stanford
- EMBL
- GCG (single sequence only)
- DNAStrider
- Fitch
- Zuker (import only)
- Olsen (import only)
- Plain or raw (single sequence only)
- PIR/CODATA
- MSF (multiple sequence format)
- ASN.1 (NCBI)
- PAUP/NEXUS

Documentation for the ReadSeq utility can be found in the file ReadSeq.txt in the /apps folder of the BioEdit installation directory. Use of this utility within BioEdit is automatic when opening sequences. If a file is opened which is not one of the formats read by BioEdit, you be prompted to try to open it with ReadSeq. If ReadSeq can open it, it will be imported into BioEdit as a GenBank file, otherwise it will be opened as text. To save a file in one of these formats, choose File->Export->Sequence alignment from an open document.

BioEdit Project File Format

BioEdit provides a specialized binary alignment format for very fast opening and saving of large alignment files (20 Mb+ file sizes -- even up to 100 Mb or larger). Reading and figuring out raw text becomes very slow in large alignments of formats such as GenBank, where there is no header telling the program how may sequences there are or how big they are.

The structure of a BioEdit Project file is as follows:

- Header
    1. offset 0x00000000: the string "**BioEdit Project File**" identifies the file as a BioEdit Project file (first version).
    2. The string "**BioEdit Project File02" at offset 0x00000000 identifies the file as version 2 of the BioEdit format (the current version). Previous versions of BioEdit will not read the current BioEdit format, but BioEdit v5.0.0 or above will read the old BioEdit format.
    3. offset 0x00000018: the number of sequences in the file.
    4. offset 0x0000001C: the index of the mask sequence (if there is one).
    5. offset 0x00000020: the index of the numbering mask (if there is one).
- Offset 0x000000C8: The offsets for each sequence data structure.

Each sequence structure consists of a title, a sequence, the sequence type, and all of the same GenBank fields included with a GenBank file in BioEdit. In addition, in BioEdit v5.0.0 or above, graphical sequence annotations, sequence grouping information, consensus sequence information, sequence locking status, and positional flags are saved. None of these latter additions are saved in any of the other, standard, formats Each field is preceded by a long integer specifying the length of the data, so each piece of data may be read from the file as a single chunk, which allows a file to be read very quickly.

GenBank Format

GenBank files written by BioEdit have the following minimal format:

```
LOCUS       Escherichi       119 amino acids
DEFINITION  Escherichi     119 amino acids
ORIGIN

     1    MVKLA FPREL RLLTP SQFTF VFQQP QRAGT PQITI LGRLN SLGHP RIGLT
     51   VAKKN VRRAH ERNRI KRLTR ESFRL RQHEL PAMDF VVVAK KGVAD LDNRA
     101  LSEAL EKLWR RHCRL ARGS
//
LOCUS       Proteus_mi       119 amino acids
DEFINITION  Proteus_mi     119 amino acids
ORIGIN

     1    MVKLA FPREL RLLTP KHFNF VFQQP QRASS PEVTI LGRQN ELGHP RIGLT
     51   IAKKN VKRAH ERNRI KRLAR EYFRL HQHQL PAMDF VVLVR KGVAE LDNHQ
     101  LTEVL GKLWR RHCRL AQKS
//
```

etc...

The LOCUS, DEFINITION and ORIGIN keywords are looked for in detecting GenBank files.

GenBank files may also contain additional information. The following fields may be included in any GenBank sequence entry, and are looked for when opening a GenBank file:

LOCUS: The locus of the sequence (often the position in the genome). This field is generally a single line and contains the Locus name, length of the sequence, and often the date of submission. Previous versions of BioEdit used the LOCUS as the sequence title.

DEFINITION: A description the sequence, usually one-line. The definition field is used as the default title in the absence of a BioEdit-specific “TITLE” field.

TITLE: This is a BioEdit-specific field and should be ignored by other programs that read GenBank format. The title field allows you to save sequence titles that are different from either the LOCUS or DEFINITION field entries. This is included so that user-defined titles may be given to sequences downloaded via Entrez without changing the original data in the sequence file. The TITLE field is not a part of a standard GenBank file and is used only by BioEdit. If this field is a problem with a sequence when trying to open it with another program, open the sequence as text and delete this field before using the file with the other program.

ACCESSION: the GenBank accession number for the sequence.

PID or NID: Protein or Nucleic Acid ID.

DBSOURCE: The database from which the sequence was obtained.

KEYWORDS

SOURCE: The source of the sequence (usually the organism from which it was obtained). This field often contains the subfield ORGANISM, which gives a description of the organism (often the taxonomic classification).

REFERENCES: references associated with the sequence submission.

COMMENT: miscellaneous information. A convenient place for user-defined information associated with a given sequence.

FEATURES: Sequence features including translations, promoters, more source information, etc.

ORIGIN: Marks the beginning of the actual sequence data. Two forward slashes (//) designate the end of the sequence.

The LOCUS, DEFINITION and ORIGIN fields are required for a GenBank file to be recognized by BioEdit. The other fields are optional. When GenBank files are saved, if LOCUS or DEFINITION fields are empty, they will be created with the sequence title and length. In this case, the LOCUS and DEFINITION will be identical. Other empty fields are not written into the sequence entry.

When opening a file, each field is read in as a single text block. subfields are not formally recognized, so any "unusual" formatting that may exist in the original file (non-standard spacing, for example) will be appear as is when a file is opened in BioEdit. When saving a GenBank file, however, specific subfield names are looked for and spaced as in a NCBI Entrez GenBank or GenPep report. The following subfields are looked for:

REFERENCES field(s):

reference number (format = REFERENCE <num> <description>)
AUTHORS
TITLE
JOURNAL
MEDLINE
REMARK
STRAIN

FEATURES field:
Previous versions of BioEdit only looked for a small selection of GenBank FEATURES tags. Version 5.0.0 or above looks for all of the following 67 tags:

3'clip
3'UTR
5'clip
5'UTR
-10_signal
-35_signal
-
allele
attenuator

CDS
C_region
CAAT_signal
conflict
D-loop
D_segment
enhancer
exon
Gene
iDNA
intron
J_segment
LTR
mat_peptide
misc_binding
misc_difference
misc_feature
misc_recomb
misc_RNA
misc_signal
misc_structure
modified_base
mRNA
mutation
N_region
old_sequence
polyA_signal
polyA_site
precursor_RNA
prim_transcript
primer_bind
promoter
Protein
protein_bind
RBS
Region
repeat_region
repeat_unit
rep_origin
rRNA
S_region
satellite
scRNA
SecStr
sig_peptide
Site
snRNA
source

stem_loop
STS
TATA_signal
terminator
transit_peptide
tRNA
unsure
V_region
V_segment
variation

Any data included within a field will be saved, however, in REFERENCE and FEATURES fields, data saved under a subheading not shown above may not be spaced as expected.

To edit specific fields, see Editing in an Edit Box

Fasta Format

Fasta/Pearson files written by BioEdit have the following format:

```
>Escherichi  119 amino acids
MVKLAFPRELRLLTPSQFTFVFQQPQRAGTPQITILGRLNSLGHPRIGLT
VAKKNVRRAHERNRIKRLTRESFRLRQHELPAMDFVVVAKKGVADLDNRA
LSEALEKLWRRHCRLARGS
>Proteus_mi  119 amino acids
MVKLAFPRELRLLTPKHFNFVFQQPQRASSPEVTILGRQNELGHPRIGLT
IAKKNVKRAHERNRIKRLAREYFRLHQHQLPAMDFVVLVRKGVAELDNHQ
LTEVLGKLWRRHCRLAQKS
```

etc ...

The “>” character followed by a string consistent with a title, followed by an unbroken string of characters is looked for in detecting Fasta files.

NBRF/PIR format

NBRF/PIR files written by BioEdit have the following format:

```
>P1;Escherichi
Escherichi  119 amino acids

 MVKLAFPREL RLLTPSQFTF VFQQPQRAGT PQITILGRLN SLGHPRIGLT
 VAKKNVRRAH ERNRIKRLTR ESFRLRQHEL PAMDFVVVAK KGVADLDNRA
 LSEALEKLWR RHCRLARGS*
>P1;Proteus_mi
Proteus_mi  119 amino acids

 MVKLAFPREL RLLTPKHFNF VFQQPQRASS PEVTILGRQN ELGHPRIGLT
 IAKKNVKRAH ERNRIKRLAR EYFRLHQHQL PAMDFVVLVR KGVAELDNHQ
 LTEVLGKLWR RHCRLAQKS*
```

etc ...

>P1; signifies a protein sequence, >DL; would signify a nucleic acid sequence.
The sequence is written in blocks of 10. The end of a sequence is denoted with an asterisk.
NBRF files are detected by the presence of “>P1;” or “>DL;” immediately followed by a title

Phylip 3.2/2 format

Phylip 3.2 / Phylip 2 files written by BioEdit have the following format:

```
 3 136 I
Escherichi   MVKLAFPREL RLLTPSQFTF VFQQPQRAGT PQITILGRLN SLGHPRIGLT
             VAKKNVRRAH ERNRIKRLTR ESFRLRQHEL PAMDFVVVAK KGVADLDNRA
             LSEALEKLWR RHCRLARGS- ---------- ------
Proteus_mi   MVKLAFPREL RLLTPKHFNF VFQQPQRASS PEVTILGRQN ELGHPRIGLT
             IAKKNVKRAH ERNRIKRLAR EYFRLHQHQL PAMDFVVLVR KGVAELDNHQ
             LTEVLGKLWR RHCRLAQKS- ---------- ------
Haemophilu   MLKVVKVYLH NHNSQFLVVK LNFSRELRLL TPIQFKNVFE QPFRASTPEI
             TILARKNNLE HPRLGLTVAK KHLKRAHERN RIKRLVRESF RLSQHRLPAY
             DFVFVAKNGI GKLDNNTFAQ ILEKLWQRHI RLAQKS
```

All sequences in Phylip format have the same length. The first line of the file specifies the number of sequences and the length of each sequence. The “I” here specifies that it is Phylip 3.2 format rather than Phylip 4. Each sequence is written after its title in blocks of 10. The titles are 10 characters long and the sequences are spaced three spaces after the titles.

Phylip 4 format

Phylip 4 files written by BioEdit have the following format

```
 3 136
Escherichi   MVKLAFPREL RLLTPSQFTF VFQQPQRAGT PQITILGRLN SLGHPRIGLT
Proteus_mi   MVKLAFPREL RLLTPKHFNF VFQQPQRASS PEVTILGRQN ELGHPRIGLT
Haemophilu   MLKVVKVYLH NHNSQFLVVK LNFSRELRLL TPIQFKNVFE QPFRASTPEI

             VAKKNVRRAH ERNRIKRLTR ESFRLRQHEL PAMDFVVVAK KGVADLDNRA
             IAKKNVKRAH ERNRIKRLAR EYFRLHQHQL PAMDFVVLVR KGVAELDNHQ
             TILARKNNLE HPRLGLTVAK KHLKRAHERN RIKRLVRESF RLSQHRLPAY

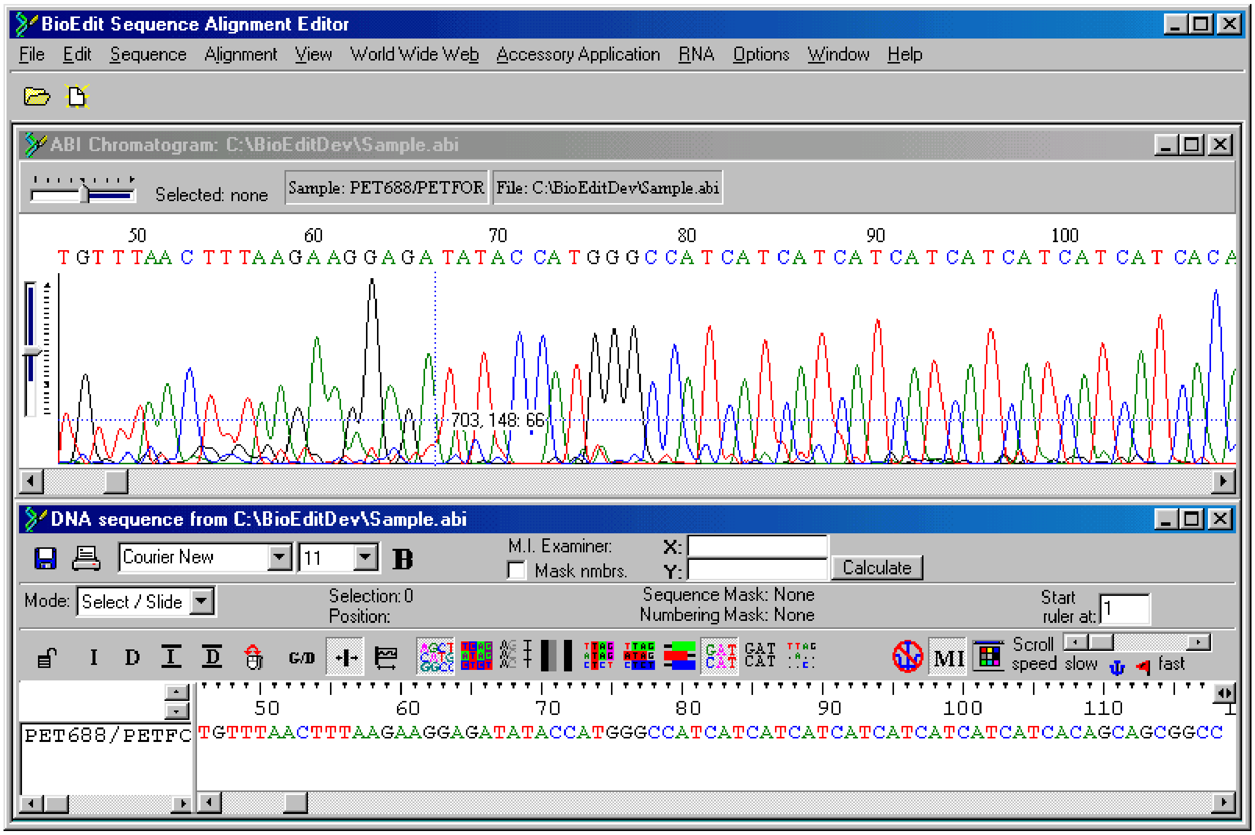
             LSEALEKLWR RHCRLARGS- ---------- ------
             LTEVLGKLWR RHCRLAQKS- ---------- ------
             DFVFVAKNGI GKLDNNTFAQ ILEKLWQRHI RLAQKS
```

The sequences are all the same length and are interleaved. The first line specifies the number of sequences and the length of the sequences. The sequences are written in blocks of 10 and interleaved with 50 residues of each sequence written per block. The titles are written before the first block. Titles are 10 characters long and sequences are spaced 3 spaces after the titles. All blocks are spaced over to the right 13 spaces.

ABI Autosequencer Trace Files

BioEdit version 4.7.0 and above will read ABI model 377 trace files. I am not yet familiar with older ABI files or .SCF files, so there is currently no support for these files. Much of the information needed to decipher ABI files was obtained from the ABIView web page (author David H. Klatte). Information for printout headers was figured out using a hex editor and the information from David Klatte as a starting point.

To open an ABI trace file, simply open the file as if you are opening any other file in BioEdit. As with alignment and plasmid files, the file format will be automatically detected (you may use the *.abi filter if the file(s) is/are named with a .abi extension). When an ABI file is opened, the (editable) sequence will be extracted into a new sequence/alignment document and the trace will be displayed in a separate window. An ABI file contains a duplicate sequence that allows both editing of the sequence and preservation of the original base-calls The non-editable sequence is displayed in the trace window upon first opening a trace. The following example shows the sample.abi file that comes with the BioEdit installation opened with the windows tiled:


BioEdit Sequence Alignment Editor
File
Edit
Sequence
Alignment
View
World Wide Web
Accessory Application
RNA
Options
Window
Help
ABI Chromatogram: C:\BioEditDev\Sample.abi
Selected: none
50
60
70
80
90
100
T GT T TAA C T T TAAGA AG GAGA TATAC CA T GGG C CA T CA T CA T CA T CA T CA T CA T CA T CA T CA C A
703, 148: 66
DNA sequence from C:\BioEditDev\Sample.abi
M.I. Examiner:
X:
Y:
Calculate
Scroll speed
slow
fast
MI
G/D
I
D
50
60
70
80
90
100
110

Sample: PET688/PETFOR

File: C:\BioEditDev\Sample.abi

Courier New

11

□ Mask nmbrs.

Mode: Select / Slide

Selection: 0

Position:

Sequence Mask: None

Numbering Mask: None

Start ruler at: 1

PET688/PETFC TGTTTAACTTTAAGAAGGAGATATACCATGGGCCATCATCATCATCATCATCATCATCATCACAGCAGCGGCC

The mouse may be used to select any part of the trace and partial sequence may be copied from the trace window. Alternatively, the entire sequence may be copied or exported as raw text or Fasta.

Vertical scaling resizes proportionately when the window is resized. The entire trace may be zoomed via the Zoom menu, and horizontal scaling may be changed separately from the Horizontal Scale menu.

To edit the sequence, you must first switch to the editable sequence by choosing View->Editable sequence. Individual basecalls may be edited by highlighting the base with the mouse and typing over it. Saving the edited sequence will not alter the non-editable sequence, and the non-editable sequence may be viewed at any time by choosing View->Non-editable sequence. The non-editable sequence is always shown by default upon first opening an ABI file. The editable sequence, however, is the one extracted to a sequence document. The edited sequence may be reverted at any time by choosing Edit->Revert edited to non-editable sequence.

You can view some of the relevant header information from the file by choosing File->info.

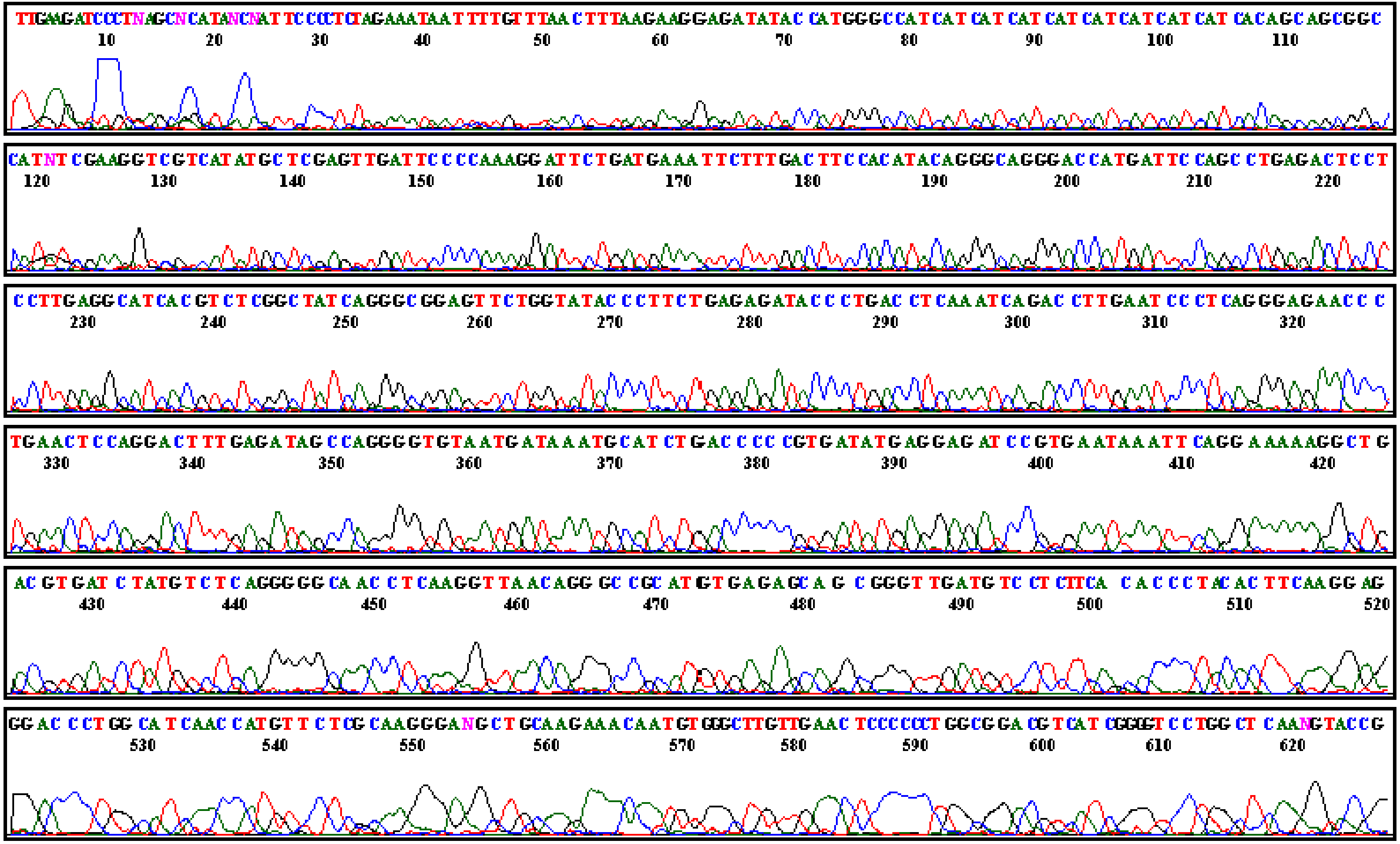
The trace and sequence may be reverse-complemented by choosing View->Reverse complement.

A printout of the trace looks similar to an ABI Prism printout. For most purposes, simply choosing "Print" from the "File" menu will produce a formatted print of desirable scaling. However horizontal and vertical scaling may be changed for printing via the "Print Scaling" menu under the "File" menu. A set of presets may be chosen, or any exact scaling may be specified (as %) by choosing "other".

The picture on the following page is similar to what can be expected for page one of a printout of the sample.abi file. A normal printout, however, will print at the ouput resolution of the printer (the image in this document is a bitmap).

BIO TRACE
BioEdit version 4.7.1

Model 377	File: Sample.abi	Signal G:90 A:90 T:61 C:83	Page 1 of 2
ABI200	Tom Hall	DT {BD Set Any-Primer}	8/12/1998
Version 3.2	PET688/PETFOR	dR Walt Matrix File	Spacing: 11.6000003814697
	Lane 34	Points 1091 to 9144	



Saving sequence annotation information

BioEdit will save much of the information contained within a standard GenBank formatted file.

The following fields may be included in any GenBank or BioEdit file:

LOCUS
DEFINITION
TITLE (BioEdit specific -- not standard in GenBank format)
ACCESSION
PID or NID
DBSOURCE
KEYWORDS
SOURCE
REFERENCES
COMMENT
FEATURES

In addition to the text information retained in the "FEATURES" field, sequences may be graphically annotated independently of these GenBank fields either manually or automatically using the standard tags from the GenBank FEATURES field. Graphical annotation information, however, will only be saved in BioEdit file format.

For a description of the above fields, see "GenBank file format".

For a description of the graphical sequence annotations, see "Graphical Feature Annotations".

Note that information other than sequence, title and length will only be saved in GenBank and BioEdit format. It may be easiest to keep most sequence files in GenBank format and only use other formats when a specific conversion is needed.

Reading Files saved with BioEdit with a Macintosh program

Macintosh computers use a different carriage return character than PC-compatibles. If you need to use a file created in BioEdit with a Macintosh program such as SeqApp or DNA Strider, you may need to first open the file with a word processor such as Microsoft Word or WordPerfect, then save it again to produce the correct carriage returns. BioEdit *will* correctly read a file that was created on a Macintosh or a UNIX machine.

Toggling between nucleotide and protein views

To control the way translation handles gaps , make sure the “Toggle Translation Control” option is checked from the “View” menu, and that the “Force contiguous codons” and “Ignore gaps that split codons” checkboxes are visible on the control bar of an alignment document.

When working with nucleotide sequences that code for proteins, BioEdit allows the toggling back and forth between nucleotide and protein sequences, with each view reflecting any gaps inserted or deleted in either view. The nucleotide information is retained when toggling back from a protein view (it is not re-translated degenerately).

To switch back and forth between nucleotide and protein views of protein-encoding nucleic acids, first trim the 5’ end of the sequence(s) to the start codon and make sure the coding region is in frame 1. Then either select the sequences to toggle and choose “Toggle Translation” from the Sequence” menu, or choose “Toggle Translation” from the “Alignment” menu (in which case all sequences will be automatically selected and toggled). The sequences may be aligned in either view. Additionally, if the protein view is Clustal-aligned, the underlying nucleotide sequences will be updated with the proper gaps.

Note 1: When saving an alignment, if the sequences are toggled on the protein view, the nucleotide sequences, not the proteins, are saved.

Note 2: This feature is only functional when the starting sequences are nucleic acid. If the starting sequences are protein, then this feature does not do anything when chosen, because the coding region of a protein cannot be known by examining the amino acid sequence alone. The feature can be used on degenerate reverse translations by first reverse-translating the sequences, then choosing this menu option.

Note 3: ***There are three available modes for handling gaps placed in nucleotide sequences which either split codons internally or occur as singles or pairs.*** A gap in a protein sequence will correspond to three gaps in the encoding nucleotide sequence. However, if a one or two gaps are placed in a nucleotide sequence, or gaps are placed directly within a codon (in frame 1), there is a problem. BioEdit handles this in one of three ways, depending upon the options chosen:

To alter the options for translation toggling, the “Translation Toggle Control” option under the “View” menu must be checked. When this menu item is checked, there will be two checkboxes on the right side of the top panel of the alignment window. The available options are:

1. Force all gaps to occur in groups of three and to only occur between codon (not within codons). In this mode, if a gap is introduced inside a codon, the nucleotides downstream are shifted left until a full codon is produced. If this results in a single or double gap (or if one is manually put between two codons), the gap is extended to three places to make a single amino acid size gap. This will cause gap positions to automatically change if they are not introduced as triplets between codons. It is easiest to simply align the sequences by their protein translations.

** This mode is active when the “Force contiguous codons” checkbox is checked.

2. Ignore gaps that split codons. In this mode, rather than trying to “fix” the sequences, any gaps that occur within codons or occur don’t make a whole amino acid gap are simply ignored in the protein translation. They are still retained in the nucleotide sequence, however, and will still be there when the proteins are toggled back to the nucleotides.

** This mode is active when the “Ignore gaps that split codons” checkbox is checked

Mode 1 and 2 cannot be active at the same time.

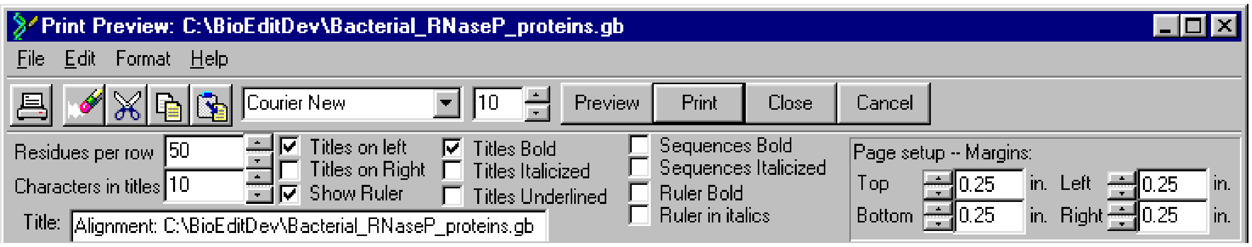
3. Neither mode. This is active when neither checkbox is checked. In this mode, no attempt to fix sequence edits is made, but gaps are not ignored in translating. Any gap that is not a multiple of three will cause a frameshift. A gap that occurs within a codon (in frame 1) will cause the translation to see an “X” rather than a valid amino acid.

** This mode is active when neither of the above checkboxes is checked.

Printing

To print an alignment, choose “Print Alignment as Text” from the “File” menu. A preview window will appear. This preview is incorporated into a rich text editor, and you may edit the alignment on-screen if you wish. If a title is specified, it is printed at the beginning of the alignment. Pressing the preview button causes the alignment to be re-drawn in the preview window with the selected specifications. If any on-screen editing will be done, make sure that the basic format (residues per line, characters per title, etc) is set, because pressing preview again will overwrite any typing in the preview window.

The preview interface is fairly straightforward:



Courier New 10

Residues per row 50
Characters in titles 10
Title: Alignment: C:\BioEditDev\Bacterial_RNaseP_proteins.gb

☑ Titles on left
☐ Titles on Right
☑ Show Ruler
☑ Titles Bold
☐ Titles Italicized
☐ Titles Underlined
☐ Sequences Bold
☐ Sequences Italicized
☐ Ruler Bold
☐ Ruler in italics

Top 0.25 in. Left 0.25 in.
Bottom 0.25 in. Right 0.25 in.

Note: The preview window will do its best to show a preview that will let you know if you’ve overshot on residues per row with the currently chosen right margin and font size. If this happens, the individual lines of sequence will wrap. This is what will happen on the printer if you try to print with the residues per row set beyond the end of the specified right margin. If this happens, decrease the residues per row, font size, or the right margin, or print in landscape orientation.

Exporting as raw text

BioEdit provides an easy function for converting alignments into properly spaced raw text files. To export the alignment as a raw text file (no formatting), select the “Save As ...” option from the “File” menu and choose “Text Files” from the “Save as type” options in the save dialog. You will get a dialog that asks for the number of residues per line (by tens) and the number of characters per title to save:

Exporting as Rich Text

An alignment shaded for identities and similarities may be exported in rich text format, preserving the residue highlighting as long as the file is viewed with Word 97 or newer or another word processor that supports highlighting in rich text. The alignment is shaded according to the current settings for shaded graphic views, and exporting in this format may also be done directly from the shaded graphic view form. To export an alignment as rich text directly from a document, choose File->Export->Rich text with current shaded view settings.

Shaded graphic view of alignment

For a presentation of the alignment showing identities and similarities shaded, select the titles for the sequences you want to include, then choose “Graphic View” from the “File” menu of an open document. This is similar to the print preview, but allows for the shading of identical and similar residues in the alignment, and allows you to show any subset of the alignment.
The following options are currently offered:

- Variable threshold percentage for shading residues (one threshold for both identities and similarities)
- Show or hide ruler
- Show or hide titles on the left and right of sequence data
- Show or hide position numbers on the left and right
- Variable number of residues per line (by tens -- 20 to 2000)
- Variable number of characters per title (5 to 30)
- Titles may be bolded, italicized and/or underlined
- Sequences may be italicized and/or bolded
- A choice of scoring matrices is available
- Fonts: Any font can be used and will spaced approximately correctly. However, some fonts look odd in this view (most, actually, are spaced too wide because the widest character must be accommodated), and typeset fonts work best. For greater control over the font, choose “Character Font” from the “Font” menu.
- Colors for background of page, identities and similarities and colors for non-identical, identical and similar residue characters.
- A title may be added which will be placed at the beginning of each page. In this release, this creates a problem if the alignment requires more than one page, so it is best to leave the title field blank.
- Alignment may be drawn in the standard color table colors (to allow printing of the alignment in color).

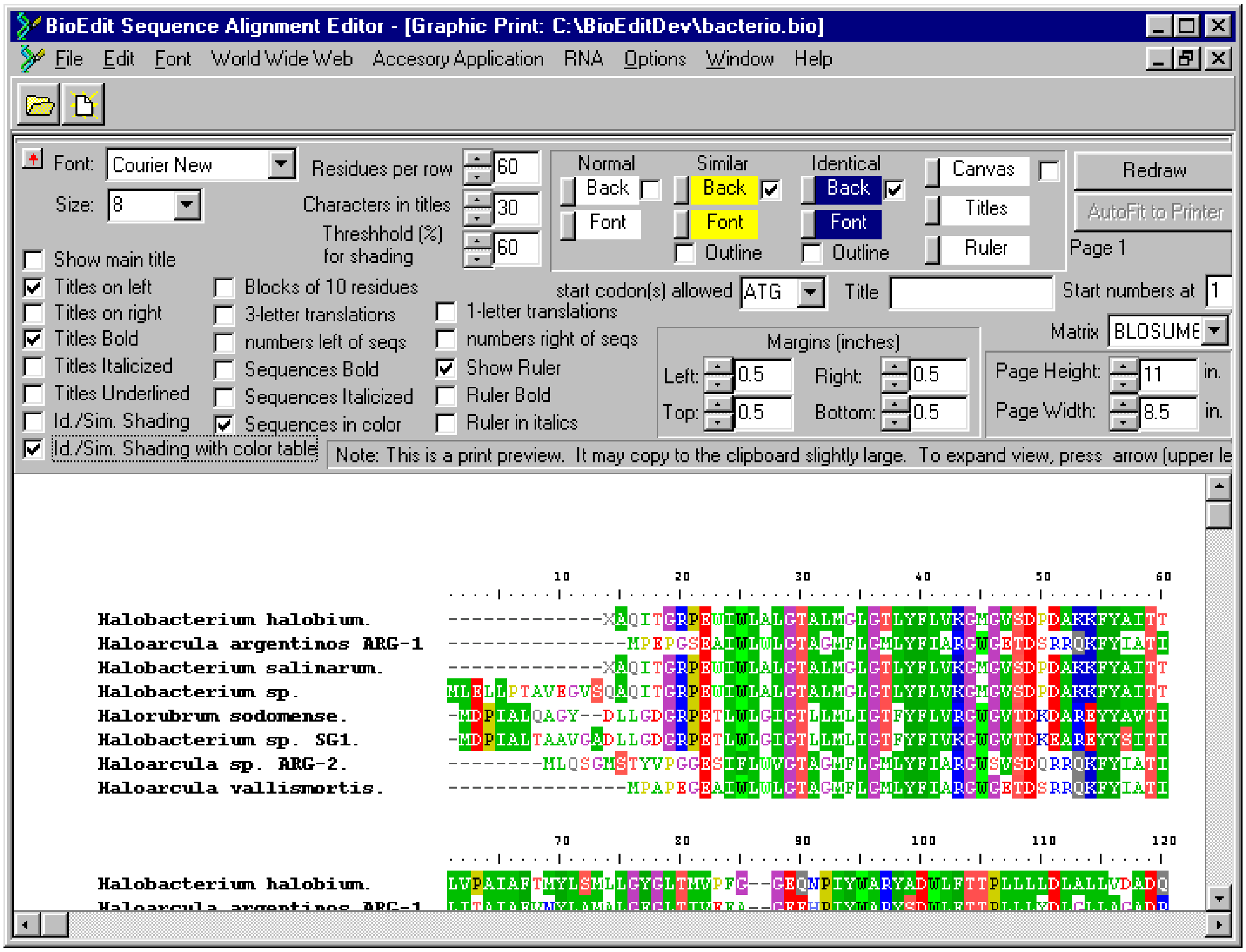
- Shading according to identity and similarity (threshold defined by “Threshold (%) for shading” on control panel) may be done with the user defined colors on the control panel, or with current alignment color table colors.
- Lines of sequence may be blocked into groups of 10 (like a Phylip file) or printed as continuous, unbroken lines.
- Translations may be shown below nucleic acid sequences in either 3-letter or one-letter codes
- Shaded alignments may be exported as rich text documents which preserve colored highlighting (Word 97 or above, or equivalent, is required to display highlighting in rich text).

When certain changes are made to the current view, the “Redraw” button must be pressed for the changes to take place on the screen. Many changes are automatically updated.
To change colors, either double-click on the labeled, colored box, or press the small button to the left of it.
To copy a page to the clipboard for pasting into another application, choose “Copy” form the “Edit” menu. A page may be copied as a bitmap or an Enhanced Windows Metafile (EMF). Copying as a metafile allows for pasting directly into an application such as PowerPoint for inclusion of a shaded alignment in a slide presentation, or into a page layout program for creation of a publication figure. A metafile offers the advantage of being a vectored graphic (the graphics are defined by formula/code rather than pixels such as in a bitmap) which can take advantage of the full resolution of the output device. At this time, only an entire page can be copied. A later version may offer annotation and selection capabilities, if there is demand for this.

Multiple pages are supported for long alignments, however, this is a serious problem with this version of BioEdit. Currently, a page is defined vertically by the page height in inches specified by the user in the graphic view window. When vertically scrolling through the graphic view, the current page is shown in the upper right of the form. When the end of one page is reached, the next page is drawn in its place. There is no continuing view from page to page, which can make viewing page transitions on-screen very tedious. Also, some image-editing is required to produce an image figure of an alignment that takes more than one page. Currently, it is easiest if the alignment can be made to fit on one page. The height of a page can be set to up to 100 inches, but this will take an enormous amount of memory and is not recommended (each page is a bitmapped image). To create an image longer than a piece of paper, you can increase the page height and copy the image to the clipboard. On a slow computer, there is a delay as each new page is drawn.

Previous versions of BioEdit determined the page size only vertically. The current version calculates the specified page settings (from the print setup and the margin and page size settings) and will clip the graphical image if it runs over the right side of the page. This should correspond rather closely (may not always be perfect) with what will print on the currently chosen printer with the current settings. Also, margins are now shown in the graphic view to reflect a fairly accurate print preview for each page.

The following are two shaded views of a ClustalW alignment of bacteriorhodopsin protein sequences from representative halophilic Archaea:



Font: Courier New

Size: 8

Residues per row 60

Characters in titles 30

Threshhold (%) for shading 60

Normal: Back □ Font

Similar: Back ☑ Font □ Outline

Identical: Back ☑ Font □ Outline

Canvas □

Page 1

□ Show main title

☑ Titles on left

□ Titles on right

☑ Titles Bold

□ Titles Italicized

□ Titles Underlined

□ Id./Sim. Shading

☑ Id./Sim. Shading with color table

□ Blocks of 10 residues

□ 3-letter translations

□ numbers left of seqs

□ Sequences Bold

□ Sequences Italicized

☑ Sequences in color

□ 1-letter translations

□ numbers right of seqs

☑ Show Ruler

□ Ruler Bold

□ Ruler in italics

start codon(s) allowed ATG

Title

Start numbers at 1

Matrix BLOSUM6

Margins (inches) Left: 0.5 Right: 0.5 Top: 0.5 Bottom: 0.5

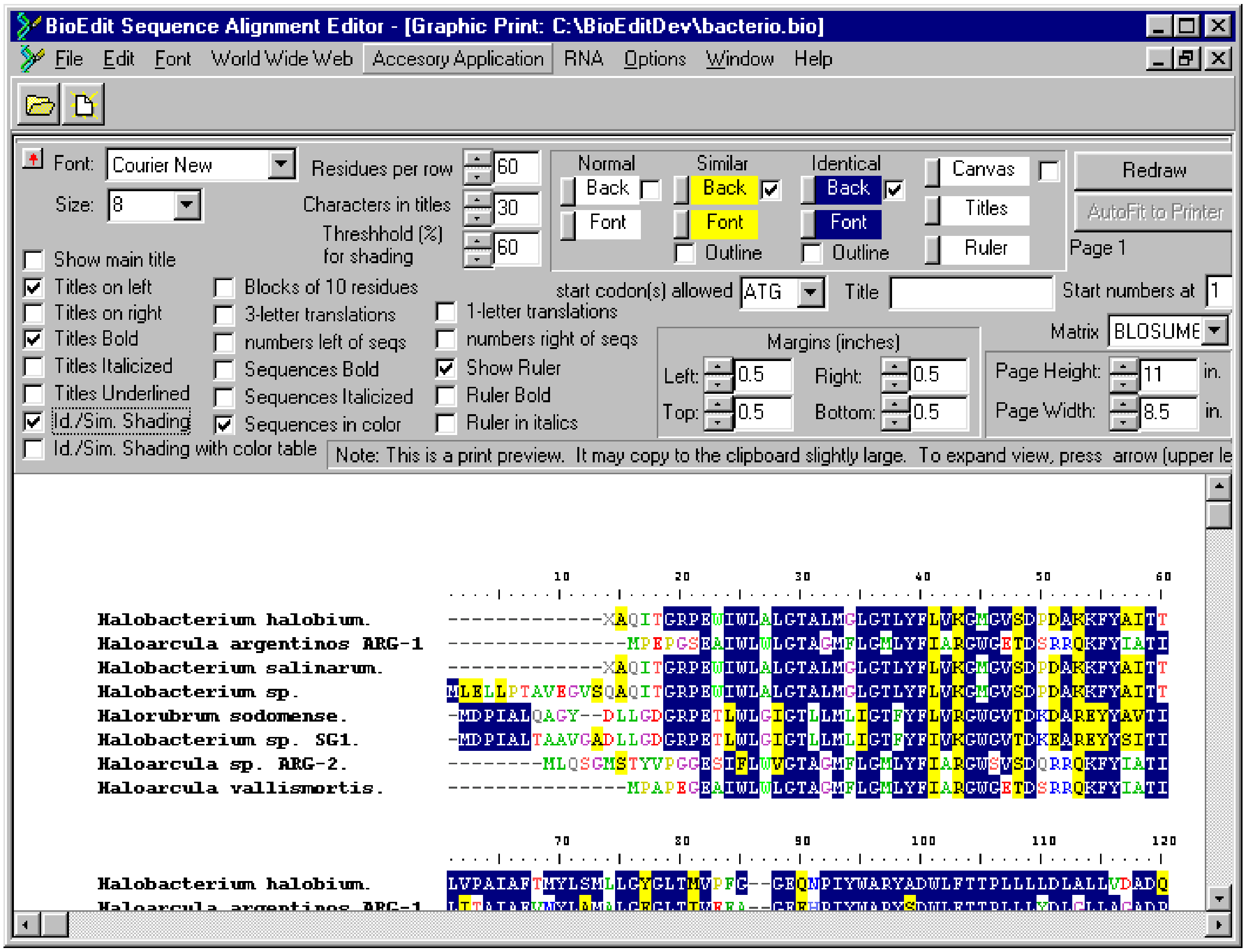
Page Height: 11 in.

Page Width: 8.5 in.

Note: This is a print preview. It may copy to the clipboard slightly large. To expand view, press arrow (upper le

```
                                         10        20        30        40        50        60
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
Halobacterium halobium.          -------------XAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT
Haloarcula argentinos ARG-1      ---------------MPEPGSEAIWLWLGTAGMFLGMLYFIARGWGETDSRRQKFYIATI
Halobacterium salinarum.         -------------XAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT
Halobacterium sp.                MLELLPTAVEGVSQAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT
Halorubrum sodomense.            -MDPIALQAGY--DLLGDGRPETLWLGIGTLLMLIGTFYFLVRGWGVTDKDAREYYAVTI
Halobacterium sp. SG1.           -MDPIALTAAVGADLLGDGRPETLWLGIGTLLMLIGTFYFIVKGWGVTDKEAREYYSITI
Haloarcula sp. ARG-2.            --------MLQSGMSTYVPGGESIFLWVGTAGMFLGMLYFIARGWSVSDQRRQKFYIATI
Haloarcula vallismortis.         ---------------MPAPEGEAIWLWLGTAGMFLGMLYFIARGWGETDSRRQKFYIATI

                                         70        80        90       100       110       120
                                 ....|....|....|....|....|....|....|....|....|....|....|....|
Halobacterium halobium.          LVPAIAFTMYLSMLLGYGLTMVPFG--GEQNPIYWARYADWLFTTPLLLLDLALLVDADQ
Haloarcula argentinos ARG-1      LLTAIAFWNYLAMALGFGLTIVEFA--GEEHPIYWARYSDWLFTTPLLLYDLGLLAGADR
```



Font: Courier New
Size: 8
Residues per row 60
Characters in titles 30
Threshhold (%) for shading 60
start codon(s) allowed ATG
Title
Start numbers at 1
Matrix BLOSUM62
Left: 0.5 Right: 0.5
Top: 0.5 Bottom: 0.5
Page Height: 11 in.
Page Width: 8.5 in.

☐ Show main title
☑ Titles on left
☐ Titles on right
☑ Titles Bold
☐ Titles Italicized
☐ Titles Underlined
☑ Id./Sim. Shading
☐ Id./Sim. Shading with color table
☐ Blocks of 10 residues
☐ 3-letter translations
☐ numbers left of seqs
☐ Sequences Bold
☐ Sequences Italicized
☑ Sequences in color
☐ 1-letter translations
☐ numbers right of seqs
☑ Show Ruler
☐ Ruler Bold
☐ Ruler in italics

Note: This is a print preview. It may copy to the clipboard slightly large. To expand view, press arrow (upper le

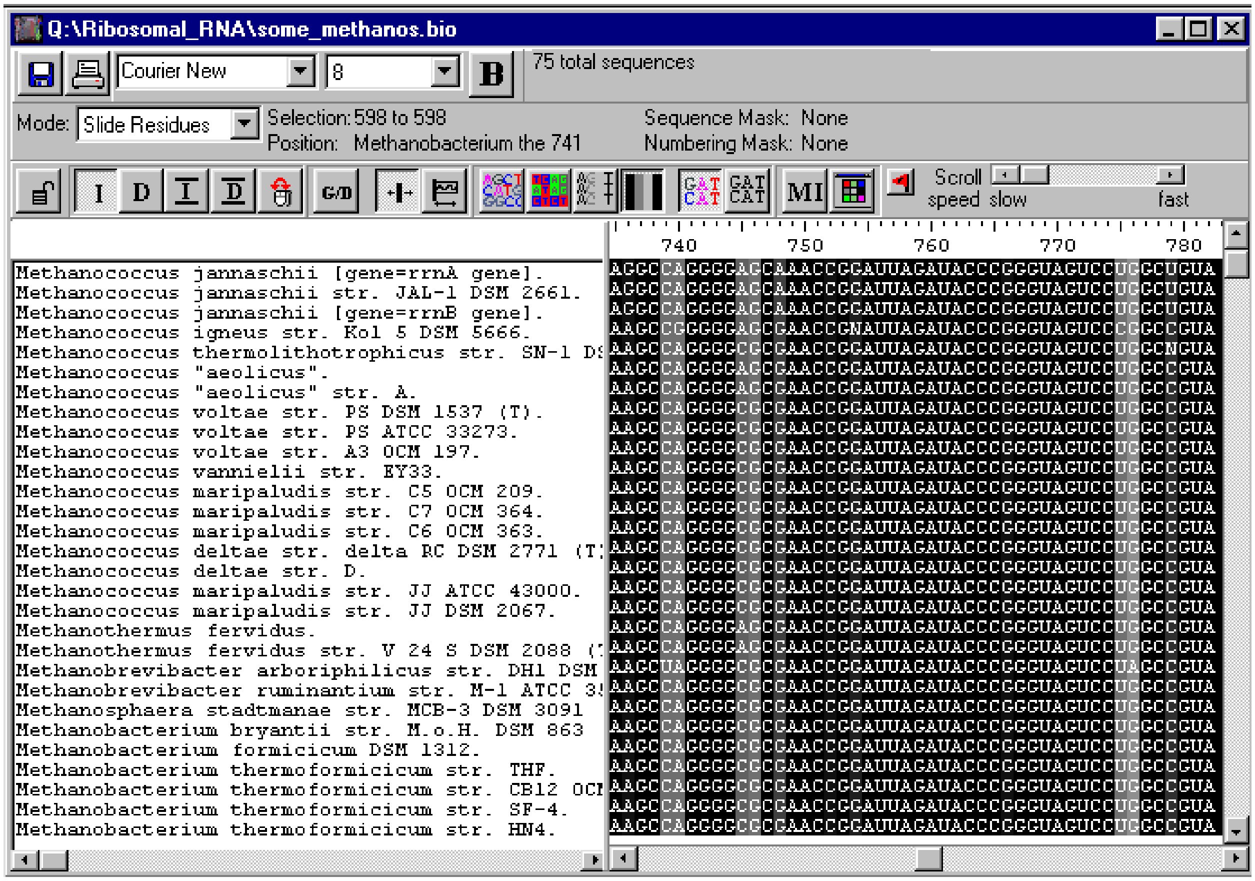
```
                                         10        20        30        40        50        60
                                ....|....|....|....|....|....|....|....|....|....|....|....|
Halobacterium halobium.         -------------XAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT
Haloarcula argentinos ARG-1     ---------------MPEPGSEAIWLWLGTAGMFLGMLYFIARGWGETDSRRQKFYIATI
Halobacterium salinarum.        -------------XAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT
Halobacterium sp.               MLELLPTAVEGVSQAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITT
Halorubrum sodomense.           -MDPIALQAGY--DLLGDGRPETLWLGIGTLLMLIGTFYFLVRGWGVTDKDAREYYAVTI
Halobacterium sp. SG1.          -MDPIALTAAVGADLLGDGRPETLWLGIGTLLMLIGTFYFIVKGWGVTDKEAREYYSITI
Haloarcula sp. ARG-2.           --------MLQSGMSTYVPGGESIFLWVGTAGMFLGMLYFIARGWSVSDQRRQKFYIATI
Haloarcula vallismortis.        ---------------MPAPEGEAIWLWLGTAGMFLGMLYFIARGWGETDSRRQKFYIATI

                                         70        80        90       100       110       120
                                ....|....|....|....|....|....|....|....|....|....|....|....|
Halobacterium halobium.         LVPAIAFTMYLSMLLGYGLTMVPFG--GEQNPIYWARYADWLFTTPLLLLDLALLVDADQ
Haloarcula argentinos ARG-1     LITAIAFVMYLAMALGFGLTIVEFA--GEEHPIYWARYSDWLFTTPLLLYDLGLLAGADR
```

Information-based shading in the alignment window

The following view shows an information-based shaded view of an alignment of 75 16S rRNA sequences from methanogenic Archaea. The region shown was picked by an entropy/information-based search for conserved regions.

Compare this view to the split-window view below it showing the same alignment with this region compared to a less well-conserved region (on the next page).

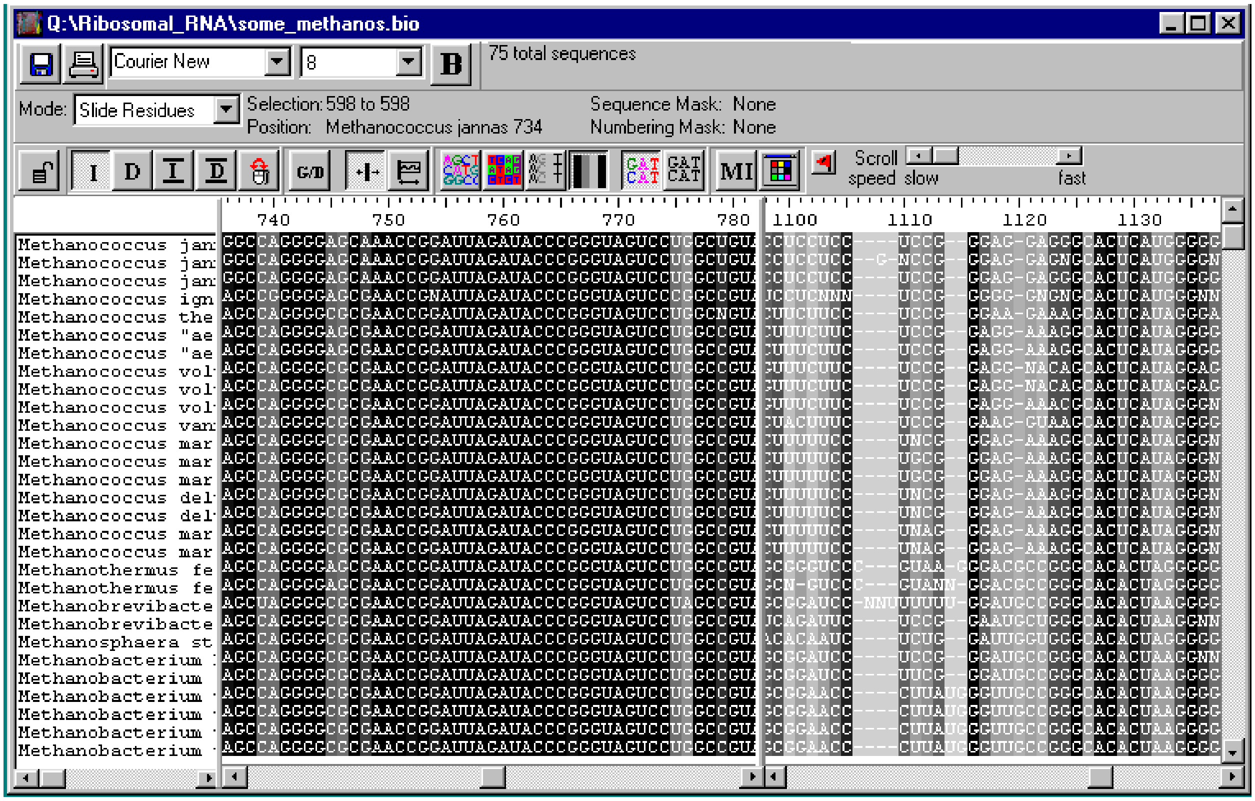


Q:\Ribosomal_RNA\some_methanos.bio

Courier New 8 B 75 total sequences

Mode: Slide Residues Selection: 598 to 598 Position: Methanobacterium the 741 Sequence Mask: None Numbering Mask: None

```
Methanococcus jannaschii [gene=rrnA gene].       AGGCCAGGGGAGCAAACCGGAUUAGAUACCCGGGUAGUCCUGGCUGUA
Methanococcus jannaschii str. JAL-1 DSM 2661.    AGGCCAGGGGAGCAAACCGGAUUAGAUACCCGGGUAGUCCUGGCUGUA
Methanococcus jannaschii [gene=rrnB gene].       AGGCCAGGGGAGCAAACCGGAUUAGAUACCCGGGUAGUCCUGGCUGUA
Methanococcus igneus str. Kol 5 DSM 5666.        AAGCCGGGGGAGCGAACCGNAUUAGAUACCCGGGUAGUCCCGGCCGUA
Methanococcus thermolithotrophicus str. SN-1 DS  AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCNGUA
Methanococcus "aeolicus".                        AAGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus "aeolicus" str. A.                 AAGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus voltae str. PS DSM 1537 (T).       AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus voltae str. PS ATCC 33273.         AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus voltae str. A3 OCM 197.            AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus vannielii str. EY33.               AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus maripaludis str. C5 OCM 209.       AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus maripaludis str. C7 OCM 364.       AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus maripaludis str. C6 OCM 363.       AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus deltae str. delta RC DSM 2771 (T   AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus deltae str. D.                     AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus maripaludis str. JJ ATCC 43000.    AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanococcus maripaludis str. JJ DSM 2067.      AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanothermus fervidus.                         AAGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanothermus fervidus str. V 24 S DSM 2088 (   AAGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobrevibacter arboriphilicus str. DH1 DSM   AAGCUAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUAGCCGUA
Methanobrevibacter ruminantium str. M-1 ATCC 3   AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanosphaera stadtmanae str. MCB-3 DSM 3091    AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobacterium bryantii str. M.o.H. DSM 863    AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobacterium formicicum DSM 1312.            AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobacterium thermoformicicum str. THF.      AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobacterium thermoformicicum str. CB12 OC   AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobacterium thermoformicicum str. SF-4.     AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
Methanobacterium thermoformicicum str. HN4.      AAGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA
```



Courier New 8

75 total sequences

Mode: Slide Residues

Selection: 598 to 598

Position: Methanococcus jannas 734

Sequence Mask: None

Numbering Mask: None

Scroll speed slow fast

```
                          740       750       760       770       780     1100      1110      1120      1130
Methanococcus jan GGCCAGGGGAGCAAACCGGAUUAGAUACCCGGGUAGUCCUGGCUGUA CCUCCUCC----UCCG--GGAG-GAGGGCACUCAUGGGGG
Methanococcus jan GGCCAGGGGAGCAAACCGGAUUAGAUACCCGGGUAGUCCUGGCUGUA CCUCCUCC--G-NCCG--GGAG-GAGNGCACUCAUGGGGN
Methanococcus jan GGCCAGGGGAGCAAACCGGAUUAGAUACCCGGGUAGUCCUGGCUGUA CCUCCUCC----UCCG--GGAG-GAGGGCACUCAUGGGGG
Methanococcus ign AGCCGGGGGAGCGAACCGNAUUAGAUACCCGGGUAGUCCCGGCCGUA UCCUCNNN----UCCG--GGGG-GNGNGCACUCAUGGGNN
Methanococcus the AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCNGUA CUUCUUCC----UCCG--GGAA-GAAAGCACUCAUAGGGA
Methanococcus "ae AGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUCUUC----UCCG--GAGG-AAAGGCACUCAUAGGGG
Methanococcus "ae AGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUCUUC----UCCG--GAGG-AAAGGCACUCAUAGGGG
Methanococcus vol AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GUUUCUUC----UCCG--GAGG-NACAGCACUCAUAGGAG
Methanococcus vol AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GUUUCUUC----UCCG--GAGG-NACAGCACUCAUAGGAG
Methanococcus vol AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GUUUCUUC----UCCG--GAGG-AAACGCACUCAUAGGGN
Methanococcus van AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUACUUUC----UCCG--GAAG-GUAAGCACUCAUAGGGG
Methanococcus mar AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UNCG--GGAG-AAAGGCACUCAUAGGGN
Methanococcus mar AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UGCG--GGAG-AAAGGCACUCAUAGGGN
Methanococcus mar AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UGCG--GGAG-AAAGGCACUCAUAGGGN
Methanococcus del AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UNCG--GGAG-AAAGGCACUCAUAGGGN
Methanococcus del AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UNCG--GGAG-AAAGGCACUCAUAGGGN
Methanococcus mar AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UNAG--GGAG-AAAGGCACUCAUAGGGN
Methanococcus mar AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA CUUUUUCC----UNAG--GGAG-AAAGGCACUCAUAGGGN
Methanothermus fe AGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCGGGUCCC---GUAA-GGGACGCCGGGCACACUAGGGGG
Methanothermus fe AGCCAGGGGAGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCN-GUCCC---GUANN-GGACGCCGGGCACACUAGGGGG
Methanobrevibacte AGCUAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUAGCCGUA GCGGAUCC-NNUUUUUU-GGAUGCCGGGCACACUAAGGGG
Methanobrevibacte AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA UCAGAUUC----UCCG--GAAUGCUGGGCACACUAAGGNN
Methanosphaera st AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA ACACAAUC----UCUG--GAUUGGUGGGCACACUAGGGGG
Methanobacterium   AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCGGAUCC----UCCG--GGAUGCCGGGCACACUAAGGNN
Methanobacterium  AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCGGAUCC----UUCG--GGAUGCCGGGCACACUAAGGGG
Methanobacterium  AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCGGAACC----CUUAUGGGUUGCCGGGCACACUAAGGGG
Methanobacterium  AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCGGAACC----CUUAUGGGUUGCCGGGCACACUAAGGGG
Methanobacterium  AGCCAGGGGCGCGAACCGGAUUAGAUACCCGGGUAGUCCUGGCCGUA GCGGAACC----CUUAUGGGUUGCCGGGCACACUAAGGGG
```

Restriction Maps

BioEdit offers two ways to generate restriction maps of nucleic acid sequences. An internal restriction map utility allows generation of maps for sequences up to 65,536 nucleotides. It has only really been tested up to about 35 Kb, and it takes a while on a slower computer for a large sequence. You can also link directly to WebCutter restriction mapping via the World Wide Web.

WebCutter: Highlight the title of the sequence you wish to map and choose "Auto-fed WebCutter Restriction Mapping" from the "World Wide Web" menu.

BioEdit: Highlight the title of the sequence you wish to map and choose "Restriction Map" from the "Sequence" menu. An interface window will appear with the following options:

-- Display Map: Display or omit a full map of the sequence and the complementary strand showing the cut positions of each enzyme. Default: yes
-- Alphabetical by name: Display a list of all enzymes that cut, their recognition sequences, frequency of cutting, and all positions (5' end starting at 1). Default: yes
-- Numeric by position: A list of all positions that are cut, in increasing order, and the enzymes that cut there. Enzyme cut positions are defined at the cut site, rather than the start of the recognition sequence (e.g.. if a BamHI site (G'GATC_C) started at position 1476, the cut position would be reported as 1477 -- where it actually cuts). Default: no
-- List of unique sites: List of enzymes that cut only once in the entire sequence. Default: no
-- Enzymes that cut five or fewer times. Default: yes
-- Summary table of frequencies: A table of all enzymes currently selected and the number of times they cut in the sequence. Default: no
-- Enzymes that do not cut. Default: yes
-- 4-base cutters: You may choose to omit enzymes that cut at a 4-base recognition sequence. To *include* these enzymes, the box must be *checked*. Default: no (don't include 4-base cutters).
-- 5-base cutters: Same as 4-base cutters.
-- Enzymes with degenerate recognition sequences: Many restriction enzymes recognize sequences loosely. For example, AccI recognizes the sequence "GT'mk_AC", where 'm' can be A or C and 'k' can be G or T. You may wish to exclude these on occasion. Default: include these.
-- Large recognition sites: Often for cloning, only the common 6-base recognizing enzymes are used. If you do not want a map cluttered with extra information, uncheck this box (as well as 4-base and 5-base cutters).
-- All Isoschizomers: The enzyme list file used is the GCG-format file available from ReBase. Several enzymes are in this file which cut the same recognition sequence of other enzymes in the file. To show only one enzyme for a particular recognition site, uncheck this box (Default=unchecked). If this option is chosen, the map will be very large. Isoschizomers for all enzymes which you choose to include may be examined by viewing the enzyme table from the mapping interface (press the "View Current Enzyme Table" button).
-- Three frame translation: Shows a translation along the sequence as shown in the alignment (assumed to be in the 5' to 3' orientation left to right).
-- Translation of complement: A three-frame translation of the complementary strand running the opposite direction.

Considerations for BioEdit restriction mapper.

-- Numbering is *at the nucleotide where the enzyme cuts, not the start of the recognition sequence.* This is important to keep in mind for enzymes such as AceIII, which recognizes the sequence “CAGCTCnnnnnnn'nnnn_”. AceIII actually cuts 12 bps. downstream of its recognition sequence start.

-- The interface window is not an MDI child and is designed to stay on top of the application. When a restriction map is generated, the window disappears, but the selected options remain as the default until the application is closed and reopened. To view the enzyme list, the interface window must either be closed or minimized to see the table list behind it.

-- A different enzyme file may be supplied to BioEdit, but it must be in the GCG format, must be named “enzyme.tab” *(case sensitive)*, and must be located in the \tables\ folder.

An example of the GCG format for restriction enzyme tables is:

```
REBASE version 811                                                gcgenz.811

    =-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=
    REBASE, The Restriction Enzyme Database   http://www.neb.com/rebase
    Copyright (c)  Dr. Richard J. Roberts, 1998.   All rights reserved.
    =-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=-=

Rich Roberts                                                      Oct 28 98


REBASE codes for commercial sources of restriction enzymes & methylases

                A        Amersham Life Sciences-USB (4/97)
                B        Life Technologies Inc. (1/98)
                C        Minotech Molecular Biology Products (1/98)
                D        Angewandte Gentechnologie Systeme (10/97)
                E        Stratagene (1/98)
                F        Fermentas AB (5/98)
                G        Appligene Oncor (10/97)
                H        American Allied Biochemical, Inc. (10/98)
                I        SibEnzyme Ltd. (10/98)
                J        Nippon Gene Co., Ltd. (10/97)
                K        Takara Shuzo Co. Ltd. (1/98)
                L        Kramel Biotech (7/98)
                M        Boehringer-Mannheim (10/97)
                N        New England BioLabs (8/98)
                O        Toyobo Biochemicals (1/98)
                P        Pharmacia Biotech Inc. (8/97)
                Q        CHIMERx (10/97)
                R        Promega Corporation (10/98)
                S        Sigma (7/98)
                T        Advanced Biotechnologies Ltd. (3/98)

..
AarI       3 CACCTGC        0 ? !                                 >     1667
;AatI      3 AGG'CCT        0 !  Eco147I,Pme55I,StuI,SseBI      >O      6643
AatII      5 G_ACGT'C      -4 !                                 >ADEFKLMNOPR   6643
;AauI      1 T'GTAC_A       4 !  Bsp1407I,BsrGI,SspBI           >I      6251
AccI       2 GT'mk_AC       2 !  FblI                           >ABDEGJKLMNOPQRS
;AccII     2 CG'CG          0 !  BstUI,MvnI,ThaI,Bsh1236I       >AJKQ   3992,7430
;AccIII    1 T'CCGG_A       4 !  BseAI,BsiMI,Bsp13I,BspEI,Kpn2I,MroI  >EJKQR      3994,5140
```

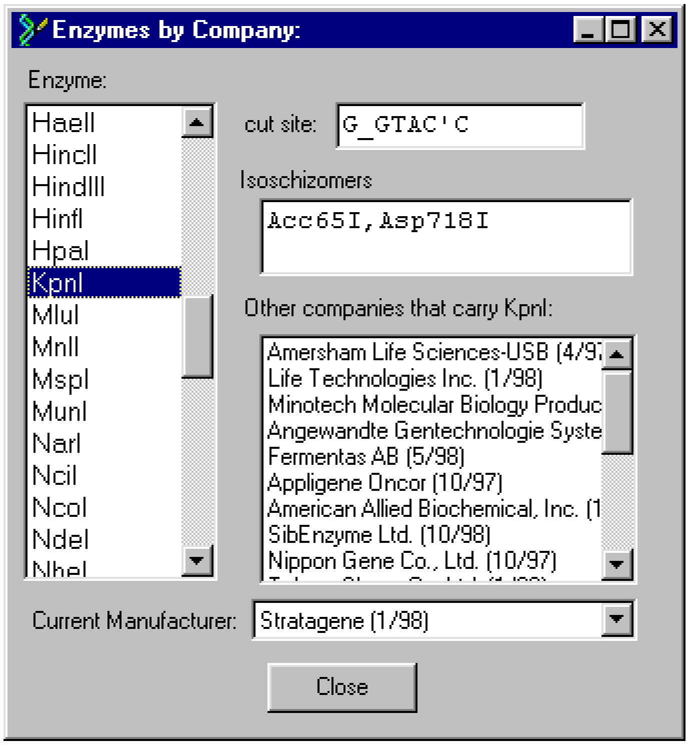
... and on like this -- the file simply ends after the last enzyme.

Restriction Enzyme Browser

When running a restriction map on a nucleic acid sequence, it may be useful to show enzymes available from a particular company. For example, many scientific departments have a contract deal with companies such as Promega or Boehringer-Mannheim with an on-site freezer from which enzymes and reagents may be obtained with no delay.

Restriction enzymes may be browsed by manufacturer by choosing a manufacturer and pressing the ➔ button on the restriction map dialog. You may also examine restriction enzymes at any time by choosing “View Restriction Enzymes by Manufacturer” from the “Options” menu.

The following dialog will appear:



Enzymes by Company:

Enzyme:

HaeII
HincII
HindIII
HinfI
HpaI
KpnI
MluI
MnlI
MspI
MunI
NarI
NciI
NcoI
NdeI
NheI

cut site: G_GTAC'C

Isoschizomers: Acc65I,Asp718I

Other companies that carry KpnI:

Amersham Life Sciences-USB (4/97
Life Technologies Inc. (1/98)
Minotech Molecular Biology Produc
Angewandte Gentechnologie Syste
Fermentas AB (5/98)
Appligene Oncor (10/97)
American Allied Biochemical, Inc. (1
SibEnzyme Ltd. (10/98)
Nippon Gene Co., Ltd. (10/97)

Current Manufacturer: Stratagene (1/98)

In this example, all restriction enzymes available from Stratagene are listed on the left and KpnI is highlighted. The recognition sequence for KpnI is shown on top, isoschizomers are shown below that, and other companies are shown which also carry KpnI. The numbers in parentheses next to each company name specify the month and year in which the information for that company was last updated. BioEdit uses the gcgenz table supplied by ReBase, the restriction enzyme database on the World Wide Web: `http://www.neb.com/rebase/`
This table may be updated by downloading the most recent version of the gcgenz.* table from rebase, naming it “enzyme.tab”, and replacing the old table file in the “tables” directory of the BioEdit installation folder.

Note: The table must be in the gcgenz format. You may open the “ enzyme.tab” file from the tables folder to see what the format looks like, or see Restriction Maps. The restriction enzyme table file must be named “enzyme.tab” and must be located in the “tables” folder in order to be recognized by BioEdit.

Codon Tables

BioEdit uses only codon tables with the format produced by the GCG program CodonFrequency. The default codon table that comes with BioEdit is the E. coli codon usage table produced by J. Michael Cherry (cherry@frodo.mgh.harvard.edu). The default codon table is shown below as an example of the format:

```
Escherichia coli

681 genes found in GenBank 63.

 Produced by J. Michael Cherry (cherry@frodo.mgh.harvard.edu) with the
 GCG program CodonFrequency.

 Duplicates, pseudogenes, mutant and synthetic genes were not included.
 Coding regions were specified using the Feature Table of each entry, then
 checked for accuracy. If more than one stop codon was found the sequence
 was not included.

This table was taken directly from the SeqPup distribution (Don Gilbert).
The following note is left in:
_________________________________________________________________________
    Note for SeqPup usage ----
        The start codon needs to be in >>lower case<< to
   be recognized by SeqPup as the start codon.  Otherwise,
   Met/atg will be used as the start codon for ORF searching.
_________________________________________________________________________

BioEdit v1.0 alpha has no ORF-searching, but later versions will, and the
same convention will be followed.


AmAcid  Codon     Number    /1000     Fraction   ..

Gly     GGG     1743.00      9.38      0.13
Gly     GGA     1290.00      6.94      0.09
Gly     GGT     5243.00     28.22      0.38
Gly     GGC     5588.00     30.08      0.40

Glu     GAG     3527.00     18.98      0.30
Glu     GAA     8101.00     43.61      0.70
Asp     GAT     6103.00     32.85      0.59
Asp     GAC     4244.00     22.84      0.41

Val     GTG     4429.00     23.84      0.34
Val     GTA     2231.00     12.01      0.17
Val     GTT     3744.00     20.15      0.29
Val     GTC     2601.00     14.00      0.20

Ala     GCG     5946.00     32.01      0.34
Ala     GCA     3899.00     20.99      0.22
Ala     GCT     3266.00     17.58      0.19
Ala     GCC     4274.00     23.01      0.25

Arg     AGG      286.00      1.54      0.03
Arg     AGA      464.00      2.50      0.04
```

```
Ser     AGT     1366.00      7.35       0.13
Ser     AGC     2871.00     15.45       0.27

Lys     AAG     2238.00     12.05       0.24
Lys     AAA     7102.00     38.23       0.76
Asn     AAT     3047.00     16.40       0.39
Asn     AAC     4755.00     25.59       0.61

Met     atg     4756.00     25.60       1.00
Ile     ATA      738.00      3.97       0.07
Ile     ATT     4970.00     26.75       0.47
Ile     ATC     4955.00     26.67       0.46

Thr     ACG     2375.00     12.78       0.23
Thr     ACA     1263.00      6.80       0.12
Thr     ACT     2160.00     11.63       0.21
Thr     ACC     4437.00     23.88       0.43

Trp     TGG     2504.00     13.48       1.00
End     TGA      180.00      0.97       0.30
Cys     TGT      887.00      4.77       0.43
Cys     TGC     1173.00      6.31       0.57

End     TAG       52.00      0.28       0.09
End     TAA      371.00      2.00       0.62
Tyr     TAT     3017.00     16.24       0.53
Tyr     TAC     2629.00     14.15       0.47

Leu     TTG     2046.00     11.01       0.11
Leu     TTA     1879.00     10.11       0.11
Phe     TTT     3443.00     18.53       0.51
Phe     TTC     3328.00     17.91       0.49

Ser     TCG     1434.00      7.72       0.13
Ser     TCA     1274.00      6.86       0.12
Ser     TCT     1992.00     10.72       0.19
Ser     TCC     1794.00      9.66       0.17

Arg     CGG      851.00      4.58       0.08
Arg     CGA      580.00      3.12       0.05
Arg     CGT     4534.00     24.41       0.42
Arg     CGC     4006.00     21.56       0.37

Gln     CAG     5389.00     29.01       0.69
Gln     CAA     2375.00     12.78       0.31
His     CAT     2145.00     11.55       0.52
His     CAC     1987.00     10.70       0.48

Leu     CTG     9749.00     52.48       0.55
Leu     CTA      565.00      3.04       0.03
Leu     CTT     1857.00     10.00       0.10
Leu     CTC     1764.00      9.50       0.10

Pro     CCG     4371.00     23.53       0.55
Pro     CCA     1559.00      8.39       0.20
Pro     CCT     1248.00      6.72       0.16
Pro     CCC      785.00      4.23       0.10
```

Six-frame translation

A DNA sequence may be translated in all six reading frames into all possible open reading frames (simple codon stretches, actually) by highlighting the sequence title in the document window and choosing either “Sorted Six-Frame Translation” or “Unsorted Six-Frame Translation” from the “Sequence” menu. You will get a dialog asking you to specify the minimum ORF size, maximum ORF size, and start codon.

Minimum ORF size: Only codon stretches equal to or greater in length than the minimum will be reported.
Maximum ORF size: Only codon stretches equal to or lesser in length than the maximum will be reported. Leave this entry blank to allow unlimited ORF size.
Start codon: Choose ATG or Any from the drop-down box, or type in any three-base codon you wish. Only codon stretches beginning with this start codon will be reported. If “Any” is chosen, codon stretches will basically go from stop to stop.

Differences between sorted and unsorted translations:

Sorted: ORFs will be reported in order of start position. Negative-frame sequences are sorted according to their end positions (first position along the positive sequence). The number of sequences which can be translated and sorted is limited to something above 10,500 sequences. The exact number, I am not sure of. If a sorted translation becomes too large, resources for storing the sequences to be sorted runs out. If this happens, BioEdit will tell you, then present the sequences it was able to translate. Multiple sequences may be translated into a single ORF list suitable for BLAST database creation.

Unsorted: Sequences are reported in the order that their stop codons are encountered in a once-through, 6-frame simultaneous pass through the entire sequence. The codon stretches are written into a file as they are encountered and therefore do not need to be stored in memory. Very long lists can thus be generated. Currently, only one sequence at a time may be translated this way.

No sophisticated ORF identification is currently implemented. sequences are simply translated into raw codon stretches. A future addition may allow the user to require threshold matches to consensus promoters and/or ribosome-binding sites for ORF reporting.

Possible open reading frames are reported as shown in the following example:

```
>ecoli.m52: 620 to 111: Frame -2       170 aa
STKVFNCASGNPGWAAASPVKSSAKIRSASLILGKASWPLMVFSIIATRWLVIL
AGAERTVATCPCLALLSRISATRRKRSAFATDVPPNFNTRMVVTSLPLVEKKSP
HCQVRAFFCVSCTRQPAPLPVVMVMVVVMVVLMRFMDVVYSVIFICLCAMPILV
KVFSDLSQ
>ecoli.m52: 292 to 2796: Frame 1       835 aa
QCGLFFSTKGNEVTTMRVLKFGGTSVANAERFLRVADILESNARQGQVATVLSA
PAKITNHLVAMIEKTISGQDALPNISDAERIFAELLTGLAAAQPGFPLAQLKTF
VDQEFAQIKHVLHGISLLGQCPDSINAALICRGEKMSIAIMAGVLEARGHNVTV
IDPVEKLLAVGHYLESTVDIAESTRRIAASRIPADHMVLMAGFTAGNEKGELVV
LGRNGSDYSAAVLAACLRADCCEIWTDVDGVYTCDPRQVPDARLLKSMSYQEAM
ELSYFGAKVLHPRTITPIAQFQIPCLIKNTGNPQAPGTLIGASRDEDELPVKGI
SNLNNMAMFSVSGPGMKGMVGMAARVFAAMSRARISVVLITQSSSEYSISFCVP
```

```
QSDCVRAERAMQEEFYLELKEGLLEPLAVTERLAIISVVGDGMRTLRGISAKFF
AALARANINIVAIAQGSSERSISVVVNNDDATTGVRVTHQMLFNTDQVIEVFVI
GVGGVGGALLEQLKRQQSWLKNKHIDLRVCGVANSKALLTNVHGLNLENWQEEL
AQAKEPFNLGRLIRLVKEYHLLNPVIVDCTSSQAVADQYADFLREGFHVVTPNK
KANTSSMDYYHQLRYAAEKSRRKFLYDTNVGAGLPVIENLQNLLNAGDELMKFS
GILSGSLSYIFGKLDEGMSFSEATTLAREMGYTEPDPRDDLSGMDVARKLLILA
RETGRELELADIEIEPVLPAEFNAEGDVAAFMANLSQLDDLFAARVAKARDEGK
VLRYVGNIDEDGVCRVKIAEVDGNDPLFKVKNGENALAFYSHYYQPLPLVLRGY
GAGNDVTAAGVFADLLRTLSWKLGV
>ecoli.m52: 2792 to 3730: Frame 2       313 aa
ESDMVKVYAPASSANMSVGFDVLGAAVTPVDGALLGDVVTVEAAETFSLNNLGR
FADKLPSEPRENIVYQCWERFCQELGKQIPVAMTLEKNMPIGSGLGSSACSVVA
ALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYDNVAPCFLGGMQLMIEEND
IISQQVPGFDEWLWVLAYPGIKVSTAEARAILPAQYRRQDCIAHGRHLAGFIHA
CYSRQPELAAKLMKDVIAEPYRERLLPGFRQARQAVAEIGAVASGISGSGPTLF
ALCDKPETAQRVADWLGKNYLQNQEGFVHICRLDTAGARVLEN
```

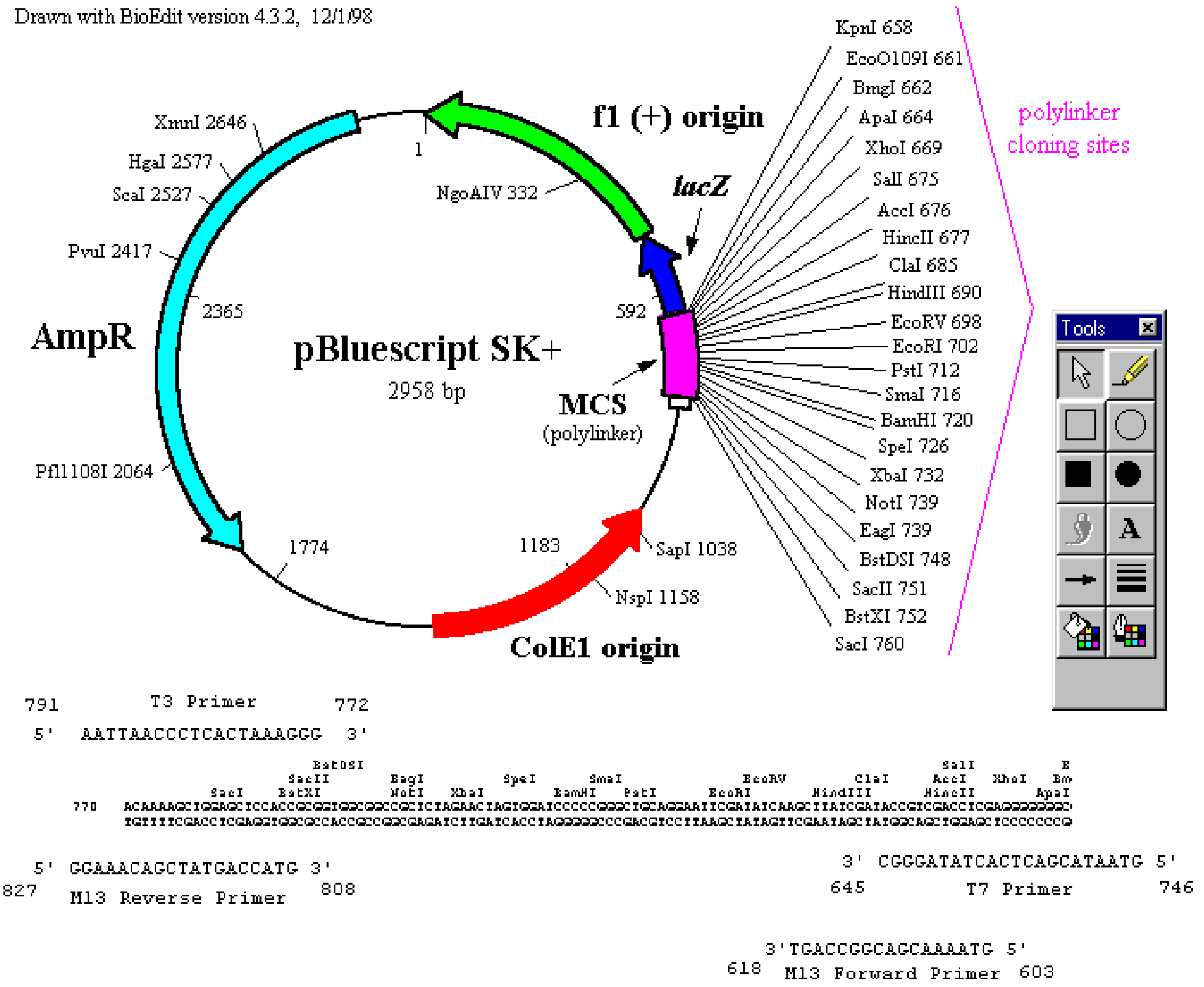
... etc.

The ORF titles are constructed as follows:

<sequence title>: <start base> to <end base>: Frame <frame number>   <sequence length>

Plasmid drawing with BioEdit

BioEdit provides tools for simple plasmid drawing and annotation in a fairly quick and easy manner. The following vector map for pBluescript SK+ (Stratagene) was drawn in a few minutes using BioEdit. The features in this particular map were basically copied from the map provided by Stratagene. Creating a new vector is just as simple, though.



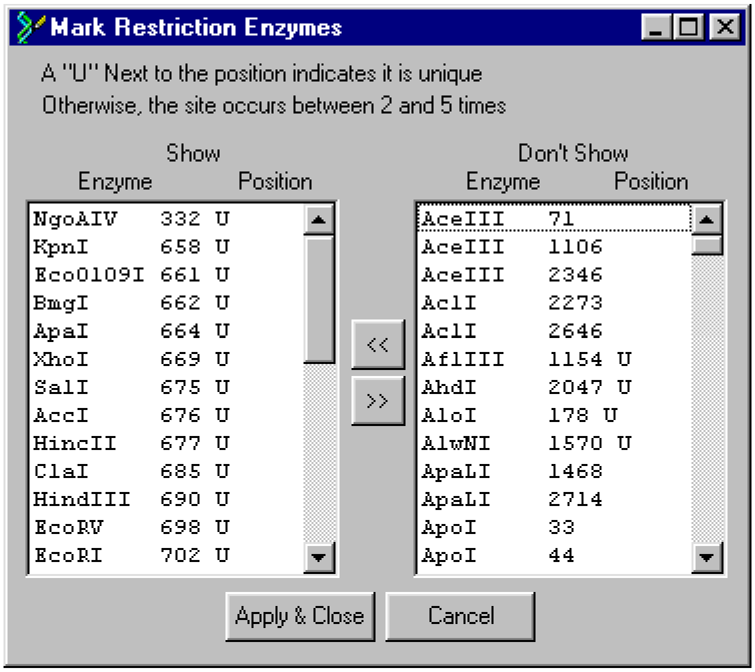
Drawn with BioEdit version 4.3.2, 12/1/98
pBluescript SK+
2958 bp
f1 (+) origin
lacZ
MCS
(polylinker)
AmpR
ColE1 origin
1
592
2365
1774
1183
XmnI 2646
HgaI 2577
ScaI 2527
PvuI 2417
Pfl1108I 2064
NgoAIV 332
SapI 1038
NspI 1158
KpnI 658
EcoO109I 661
BmgI 662
ApaI 664
XhoI 669
SalI 675
AccI 676
HincII 677
ClaI 685
HindIII 690
EcoRV 698
EcoRI 702
PstI 712
SmaI 716
BamHI 720
SpeI 726
XbaI 732
NotI 739
EagI 739
BstDSI 748
SacII 751
BstXI 752
SacI 760
polylinker
cloning sites
Tools
A
791 T3 Primer 772
5' AATTAACCCTCACTAAAGGG 3'
BstDSI
SacII EagI SpeI SmaI EcoRV ClaI SalI E
SacI BstXI NotI XbaI BamHI PstI EcoRI HindIII AccI XhoI Bm
HincII ApaI
770 ACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCC
TGTTTTCGACCTCGAGGTGGCGCCACCGCCGGCGAGATCTTGATCACCTAGGGGGCCCGACGTCCTTAAGCTATAGTTCGAATAGCTATGGCAGCTGGAGCTCCCCCCCGG
5' GGAAACAGCTATGACCATG 3'
827 M13 Reverse Primer 808
3' CGGGATATCACTCAGCATAATG 5'
645 T7 Primer 746
3'TGACCGGCAGCAAAATG 5'
618 M13 Forward Primer 603

With the BioEdit plasmid drawing utility, sequences may be automatically converted into circular plasmids with easy automated positional marking. Features, polylinker and restriction sites may be easily added through the use of dialogs. When a sequence is made into a plasmid map, a restriction map is silently run in the background, so restriction sites may be added by simply selecting from a dialog. They are added to the correct position on the map automatically. The plasmid utility also provides simple drawing and labeling tools. These need to be improved and expanded, however. Labels and drawn objects may be moved and scaled with the mouse. To edit an object's properties, double click on the object.

To create a plasmid from a DNA sequence, choose "Create Plasmid from Sequence" either from the "Sequence" menu, or from the "Nucleic Acid" submenu of the "Sequence" menu. When this option is chosen, a restriction map will be run using the common commercial enzymes and stored in memory. When the plasmid first comes up it will simply be a circle with 10 positional markings and a title in the center.

Restriction sites:

To add restriction sites, choose "Restriction Sites" from the "Vector" menu. The following dialog will appear: (There will not initially be anything in the "Show" dialog)



Mark Restriction Enzymes

A "U" Next to the position indicates it is unique
Otherwise, the site occurs between 2 and 5 times

Show

Enzyme	Position
NgoAIV	332 U
KpnI	658 U
Eco0109I	661 U
BmgI	662 U
ApaI	664 U
XhoI	669 U
SalI	675 U
AccI	676 U
HincII	677 U
ClaI	685 U
HindIII	690 U
EcoRV	698 U
EcoRI	702 U

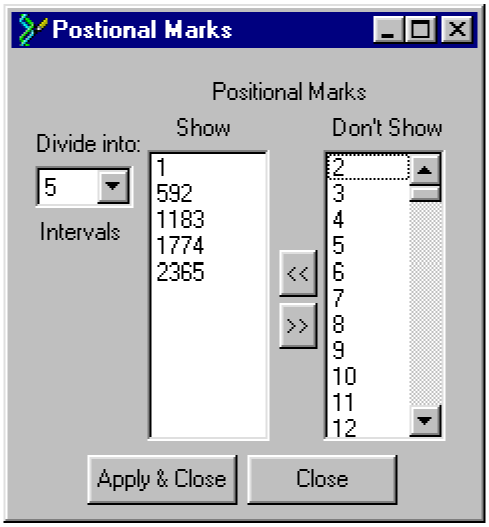
Don't Show

Enzyme	Position
AceIII	71
AceIII	1106
AceIII	2346
AclI	2273
AclI	2646
AflIII	1154 U
AhdI	2047 U
AloI	178 U
AlwNI	1570 U
ApaLI	1468
ApaLI	2714
ApoI	33
ApoI	44

To display restriction enzymes on the map, select any enzymes desired on the right side (the "Don't Show" box) and move them to the left using the << button. When "Apply & Close" is pressed, these sites will be added to the map. An enzyme is specified as being a single-cutter (unique) by a "U" after the cut site. If there is no "U", then the first cut position is shown. Only enzymes which cut 5 or fewer times are shown. To remove enzyme positions from the map, highlight the enzyme(s) in the "Show" box and press the >> button to move them to the other side.

Positional marks:

The following dialog can be brought up with the "Positional Marks" option under the "Vector" menu:



Divide into: 5 Intervals

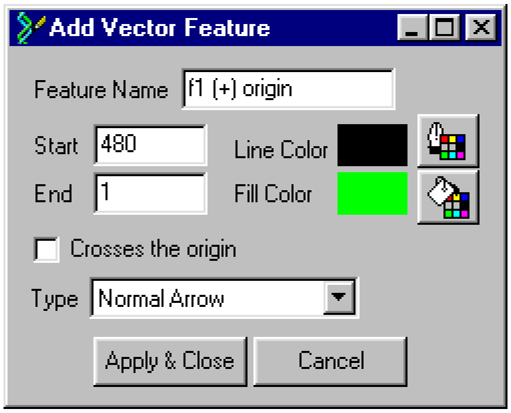
Show: 1, 592, 1183, 1774, 2365

Don't Show: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12

Positional markings may be added individually by moving them to the "Show" box, or a set number of divisional markings may be applied. To have no marks at all, choose "none" at the top of the "Divide into:" drop-down list.

Features:

To add a feature such as an antibiotic resistance marker, choose "Add Feature" from the "Vector" menu. the following dialog appears:



Feature Name: f1 (+) origin

Start: 480

End: 1

Line Color: ⬛

Fill Color: 🟩

☐ Crosses the origin

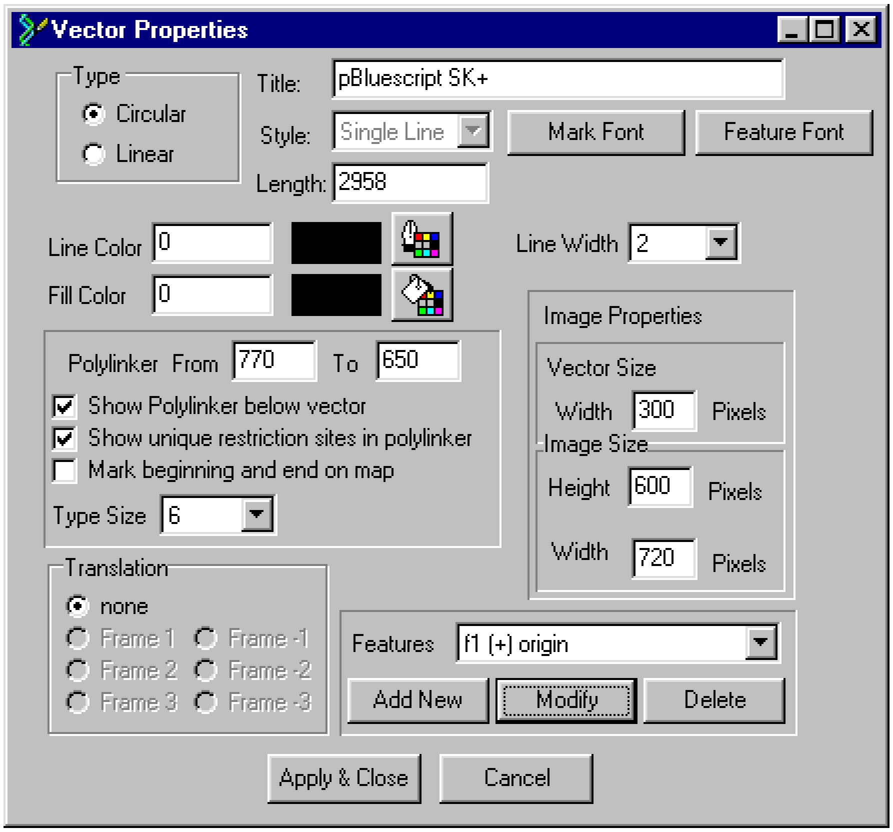
Type: Normal Arrow

The choices of type are Normal Arrow, Wide Arrow, Normal Box and Wide Box. All of the features in the above example are of the "normal" width. If the feature is an arrow, the direction of the arrow will be determined by the start and end positions, and whether or not it crosses position 1 (the origin).

When features or enzymes are added, their respective labels are added on the outside, centered as well as possible over the site. The labels may then be selected with the selector tool and moved, scaled or edited.

General Vector properties:

Properties of the vector may be modified by choosing "Properties" from the "Vector" menu:



Vector Properties

Type
● Circular
○ Linear

Title: pBluescript SK+

Style: Single Line

Length: 2958

Line Color 0

Fill Color 0

Line Width 2

Polylinker From 770 To 650

☑ Show Polylinker below vector
☑ Show unique restriction sites in polylinker
□ Mark beginning and end on map

Type Size 6

Translation
● none
○ Frame 1 ○ Frame -1
○ Frame 2 ○ Frame -2
○ Frame 3 ○ Frame -3

Image Properties

Vector Size
Width 300 Pixels

Image Size
Height 600 Pixels
Width 720 Pixels

Features f1 (+) origin

A polylinker may be added to the bottom by specifying the beginning and end positions. The polylinker is shown in "Courier New" font.

Features may be edited, added or deleted with this dialog. To edit or delete an existing feature, choose the feature in the "Features" drop-down and press the appropriate button. A new feature may be added by pressing the "Add New" button.

At this time only circular, single line plasmids are available. This will be expanded later.

The "Font" buttons change the indicated default font. The fonts of feature labels may be changed independent of each other, but positional markings are not created as individual selectable objects.

Drawing tools:

Very simple drawing tools are available which behave more or less like standard drawing tools in most programs. The order of objects may be changed in the "Arrange" menu, and objects may be grouped and ungrouped from the "Arrange" menu.

Note: Scaling grouped objects does not work well, as the objects are scaled independently of each other.

To edit an object's properties, double-click on the object, or select the object and choose "Object Properties" from the "Edit" menu.

Cut / Copy / Paste:

When an object(s) is copied in the plasmid utility, a structure is copied into memory for the use of BioEdit, and a bitmap of the object or objects is copied to the clipboard. Objects may therefore be pasted into other applications as bitmap images.

Printing:

When printing, the map is drawn to the printer at the printer's resolution to avoid the pixellation that occurs with a screen-resolution bitmap. The print interface is not very advanced, however. A left margin and top margin may be specified in the "Print Setup" dialog (from the "File" menu) At this time there is no support for scaling output to the printer to defined print dimensions. The size of the printed figure is determined by the ratio of printer resolution to screen resolution. The full width of a screen set at 800 x 600 resolution corresponds to roughly 8.3 inches, which is pretty close to the width of a normal paper page (8.5 inches). The plasmid itself scales slightly small on the printer, and I’m currently trying to figure out why. The size relative to an 800 x 600 resolution screen is fairly close, however.

Moving the vector:

The vector may be moved around the page by first selecting it with the mouse (a dotted box will be drawn around it to indicate it is selected), then dragging it with the mouse to its new location. All of the labels and objects on the page will be moved accordingly.

Searching functions

The following search options are available under the Edit menu. These functions were never originally conceived very well and have evolved sort of sloppily. Searching functionality is lacking in BioEdit at this time and is in need of improvement.

Simple search: Find and Find Next

This is a very simple search function and needs to be improved. The menu option for simple searching is found under Edit->Find. A standard search dialog is presented which allows for searching of exact text strings (either case sensitive or insensitive) within selected sequences. The search is always performed downwards from the beginning of the document, and only includes sequences whose titles are selected (the search is performed only upon the sequences, and does not include titles). When the text is found, the first instance encountered is highlighted in the document window. The current search position is remembered. To continue the search to find the next instance, select Edit->Find Again (F3, by default). If Edit->Find is chosen again, the search position is reset to the beginning of the document.

Find in Titles and Find in Next Title

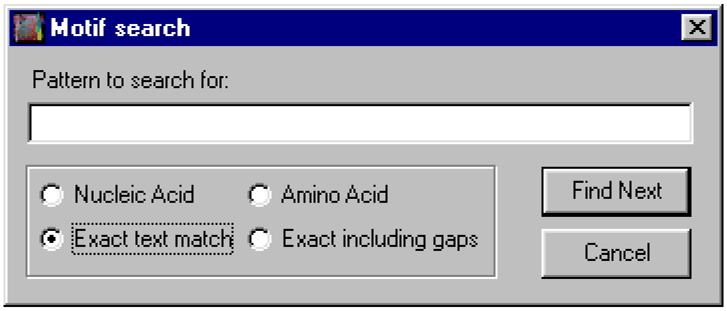
To highlight *all* titles containing specific text, choose Edit->Find in Titles. To highlight the next title (either up or down) containing specific text, choose Edit->Find in Next Title. The search is started at the last selected title in the specified direction.

Find Next ORF

When searching for ORFs, only sequences with selected titles are searched, and the search begins after the last selected nucleotide. To search, choose Edit->Find Next ORF, or Sequence->Nucleic Acid->Find Next ORF. The search is performed according to the parameters specified in the ORFs page of the preferences dialog. When an ORF is found, the sequence is highlighted in the document window.

Search for user-defined motif

BioEdit 4.7.8 and above allows searching for user-defined sequence patterns according to single-letter designations of nucleotides and amino acids. To search for a sequence within selected sequences, choose Edit->Search for user-defined motif. The following dialog appears:



Enter the text to search for in the input box and choose the type of search.

In all four search types, a '*' is a wildcard and can be used to specify a residue of any identity. A gap is specified by '-', '~' or '.'.

Search type:

Nucleic Acid: Assumes that the sequences being searched are all nucleic acid sequences. The search is case *in*sensitive, and depends only upon residue identity. DNA and RNA are treated identically and a T is seen as identical tot a U. Gaps are ignored. The following convention is followed for degenerate residue specifications:

R = A or G
Y = C or T/U
K = G or T/U
S = G or C
M = A or C
W = A or T/U
B = G, C or T/U
V = A, G or C
D = A, G or T/U
H = A, C or T/U
N = A, G, C or T/U

Degenerate matching is one-way. An 'R' in the query will match an 'R', 'A' or 'G' in the target, but neither an 'A' nor a 'G' in the query will match an 'R' in the target (an 'R' will always match an 'R', however). For example: the query 'aggryknncc**u' will match all of the following sequences:

aggacgttccttt
aggguuuuccuuu
agggcgccccttt

Amino Acid: Assumes that the sequences being searched are all amino acid sequences. The search is case *in*sensitive, and depends only upon residue identity. Gaps are ignored. The following convention is followed for degenerate residue specifications:

X = any of the twenty standard amino acids
B = D or N
Z = E or Q

Like for nucleic acids, degenerate matching is one-way. A ‘B’ in the query will match a ‘B’, ‘D’ or ‘N’ in the target, but neither a ‘D’ nor a ‘N’ in the query will match an ‘B’ in the target (a ‘B’ will always match an ‘B’, however).

Standard one-letter amino acid codes are as follows:

A = Ala = alanine
C = Cys = cysteine
D = Asp = aspartate
E = Glu = glutamate
F = Phe = phenylalanine
G = Gly = glycine
H = His = histidine
I = Ile = isoleucine
K = Lys = lysine
L = leu = leucine
M = Met = methionine
N = Asn = asparagine
P = Pro = proline
Q = Gln = glutamine
R = Arg = arginine
S = Ser = serine
T = Thr = threonine
V = Val = valine
W = Trp = tryptophan
Y = Tyr = tyrosine

Exact text match:

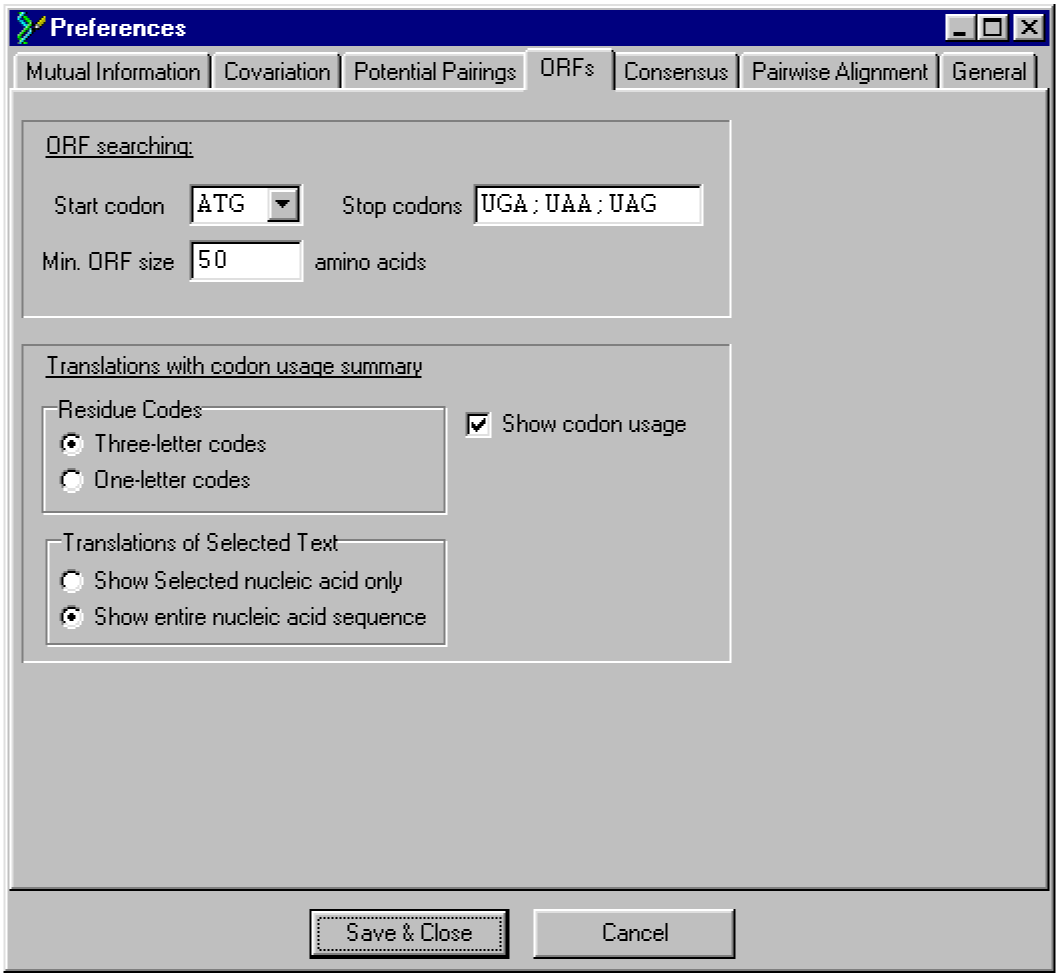
A case *in*sensitive search is performed, however, gaps (‘-’, ‘~’ or ‘.’) are ignored and ‘*’ represents any character. Note that a ‘T’ and a ‘U’ are different, even if the sequence type is nucleic acid, and no degenerate identities are considered.

Exact including gaps:

Like an exact text match, but gaps are not ignored. A gap is still a ‘-’, ‘~’ or ‘.’, however, and the search does not have to exactly specify the gap character present. A ‘*’ is still a wildcard in this search and may be used to specifiy a character of any identity.

Preferences for translation output and ORF searching

To set parameters for ORF searching or the format of translations of nucleic acid sequences via the Sequence->Nucleic Acid->Translate-> ... menu options, choose Options->Preferences->ORFs:



ORF searching:

Start codon ATG

Stop codons UGA ; UAA ; UAG

Min. ORF size 50 amino acids

Translations with codon usage summary

Residue Codes

● Three-letter codes

○ One-letter codes

☑ Show codon usage

Translations of Selected Text

○ Show Selected nucleic acid only

● Show entire nucleic acid sequence

ORF searching: The start codon used for ORF searching will generally be ATG, however, you may wish to search allowing for alternative start codons. To allow more than one start codon at a time, type in the codons separated by a ";". For example, to allow ATG and TTG, type "ATG;TTG" in the start codon box. The same syntax is used for stop codons. If you would like to allow read-through of a codon (for example, UGA), remove It from the list. The preferences will be saved for all subsequent searches.

When searching for ORFs, only sequences with selected titles are searched, and the search begins after the last selected nucleotide. To search, choose Edit->Find Next ORF, or Sequence->Nucleic Acid->Find Next ORF.

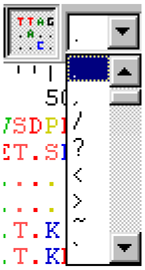
Formatted nucleic acid translations are performed by choosing Sequence->Nucleic Acid->Translate, then either Frame 1, Frame 2, Frame 3, or Selected. If the "Show codon usage" box is checked, a summary table is reported as described in Nucleic acid translation with codon usage.

Conservation plot view

Sometimes it can be convenient to plot an alignment with reference to a standard sequence (usually the top one), where any residues down a column which are identical to the standard at that point are plotted as a specific character (usually a dot). BioEdit offers two basic ways to do this:

1. Choose "Alignment->Plot identities to first sequence with a dot" to create a whole new sequence alignment document that has identities to the first sequence plotted as a dot. In this new document, the sequence data for residues converted to dots is not retained, and the new alignment doc is intended only for generating a picture of an alignment. You may then choose File->Graphic view and uncheck the option for similarity and identity shading to make a figure out of the plot.

2. For a dynamic view of the alignment which plots identities to a standard sequence as a specific character, press the button on the toolbar, or choose the menu item "View->Conservation Plot". When the conservation plot button is down, you have the option to specify the character to be used for plotting identities:



The default is a dot, but theoretically any character can be used. Realistically, though, a period or space (blank) is generally the easiest to see.

You can at any time change the reference sequence by right-clicking the title of the sequence you would like to have as reference. The reference sequence is handled internally by its number in the list, though, so if you move the sequence up or down, you will have to right-click its title again.

Basic Analysis Tools

BioEdit comes with a small (and somewhat uncoordinated) set of analysis functions and tools, which are the focus of the rest of the docuemntation. Analysis features are split into two categories:

1. External, independent programs which are written by other authors and are either distributed with BioEdit or may be obtained from an outside source and can be run from the BioEdit interface. BioEdit offers a somewhat general command line generator that may be configured through a graphical interface to launch external analysis programs and feed sequence data to them to facilitate an easier analysis environment from a single interface.
2. Functions which are built directly into BioEdit.

External Accessories

Installing TreeView:

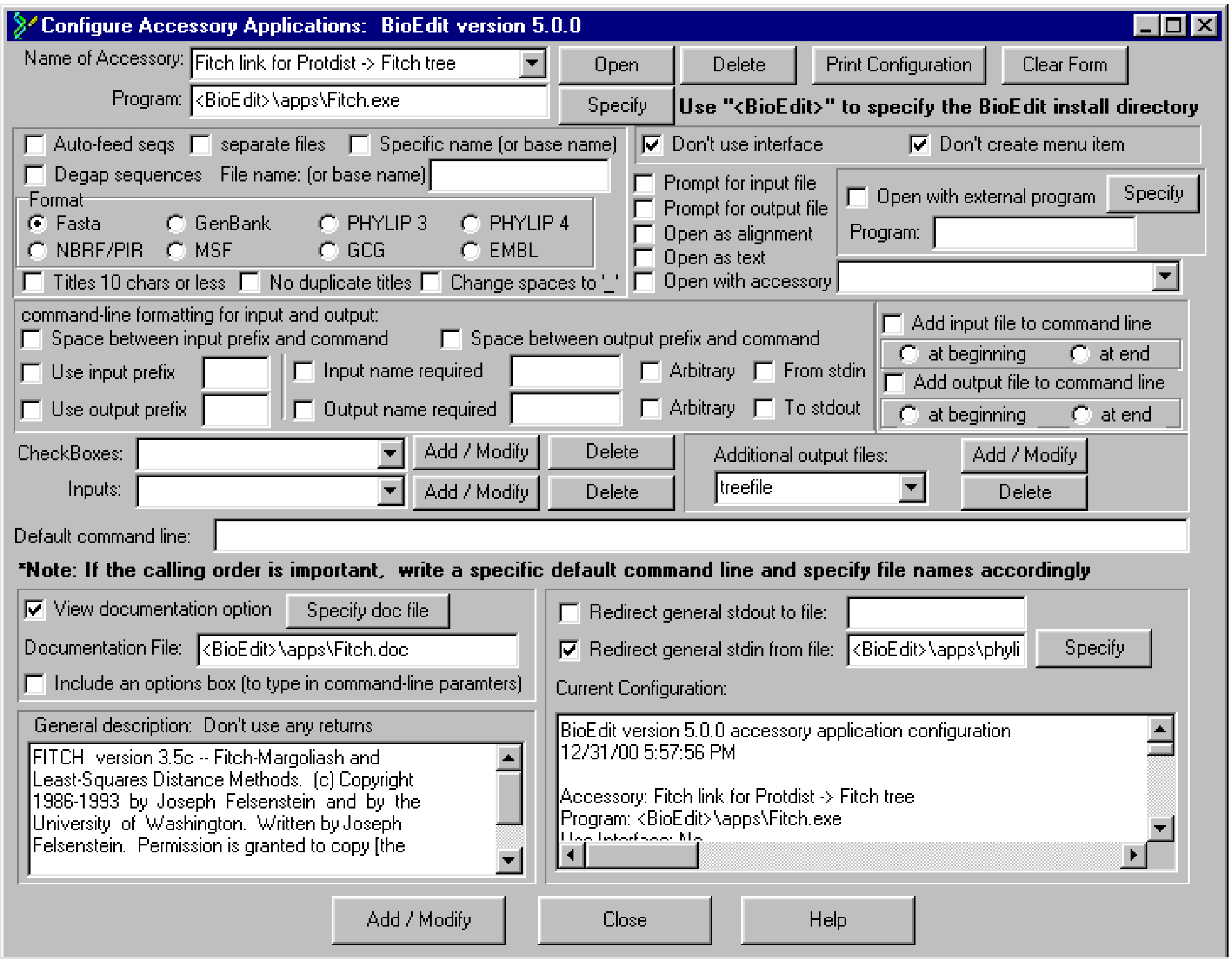
TreeView is a phylogenetic tree viewing program written by Roderic D.M. Page. Previous versions of BioEdit included a distribution of the TreeView executable and supporting libraries in the apps folder. At the request of the author full TreeView installation is now distributed with BioEdit. This installation is contained within the file called TreeView.zip.

To install TreeView, unzip the file to a temporary directory, then run the program called "setup.exe" which will be created. TreeView will install itself on your system.

To configure TreeView to run through the accessory apps menu of BioEdit, choose Add/Remove/Modify an Accessory Application from the Accessory Application menu. In the "Name of Accessory" box, type “TreeView”. Press "Specify" next to the “Program” Box and browse to the new location for the TreeView.exe program. Check the box called “Prompt for input file”. In the “General Description” box, type “TreeView version 1.5.2. Copyright Roderic D.M. Page, 1998. r.page@bio.gla.ac.uk. http://www.taxonomy.zoology.gla.ac.uk/rod/rod.html” without any carriage returns. Then press “Add / Modify” at the bottom of the dialog. Upon closing the window, you will be prompted to have BioEdit close and restart. For more information on installing accessory applications, see Configuring and Using External Applications.

Configuring and Using External Applications

BioEdit provides an interface to add and configure external applications which will be added to the “Accessory Application” menu of alignment documents. Once an application is properly configured, it can be run via a graphical interface created by BioEdit when its menu option is selected. Although any application may be configured to be launched through BioEdit, DOS and Win32 programs which can accept command line parameters to fully perform an analysis are most convenient. BioEdit may be configured to automatically feed sequences to the application, then automatically load the output when the application is finished. Multiple output files may be opened, and the output of one program may be configured to be automatically opened by another program.



Configure Accessory Applications: BioEdit version 5.0.0

Name of Accessory: Fitch link for Protdist -> Fitch tree

Program: <BioEdit>\apps\Fitch.exe

Use "<BioEdit>" to specify the BioEdit install directory

□ Auto-feed seqs □ separate files □ Specific name (or base name)

□ Degap sequences File name: (or base name)

Format

● Fasta ○ GenBank ○ PHYLIP 3 ○ PHYLIP 4

○ NBRF/PIR ○ MSF ○ GCG ○ EMBL

□ Titles 10 chars or less □ No duplicate titles □ Change spaces to '_'

☑ Don't use interface ☑ Don't create menu item

□ Prompt for input file

□ Prompt for output file

□ Open as alignment

□ Open as text

□ Open with accessory

□ Open with external program

Program:

command-line formatting for input and output:

□ Space between input prefix and command □ Space between output prefix and command

□ Use input prefix □ Input name required □ Arbitrary □ From stdin

□ Use output prefix □ Output name required □ Arbitrary □ To stdout

□ Add input file to command line ○ at beginning ○ at end

□ Add output file to command line ○ at beginning ○ at end

CheckBoxes:

Inputs:

Additional output files: treefile

Default command line:

*Note: If the calling order is important, write a specific default command line and specify file names accordingly

☑ View documentation option

Documentation File: <BioEdit>\apps\Fitch.doc

□ Include an options box (to type in command-line paramters)

General description: Don't use any returns

FITCH version 3.5c -- Fitch-Margoliash and Least-Squares Distance Methods. (c) Copyright 1986-1993 by Joseph Felsenstein and by the University of Washington. Written by Joseph Felsenstein. Permission is granted to copy (the

□ Redirect general stdout to file:

☑ Redirect general stdin from file: <BioEdit>\apps\phyli

Current Configuration:

```
BioEdit version 5.0.0 accessory application configuration
12/31/00 5:57:56 PM

Accessory: Fitch link for Protdist -> Fitch tree
Program: <BioEdit>\apps\Fitch.exe
Use Interface: No
```

Adding and configuring a new application

BioEdit v2.0 and later offers a graphical interface for configuring external applications to be run from a BioEdit alignment document. Unfortunately, there is no way to do this without knowing how to run the application independently of BioEdit. A few programs come with the BioEdit installation and are configured already. Permission has been granted by Joe Felsenstein to use PHYLIP programs with BioEdit (as long as no money changes hands). Permission has also been obtained from Roderic D.M. Page to distribute TreeView. TreeView is no longer pre-configured, however. At the request of the author, BioEdit now comes with the TreeView install package, which is extracted into the main installation folder upon installing BioEdit. For more information, see Installing TreeView.

To add a new application to be included in the "Accessory Applications" menu, choose "Add / Modify / Remove an Accessory Application" from the "Accessory Applications" menu.

There are several settings which must be specified for an accessory to be run successfully through BioEdit. Many settings will not be required for many applications and each configuration will be different, as programs are written differently by different people. One must know how to run the program via a command line in order to configure BioEdit to run the program. Refer to the documentation of your accessory application to learn how to run it before trying to configure it as an accessory. The following options are present in the configuration interface. Only the first two are universally required for all applications.

- Name of Accessory: This is the name that will appear in the "Accessory Applications" menu. This can be any name you want. It is recommended to keep it relatively short, however, as it will be a menu option in all alignment documents.
- Program: The absolute or relative path to the program, including the program name (usually an .exe file, but could be a .com or a .bat file). To specify the path relative to the BioEdit installation directory, specify the main installation directory as "<BioEdit>" (not case sensitive). For example, an application named "MyApp.exe" might be placed in the "apps" directory. To allow the whole BioEdit directory to be moved without causing a problem with finding the application, specify the path as "<BioEdit>\apps\MyApp.exe". (Note: do not include the quote marks). Alternatively, if the absolute path will be specified, you may browse the disk to find the application by pressing "Specify".
- Automatically feed sequences to App: If the program analyzes sequence or alignment data (such as ClustalW or certain PHYLIP programs), you may choose to have the sequences automatically fed to the application. This is one of the most useful benefits of running the program directly from an alignment editor. An application that takes only one or several sequences may be done this way also, as BioEdit will only feed *selected* sequences at runtime (if no sequences are selected, they will all be selected automatically).
- Specific File name required (for auto-fed sequence data): Some applications expect a specific input file name. For example, the PHYLIP programs all expect to process a file called "infile". If this is the case, check this box and enter the expected name in the "File name" input box. Don't include a path, since the file will be automatically saved to the directory containing the application.
- Degap sequences: Some applications require alignment data (such as DNAml, Protdist, DNAdist, etc.) and gaps will be included in the input. Other programs (e.g. search programs

such as BLAST) may take simple sequence data rather than alignment data. In these cases, gaps must be removed or they will be viewed as residues.

- Format (for auto-fed sequences): Eight file formats are available for auto-feeding alignment / sequence data to programs: (if you have an application that requires a different format, you may have to configure the application to take a certain file name, choose not to auto-feed the sequences, and convert the file to the correct format before running the program -- or simply run the program separately. If it would very convenient to have it run through BioEdit, simply email me at tahall2@unity.ncsu.edu with file format name and specifications and it would be a minor thing to write an export filter and add it to the accessory applications method of BioEdit. If you need this, feel free to email me and I will mail back with an address where the new copy of the program may be picked up and when (or if I can't for some reason, I will mail back and tell you that). Currently available formats are:
    - Fasta
    - GenBank
    - PHYLIP 2 / 3.2 (PHYLIP 3)
    - PHYLIP 4
    - NBRF/PIR
    - MSF (via ReadSeq.exe: Don Gilbert's sequence conversion filter).
    - GCG (via ReadSeq.exe)
    - EMBL (via ReadSeq)
- Prompt for input file: you may want to be prompted at runtime for an input file. BioEdit will produce an open file dialog. The file name will be fed to the external program.
- You may want BioEdit to prompt you at runtime to specify an output file name to feed to the program.
- Open as alignment: The main output of the program may be opened as a new alignment document. (By default, there is expected to be one main output file, however, this is often not the case, and additional output files may be specified in the box titled "Additional output files:" -- this entry could be left blank and a single output could be specified as an additional output. Functionally, this would make no difference).
- Open as text. If the output is a text data file, it may be opened as text in the BioEdit rich text editor.
- Open with external program: Perhaps the program exports tabular or matrix data that you would like to view in a spreadsheet program such as Microsoft Excel. You may specify any external program to be launched and open the output automatically. You may browse the disk by pressing "Specify". You may also specify a path relative to the BioEdit installation directory with "<BioEdit>".

    Note: An output file may be opened as a new alignment, as text and by an external program all at the same time if you want.
- Use input prefix: Some programs expect a specific prefix at the command line to specify the input file, output file, the input of specific parameters, or all of these things. Other programs may expect the input, output and parameters simply typed in a specific order. If you program requires a prefix to specify the input file, check this box and enter the prefix exactly in the associated edit box. Note: ***If the prefix and the file name will be separated by a space, type that space after the prefix when configuring the application***. For example, one application may expect to see "-i inputFile" while another may expect "input=inputFile". Depending on how the applications are written, the first one may not work if the space is not included, and the second one may not work if a space *is* included.

- Use output prefix: Same as input prefix.
- Input name required: Some applications require that an input file name be specified in the command line (e.g. ClustalW). Others may expect a specific file name which therefore is not specified at the command line (e.g. PHYLIP programs). If the application expects to be given the name of the input file at the command line, check this option.
- Output name required: Same as “Input name required”
- Input or Output name arbitrary (check boxes labeled “Arbitrary”): Sometimes a file name is required at the command line, but can be any file name, as long as it is specified correctly. If this is the case, you may simply check the “Arbitrary” option(s) and BioEdit will assign input and/or output files arbitrary names. For example, ClustalW is configured to automatically feed sequences to the application, then automatically open the output as an alignment. The “Input name required” option is checked, and so is the “Arbitrary” option. When ClustalW is run through this interface, input and output files are given the names “~inTemp.tmp” and “~outTemp.tmp”, respectively.
- From stdin and To stdout: Some programs (such as FastDNAml”) expect to get input from stdin and/or send output to stdout. Stdin and stdout are the standard system input and output streams and the defaults are from the keyboard and to the screen, respectively. To redirect the input or output from stdin or stdout to a file, which is necessary to run the program automatically, choose the appropriate checkbox(es). Most programs that expect data from stdin expect it to be redirected from a file anyway. Stdout must be redirected to a file in order to be able to save the data and/or open it up in BioEdit or another program.
- Checkboxes: The Interface created by BioEdit to run external applications may include yes/no choices in the form of checkboxes so that certain program options may be set easily at runtime. These checkboxes will be drawn on the interface created by BioEdit to run the program. Theoretically, up to 50 checkbox options may be included per application. This limit was set because I had a hard time imagining an application that would allow anywhere close to that many options and not already have its own graphical interface. Most programs will probably have none, and those that do will usually have fewer than 5. To add a checkbox, type the Caption you want to appear on the interface in the Checkboxes” drop-down list box, then press the “Add / Modify” button. A dialog will appear in which you may specify the default state of the check box (checked or unchecked), and also the command line action to specify for each choice. If no command line parameters will be added for a particular choice, leave it blank. Press OK for the changes to be entered. To modify an existing checkbox, choose it in the drop-down list and press “Add / Modify”. To delete a checkbox, choose it from the list and press “Delete”.
- Inputs: Some programs may allow or expect specific data to be entered which affects program execution (for example, CAP assembly asks the user for the minimum base overlap and the minimum percent match). For this purpose, input boxes may be included on the accessory application interface. Add, modify or delete an input the same way you would a checkbox. Each input may also have associated with it a checkbox, which allows the option to choose whether or not to use the input at runtime. In the configuration dialog, you may specify a command prefix for the parameter in the command line (may or may not be required -- if not, leave it blank), and a default value that will appear as the input text in the interface. If you want an associated checkbox, you must check this option and enter the caption for the checkbox, as well as the default state of the checkbox. Up to 50 inputs are theoretically allowed, all with the option for associated checkboxes.

- Additional output files: Up to 10 different output files may be specified and specifically dealt with, in addition to the “main” default output file. *** Note: It is assumed here that the additional outputs are automatically generated by the program and the file names are not specified at the command line ***. If this is not the case, this is not really a problem. Simply either add the appropriate parameters to the default command line or include an edit control on the program interface to enter them at runtime (see below). Add, modify or delete additional output configurations the same as checkboxes and inputs. Each additional output configuration expects a specific file name. In most cases, do not enter a path (just the file name), as most programs will simply save the output in the directory they are in. If a specific path is required, it may be included. Some programs (such as ClustalW) may produce an output file which is given the same name as the input file, only with a different extension. In this case, specify the filename as “<infile>.ext” (for example, if the input file name is “temp.tmp” and the output is specified as “<infile>.out”, then the file should be named “temp.out”). The output may be opened as an alignment, as text, by an external application, or any combination of these three options. If the output is not opened by an external application, the temporary file which contains it will automatically be deleted (you must save any information you wish to keep).
- Default command line: Certain parameters may be desired for all runs of the program. In this case, specify these in the default command-line box. For example, ClustalW allows output in GCG, GDE, PHYLIP or PIR (NBRF) formats. Since BioEdit read NBRF/PIR files internally, “/output=PIR” may be specified as the default command line to provide an output that BioEdit quickly recognizes as an alignment file.
- Add input file to command line: If this box is checked, BioEdit will automatically construct a command line that includes the input file and command prefix (if there is one), depending on the configuration for the input file. It may be necessary to leave this box unchecked and write the input file specs right into a default command line if the absolute position in the command line is important and it is not at the beginning or end. Otherwise, select either the “at beginning” or “at end” option to specifiy where to place the input file name in the command line.
- Add output file to command line: same as for input file
- View documentation option: If documentation comes with the program, or you are configuring the program for other people who are less familiar with it, you may want to include an automatic link to the documentation. This will work if the documentation is in a single text or rich text file. If this option I chosen, you may specify the doc file by pressing “Specify” or by entering the path in the “Documentation file:” box. The designation “<BioEdit>” may be used to specify a path relative to the BioEdit installation directory. If this option is chosen, a button will appear on the interface with the label “View Documentation”.
- Include an options box (to type in command-line parameters). If you would like an input box to appear on the interface which allows you to enter additional command-line parameters at runtime, check this option. If you have an application which requires a very unique arrangement of command line options, but is still convenient to run through BioEdit, you may create an interface that has only this input box and simply enter the command-line at runtime.
- Redirect stdout. Some programs may print data or progress information to the screen when running. If you would rather have this information saved to a file and opened by BioEdit for

viewing later, choose redirect stdout, specify a file name, and configure that file to be opened as an additional output file (see above).

- Redirect stdin: Some programs, such as programs in the PHYLIP package, provide a menu when launched that allows settings to be specified. If a specific set of settings will be used all of the time, a file may be created with the exact series of keystrokes that would specify these settings from the menu(s), then stdin may be redirected to this file rather than the keyboard. So far, this does not seem to work when programs are launched from within BioEdit. There does not seem to be any good reason that it should not work however, and this option has not been completely removed because I plan to fix it in the future. For now, I'm not sure if the option will work in some cases and not in others, but it is probably best to not use this option until it is figured out.
- General description: In this box, type a description of the program that will be printed on the interface at runtime. The description may be as long as you want, but it must be entered as a single line of text. If any carriage returns are entered, the description will be truncated at the first return character. This is because of the way the configuration data is stored. This description will often contain a short description of the program and a reference to the author(s).
- "Add / Modify": Pressing this button will save the entered information and list the current configuration in the "Current configuration" box.

Pressing Close will close the dialog without updating the information.
To print the current configuration, press the "Print Configuration" button.

Modifying an existing configuration

To modify an existing application configuration, first bring up the configuration dialog by choosing the “Add / Remove / Modify an Accessory Application” from the “Accessory Application” menu. Press the arrow on the “Name of Accessory” drop-down box to drop down a list of currently configured applications. Choose the application you would like to modify and press “Open”. Reconfigure the application the way you want.

You may modify the information associated with checkboxes and inputs at any time by choosing the title of the checkbox or input you would like to modify from the drop down lists associated with them, then pressing the appropriate “Add / Modify” button. You may delete any checkbox or input by highlighting its name (or typing it) in the appropriate drop-down box and pressing the associated “Delete” button. Likewise, additional output file handling may be configured in the same manner.

Removing an accessory application

To remove an accessory application from the configuration, simply bring the accessory applications dialog up, then choose the name of the application you would like to remove from the drop down list labeled “Name of Accessory”. Press the “Delete” button to remove the accessory.

Storage of Accessory Application Configuration Information

BioEdit accessory application information is stored in a file called “accApp.ini” which is found in the “apps” folder of the BioEdit installation. This file is organized in the same manner as the BioEdit.ini file found in your Windows directory. The configuration information for the ClustalW Sample configuration is shown below. As you can probably see, the information is a little cryptic as is, though it can be deciphered with a minimal effort. It is much easier to configure applications using the graphical interface than directly editing this configuration file, and a more meaningful summary of each configuration showing the same information as below is displayed on the interface and may be printed from the dialog as well. This file can be directly edited, however, following the general format laid out below. Checkboxes are designated as c<checkbox #>, starting at 0, and inputs are i<input #>, starting at 0. All of the categories of data are written as shown below. Parameters which are not used or have no value are written into the configuration as blanks. Values which may be either true or false are written as 1 or 0, respectively.

```
[ClustalW multiple alignment program]
Program=<BioEdit>\apps\clustalw.exe
Auto-Feed=1
Degap Sequences=0
Auto-Feed File Format=1
Auto-Feed File Name Required=0
Specific File Name=
Prompt for Input File=0
Prompt for Output File=0
Open Output as Alignment=1
Open Output as Text=0
Open Output with External Program=0
External Program Name=
Input File Prefix=/INFILE=
Output File Prefix=/OUTFILE=
Specify Input File Name=1
Specify Output File Name=1
Input File Name=
Output File Name=
Input File Prefix Required=1
Output File Prefix Required=1
Input File Name Arbitrary=1
Output File Name Arbitrary=1
Redirect input from stdin=0
Redirect output from stdout=0
Default Command Line=/output=PIR
View Documentation Option=1
Documentation File=<BioEdit>\apps\clustalw.txt
Description=ClustalW:  Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994).  CLUSTAL W:
improving the sensitivity of progressive multiple sequence alignment through sequence
```

```
weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research,
submitted, June 1994.
Include Additional Options Box=1
Redirect General stdout=1
Stdout Redirected Filename=clustal.sto
Redirect General stdin=0
Stdin Redirected Filename=
c0 Title=Full Multiple Alignment
c0YES=/ALIGN
c0NO=
c0 Default=1
c1 Title=Calculate NJ Tree
c1YES=/TREE
c1NO=
c1 Default=0
c2 Title=FAST Algorithm for Guide Tree
c2YES=/QUICKTREE
c2NO=
c2 Default=0
i0 Title=Number of Bootstraps
i0 Prefix=/BOOTSTRAP=
i0 Default=1000
i0 CheckBox=1
i0 CheckBox Title=Bootstrap NJ Tree
i0 CheckBox Default=1
Additional Output 0 Name=<infile>.dnd
Additional Output 0 Open as Text=0
Additional Output 0 Open as Alignment=0
Additional Output 0 Open with External Program=1
Additional Output 0 External Program Name=<BioEdit>\apps\treev32.exe
Additional Output 1 Name=clustal.sto
Additional Output 1 Open as Text=1
Additional Output 1 Open as Alignment=0
Additional Output 1 Open with External Program=0
Additional Output 1 External Program Name=
```

Accessory Application Example: Configuring ClustalW to run through a custom BioEdit interface

Below is a step-by-step example of configuring an external application to run from and work with BioEdit. This example configures ClustalW, for which an interface was already directly programmed into BioEdit before addition of the configuration interface. You will see that, after configuring ClustalW correctly, calling the new menu option will bring up an interface that looks slightly different than the one included in BioEdit, but is functionally identical. Also, if you run ClustalW from the new interface, compared to the old one, it will run in a "thread" separate from the main BioEdit application, which means that you can continue to work on other stuff while the accessory application runs simultaneously in the background.

*** Step 0: Before step 1 of any application configuration, you must know what command line options are required and what their specific designations are. If you are dealing with a new program you have not used before, you probably need to read the documentation.

To configure ClustalW:

- Bring up the configuration dialog by choosing "Add / Remove / Modify an Accessory Application" from the "Accessory Application" menu.

- Type "ClustalW Example Application" in the input labeled "Name of Accessory".

- To specify the program executable, press the "Specify" button and choose "clustalw.exe" (the directory browser should start you off in the "apps" directory which contains clustalw.exe. Replace the path up through "BioEdit" with "<BioEdit> to specify a relative path. For example, if, after clicking on clustalw.exe, the Program box contains the text "C:\BioEdit\apps\clustalw.exe", change it to "<BioEdit>\apps\clustalw.exe".

- check the "Automatically feed sequences to App" box.

- Check "Fasta" as the output format.

- If you want the sequences degapped before running ClustalW, check the "Degap Sequences" box. Otherwise leave this box unchecked (it shouldn't really matter in this case)

- Check the box titled "Open output as new alignment" to have BioEdit automatically open the new alignment as a new document when ClustalW is finished running. Leave other boxes in this area unchecked and ignore the "open with external program" option.

- Check both "Use input prefix" and "Use output prefix". These options tell BioEdit that the command line must use specific prefixes to indicate which parameter specifies the input file name and which specifies the output file name to ClustalW.

- For the "Input file command prefix", type "/INFILE="

- For the “Output file command prefix”, type “/OUTFILE=“

- Check the boxes labeled “Input name required” and “Output name required”. These tell BioEdit that ClustalW that the names of the input and output files are needed (they are not some set file name that the program always looks for).

- For both, check the “Arbitrary” box to indicate that any arbitrary file name may be used for the input and output names, as long as ClustalW is told those names.

- Leave the boxes called “Space between input prefix and command” and Space between output prefix and command” unchecked.

- Make sure that the box for “Add input file to command line” is checked and check the “at beginning” box.

- Check the “Add output file to command line” and again check the “at beginning” box.

- Next, we will add the same checkbox options as seen on the internal ClustalW interface:

- In the “CheckBoxes input, type “Full Multiple Alignment” and press “Add / Modify”. The same name will appear as the “Title” parameter in the dialog that pops up. For the “Command if checked” parameter, type “/ALIGN”. Leave the “Command if not checked” input blank. Check the “Default checked” box.

- In the “CheckBoxes input, type “Calculate NJ Tree” and press “Add / Modify”. For the “Command if checked” parameter, type “/TREE”. Leave the “Command if not checked” input blank. Do not check the “Default checked” box.

- Create a checkbox called “FAST Algorithm for Guide Tree”. For the “Command if checked” parameter, type “/QUICKTREE”. Leave the “Command if not checked” input blank. Do not check the “Default checked” box.

- Create an input called “Number of Bootstraps”. For the “Command prefix” parameter, type “/BOOTSTRAP=”. In the “Default value input, type 1000. Check the box labeled “Associate a checkbox”. Name the checkbox “Bootstrap NJ Tree” and choose the default state as checked. This will allow you to choose whether or not to bootstrap, and, if done, the number of bootstraps.

- In the “Default command line” box, type “ /output=PIR”. This specifies that the program save the output as an NBRF/PIR file.

- Check the “View documentation option” box, then press the “Specify doc file” button. Choose “clustalw.txt” as the documentation file. Modify the beginning of the path to the doc file as <BioEdit>.

- Check the box labeled “Include an options box (to type in command-line parameters)”.

- In the “General description” box, type “ClustalW: Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research, submitted, June 1994.”, without using any return characters (type as a single continuous line of text).

- If TreeView has already been installed, in the “Additional output files” box type “<infile>.dnd” and press the “Add / Modify” button next to this box. In the dialog that appears check “Open with external program”, then Choose “specify” and browse to the file treev32.exe (the executable for TreeView -- this will likely be in C:\Program Files\Rod Page\Tree View if you chose the defaults when installing). This specifies that a file will be created by ClustalW with the same base file name as the input, but with an extension of .dnd. This file is a phylogenetic tree, and may be opened by treev32.exe, which is the executable for TreeView version 1.5.2, Copyright Roderic D.M. Page, 1998. This will cause this output file to be opened automatically with TreeView.

- In the “Additional output files” box type “clustal.sto” and press the “Add / Modify” button next to this box. In the dialog that appears check “Open as new text document”. Then press OK.

- Check the “Redirect general stdout to file” and enter “clustal.sto” in the input box. This will cause the general screen output to be directed to a file called “clustal.sto” which will be brought up as a text document in BioEdit after ClustalW is finished executing.

- Press the “Add / Modify” button at the bottom of the dialog. A configuration summary should come up in the “Current Configuration” box which looks something like this:

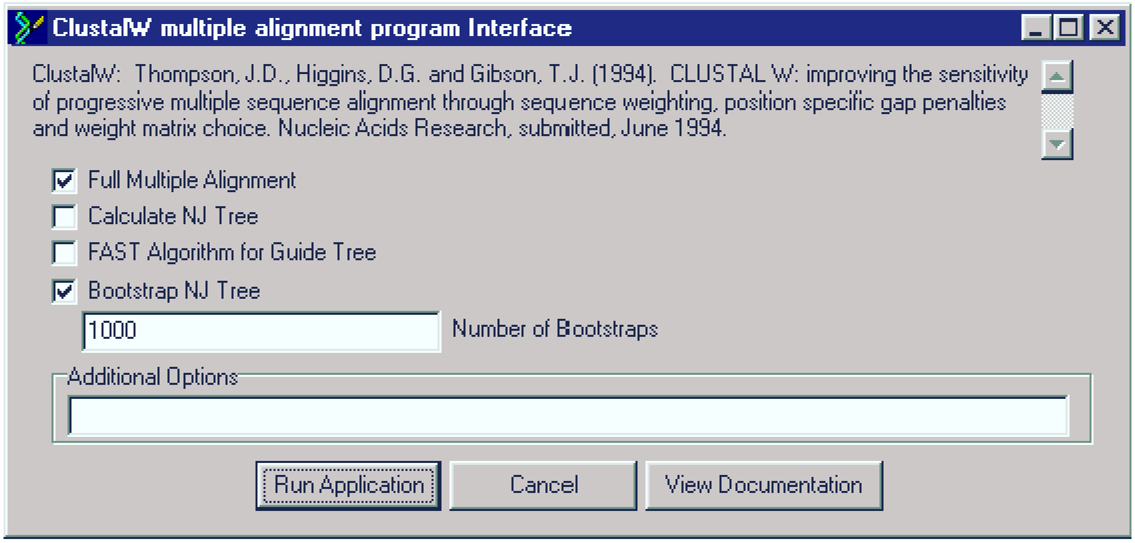
```
BioEdit version 4.7.1 accessory application configuration
7/31/99 10:40:51 PM

Accessory: ClustalW example application
Program: <BioEdit>\APPS\Clustalw.exe
Auto-Feed Sequences: Yes
Auto-Feed File Format: Fasta
Degap sequences: No
Prompt for name of input file: No
Open output as new alignment: Yes
Open output as text document: No
Open output with external program: No
Prompt for name of output file: No
Prefix required for input file: Yes
Space after input prefix: No
Prefix for input file: /INFILE=
Use arbitrary default input file name: Yes
Prefix required for output file: Yes
Space after output prefix: No
Prefix for output file: /OUTFILE=
Specify input file name: Yes
Use arbitrary default output file name: Yes
Input file name:
Specify output file name: Yes
Output file name:
Add input file to command line: Yes
   Add input file at beginning of command line.
Add output file to command line: Yes
   Add output file at beginning of command line.
Redirect input from stdin: No
Redirect output from stdout: No
```

```
Additional output file 1:
  File Name: <infile>.dnd
  Open as new text document: No
  Open as new alignment document: No
  Open with external program: Yes
  External program name: C:\Program Files\Rod Page\TreeView\treev32.exe
Additional output file 2:
  File Name: clustal.sto
  Open as new text document: Yes
  Open as new alignment document: No
  Open with external program: No
Default command line: /output=PIR
Redirect general stdout: Yes
Redirect general stdout to file: clustal.sto
Redirect general stdin: No
Add option to view documentation: Yes
Documentation file: C:\BioEdit\APPS\Clustalw.txt
Include a box for additional command-line options: Yes
Description ClustalW:  Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994).  CLUSTAL W: improving the sensitivity of
progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice.
Nucleic Acids Research, submitted, June 1994.
CheckBox 1:
  Title: Full Multiple Alignment
  Command when checked: /ALIGN
  Command when not checked:
  Default state: Yes
CheckBox 2:
  Title: Calculate NJ Tree
  Command when checked: /TREE
  Command when not checked:
  Default state: No
CheckBox 3:
  Title: FAST Algorithm for Guide Tree
  Command when checked: /QUICKTREE
  Command when not checked:
  Default state: No
Input 1:
  Title: Number of Bootstraps
  Command Prefix: /BOOTSTRAP=
  Default value: 1000
  Include associated CheckBox: Yes
     Associated CheckBox name: Bootstrap NJ Tree
     Associated CheckBox default state: Yes
```

- Press “Close” to close the dialog. You will get a message asking if you want to restart BioEdit for the new changes to take effect. Press “Yes” and wait for BioEdit to close down and restart. Now the menu option “ClustalW Example Application” should appear in the “Accessory Applications” menu.

- Open a file containing some homologous sequences to be aligned. Choose “ClustalW Example Application” from the “Accessory Applications” menu. You should see the following interface, which is functionally identical to the one incorporated directly into BioEdit.



ClustalW multiple alignment program Interface

ClustalW: Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research, submitted, June 1994.

☑ Full Multiple Alignment

□ Calculate NJ Tree

□ FAST Algorithm for Guide Tree

☑ Bootstrap NJ Tree

1000 Number of Bootstraps

Additional Options

BLAST

A BLAST (Basic Local Alignment Search Tool) search is often the most convenient method for detecting homology of a biological sequence to existing characterized sequences. BLAST looks for homology by searching for locally aligned regions of identity and/or similarity between a query sequence and sequences in a database. The algorithm works by the following general method:

1. A query sequence is divided into short sequences (called words, ca. 3 to 8 residues, depending on whether it's protein or nucleic acid).
2. A table of all sequences of the same word size which can pair with each of the words from the query sequence with a score above a defined threshold is constructed (the lookup table).
3. The database sequences are scanned for occurrence of sequences in the lookup table.
4. When a word is found in the database which can align to a word in the query over the critical threshold, the alignment is extended in both directions. This extension continues in both directions as long as the length of the extension does not exceed a defined limit without further increasing the score of the alignment.
5. If, when an extension is terminated, the total sub-alignment score is above another defined threshold, the alignment is reported.
6. When a threshold alignment is produced, that particular database sequence is re-scanned for other non-redundant high-scoring segment pairs (HSPs) which score above yet another defined cut-off (the sum of several non-significant sub-alignments within the same two sequences can, when taken together, indicate significant similarity indicative of homology).
7. A statistical measure is reported which indicates the probability that a similar-scoring HSP or set of HSPs found for a given query would result from searching the same database with a randomly-generated sequence of the same length as the query.

For a reference on BLAST, see:

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-10.

and, for the newer gapped BLAST and PSI-BLAST versions,

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

for a description of the newer PHI-BLAST method, see:

Zhang, Zheng, Alejandro A. Schäffer, Webb Miller, Thomas L. Madden, David J. Lipman, Eugene V. Koonin and Stephen F. Altschul (1998), "Protein sequence similarity searches using patterns as seeds", Nucleic Acids Res. 26:3986-3990.

BLAST Programs

BLAST offers the following programs:

blastn: Search a nucleotide database with a nucleotide query

blastp: Search protein database with a protein query

tblastn: Search a six-frame dynamic translation of a nucleotide database with a protein query

blastx: Search a protein database with a six-frame translation of a nucleotide query sequence.

tblastx: Search a six-frame translation of a nucleotide database with a six-frame translation of a nucleotide query sequence (very slow).

Local BLAST

The NCBI BLAST version 2.0.3 ([Nov-14-1997], Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.) is included in the BioEdit distribution and is found in the \apps folder of the install directory. This is particular useful if you are interested in fishing out a specific gene from a partially sequenced genome whose sequences have not yet been assigned and deposited.

To use this program, you must first create a local database. You can create as many local databases as you want, just keep in mind the following requirements:
1. Nucleotide and protein databases are separate entities.
2. Databases must be present within the \database directory of the BioEdit folder to be recognized by the BioEdit local BLAST interface (the NCBI blastall.exe program, however, is a stand-alone app that can be used entirely separately from BioEdit). When a database is created using BioEdit, it is automatically placed into the \database directory.

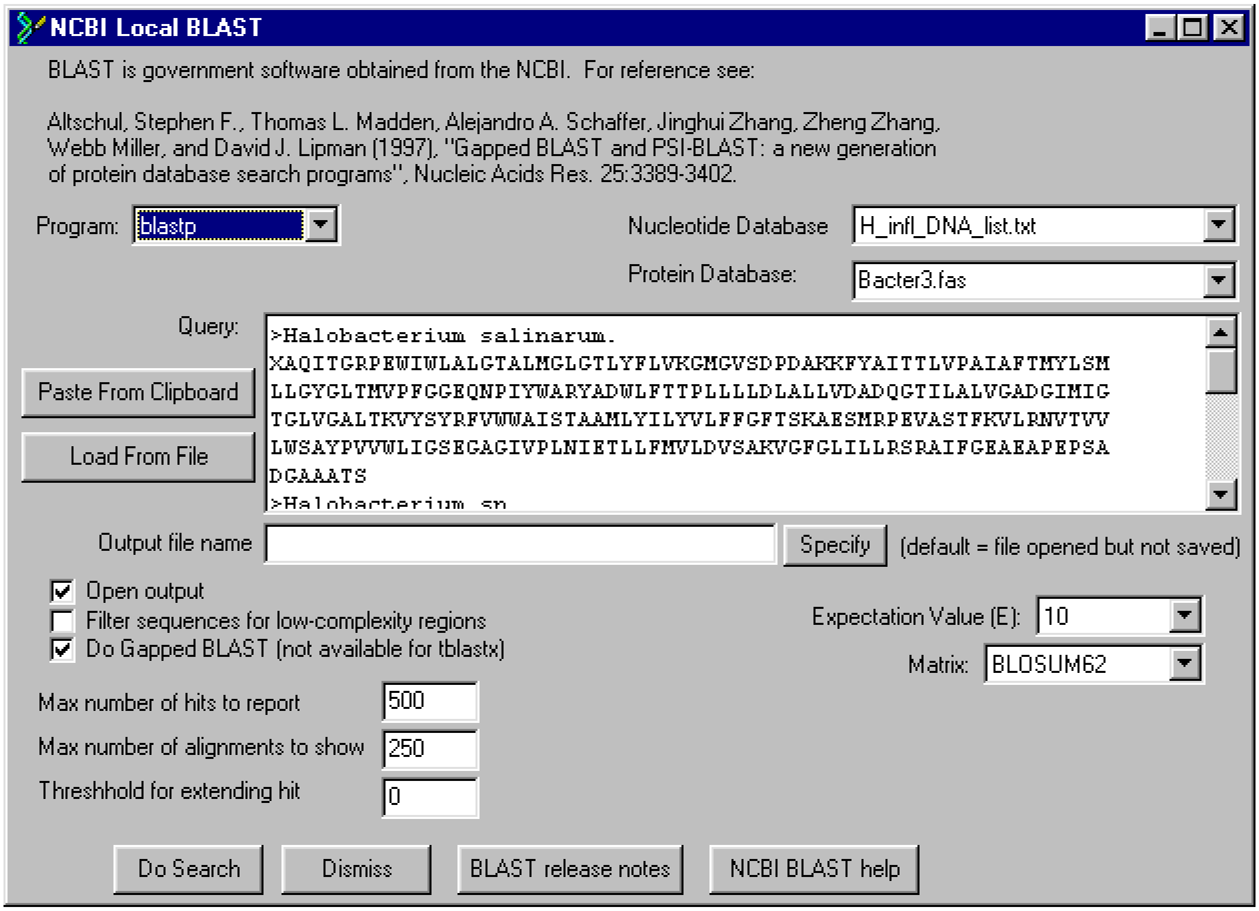
Creating a local database

To create a local protein or nucleotide database for BLAST searching, you need only have a Fasta-format file containing all of the sequences you want in the database. Nucleotide and protein sequences *cannot be mixed within the same database*. From the “Accessory Application” menu, choose “BLAST”, then “Create a local ... database file”. You will be prompted for the input Fasta file. The rest is automatic. The database will be placed in to the \database folder of the BioEdit install directory. The new database should appear in the appropriate database list box of the local BLAST interface form.

Note: If you create a database or copy one into the \database folder, and it does not appear in the choices on the BLAST search form, try quitting BioEdit and restarting. If this does not work, you may have to rename the *.pin (protein) or *.nin (nucleotide) file. You can give it the same name. I am not sure exactly why this (rarely) happens, but renaming the file to the same name seems to solve the problem.

Local BLAST Searching

To use local BLAST from within BioEdit, highlight the title of the query sequence from within a BioEdit document. Next, choose “Local Blast” from the “Blast” menu under the “Accessory apps” menu. Don’t worry about gaps, these will be removed automatically. You may also choose several sequences at once if you want to for a batch job. Choose the program you would like to use, then the database to search. In the upper right of the form, there will be a drop-down list for both nucleotide and protein databases. Choose the one you want for the appropriate type, and don’t worry about the other type (a selected choice of nucleotide database will be ignored when doing a protein search). You may choose whether to save the output to a user-named file, or simply have BioEdit create a temp file which is automatically opened when the search is done.



BLAST is government software obtained from the NCBI. For reference see:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Program: blastp

Nucleotide Database: H_infl_DNA_list.txt

Protein Database: Bacter3.fas

Query:

```
>Halobacterium salinarum.
XAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITTLVPAIAFTMYLSM
LLGYGLTMVPFGGEQNPIYWARYADWLFTTPLLLLDLALLVDADQGTILALVGADGIMIG
TGLVGALTKVYSYRFVWWAISTAAMLYILYVLFFGFTSKAESMRPEVASTFKVLRNVTVV
LWSAYPVVWLIGSEGAGIVPLNIETLLFMVLDVSAKVGFGLILLRSRAIFGEAEAPEPSA
DGAAATS
>Halobacterium sp
```

Output file name

(default = file opened but not saved)

☑ Open output
☐ Filter sequences for low-complexity regions
☑ Do Gapped BLAST (not available for tblastx)

Expectation Value (E): 10

Matrix: BLOSUM62

Max number of hits to report 500

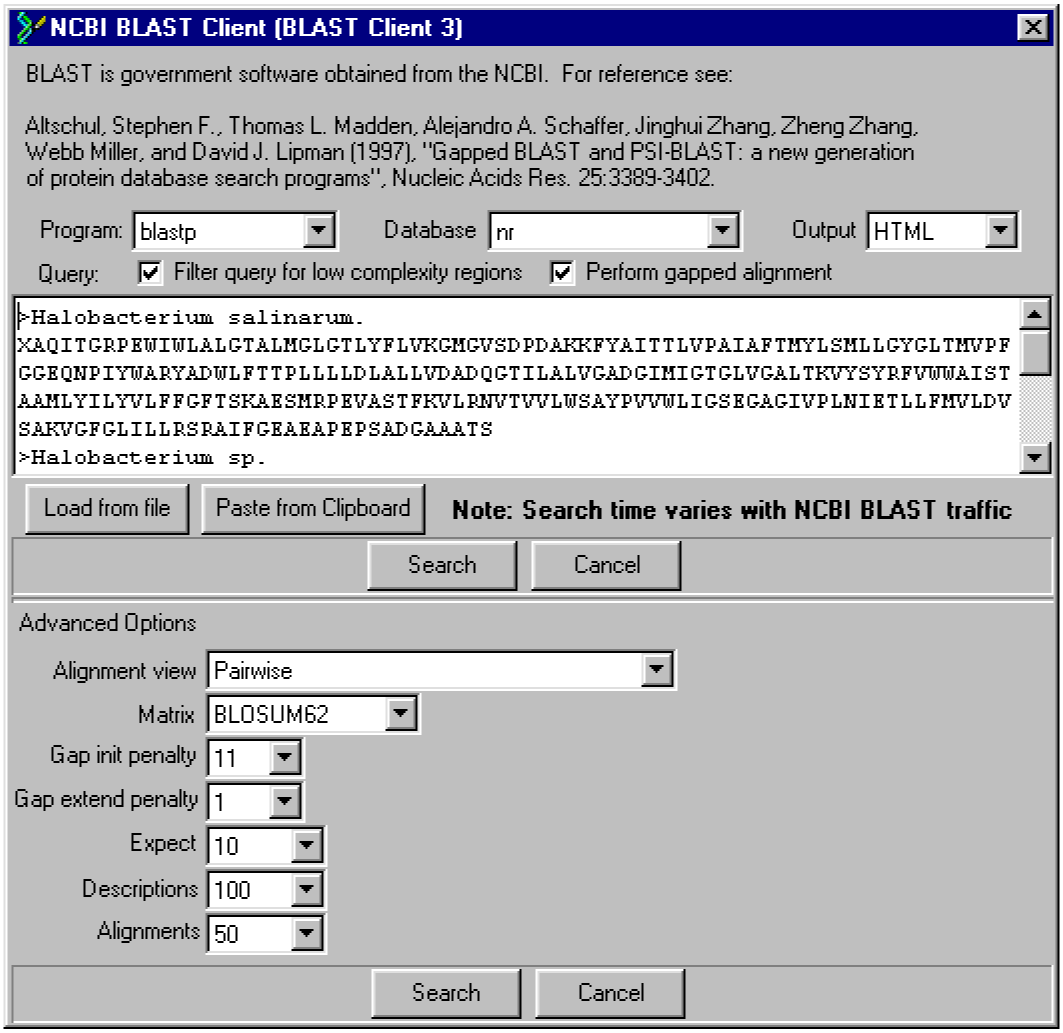
Max number of alignments to show 250

Threshhold for extending hit 0

BLAST Internet Client

Originally, the BioEdit installation packaged the NCBI BLAST client 2.0 program blatstcli.exe, which I had modified to accept an input sequence file at the command line. The NCBI BLAST 2.0 client has since been discontinued.

BioEdit now includes the NCBI's BLAST client 3 (blastcl3.exe in the "/apps" folder). If you select a sequence, or multiple sequences, from the alignment window, and choose "Accessory Applications->BLAST->NCBI BLAST over the Internet", the following interface will come up:



NCBI BLAST Client (BLAST Client 3)

BLAST is government software obtained from the NCBI. For reference see:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Program: blastp Database nr Output HTML

Query: ☑ Filter query for low complexity regions ☑ Perform gapped alignment

```
>Halobacterium salinarum.
XAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITTLVPAIAFTMYLSMLLGYGLTMVPF
GGEQNPIYWARYADWLFTTPLLLLDLALLVDADQGTILALVGADGIMIGTGLVGALTKVYSYRFVWWAIST
AAMLYILYVLFFGFTSKAESMRPEVASTFKVLRNVTVVLWSAYPVVWLIGSEGAGIVPLNIETLLFMVLDV
SAKVGFGLILLRSRAIFGEAEAPEPSADGAAATS
>Halobacterium sp.
```

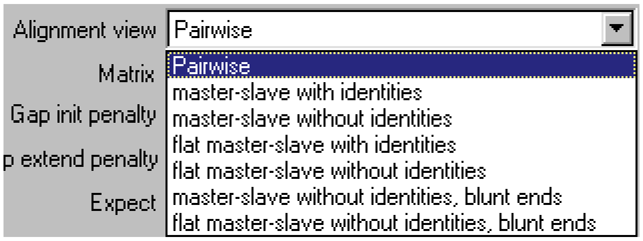
Note: Search time varies with NCBI BLAST traffic

Advanced Options

Alignment view: Pairwise
Matrix: BLOSUM62
Gap init penalty: 11
Gap extend penalty: 1
Expect: 10
Descriptions: 100
Alignments: 50

You may BLAST one or more sequences at a time. Also, you may have the output come back as HTML if you want, otherwise you may have plain text output produced. If HTML output is selected, the output will automatically be opened in the your WWW browser. Otherwise, the text output will be opened in BioEdit.

You may choose any of the standard BLAST formatting options (Pairwise is always the default):


Matrix
Gap init penalty
p extend penalty
Expect
Pairwise
master-slave with identities
master-slave without identities
flat master-slave with identities
flat master-slave without identities
master-slave without identities, blunt ends
flat master-slave without identities, blunt ends

Alignment view Pairwise

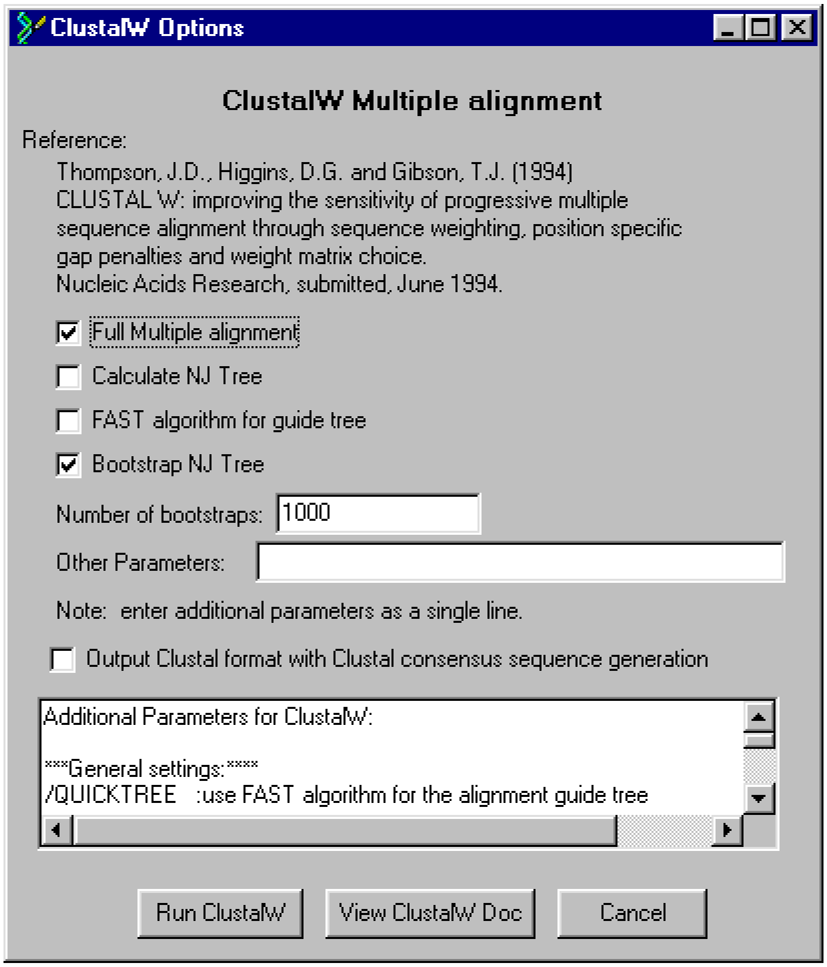
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

ClustalW

ClustalW is a program by Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) designed to construct multiple alignments of biological sequences. Clustal will automatically align many sequences with a profile-based progressive alignment procedure. This program is utilized unaltered by BioEdit and basic on-line help for this program is provided as a linked version of the original documentation distributed with the program. The original document can be found in the \apps directory and has been named “clustalw.txt”. The BioEdit interface for ClustalW is straightforward and options are described in the ClustalW documentation.

When you run a ClustalW alignment automatically from within the BioEdit interface, a new alignment document is created for you after ClustalW is finished which re-orders your sequences into their original order even if the Clustal program changed their order, then copies back your original titles and any associated GenBank and graphical annotation information, as well as user-defined sequence grouping information, so that this information is not lost and may be associated with the proper sequences with a minimum of hassle.

To run a ClustalW alignment from within a BioEdit alignment document, select the titles of all of the sequences you want to align by highlighting them with the mouse. If no titles are selected, BioEdit will assume you want to align all of the sequences. Next, choose "Accessory Applications->ClustalW Multiple alignment". You will get the following dialog:



ClustalW Options

ClustalW Multiple alignment

Reference:

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994)
CLUSTAL W: improving the sensitivity of progressive multiple
sequence alignment through sequence weighting, position specific
gap penalties and weight matrix choice.
Nucleic Acids Research, submitted, June 1994.

☑ Full Multiple alignment

□ Calculate NJ Tree

□ FAST algorithm for guide tree

☑ Bootstrap NJ Tree

Number of bootstraps: 1000

Other Parameters:

Note: enter additional parameters as a single line.

□ Output Clustal format with Clustal consensus sequence generation

Additional Parameters for ClustalW:

General settings:*
/QUICKTREE :use FAST algorithm for the alignment guide tree

Using World Wide Web tools

Automated links

Restriction Mapping with Webcutter

If you have access to the World Wide Web, there is an automatic link to WebCutter, a web tool for generating restriction maps. Simply highlight your sequence title in the edit window and Choose “Auto-fed Restriction Mapping” from the “World Wide Web” menu. There are some options to choose from, then press the “Analyze Sequence” button. In a short time your map will be returned and reformatted. You may also use this feature with your external browser (which may be necessary to fully view large maps).

BioEdit now also has an internal restriction map utility which has several nice options.

HTML BLAST with a Web Browser

This is general BLAST at NCBI. The only difference between using this feature and using Netscape or Internet Explorer is that a selected sequence is automatically degapped and entered into the query window of the BLAST form. If this feature is used with an external browser from within BioEdit, however, the sequence *is* degapped and fed directly to the form. The most obvious benefit of World Wide Web BLAST over the BLAST client program is that the resulting hits have easy links directly to ENTREZ entries and to Medline abstracts. Since the BLAST client program is just over 1 megabyte on disk, I may not include it in the next version of BioEdit to make a smaller installation.

To use WWW BLAST from a BioEdit document, select the sequence title of interest and choose “Auto-fed NCBI Standard BLAST” from the “World Wide Web” menu.

PSI-BLAST

PSI-BLAST is the newest search algorithm offered by NCBI. It is a variation on the original BLAST algorithm and is embellished to provide a search method analogous to searching with a consensus matrix defined by a set of homologous sequences in order to get a more sensitive measure of distant homology.

PSI-BLAST (Position-Specific Iterated BLAST) is an extension to standard BLAST which creates a position-specific weighted consensus matrix based upon an alignment of all high scoring segment pairs (HSPs) scoring above a defined threshold resulting from a standard BLAST search with the original query. During iterations of PSI-BLAST, the matrix is used in place of the original query. The matrix is refined at each iteration. In most cases, the matrix eventually converges to a point at which further iterations do not change the matrix. The resulting position-weighted alignments may give a strong indication of distant homologies that would be entirely missed with a single standard BLAST search.

For further reading on PSI-BLAST, see:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

PHI-BLAST

PHI-BLAST (Pattern-Hit Initiated BLAST) searches for a user-specified pattern, or motif, and reports on BLAST-like local alignments that are seeded around the pattern-matched region.

For more information on PHI-BLAST see:

http://www2.ncbi.nlm.nih.gov/BLAST/phiblast.html

For the paper describing PHI-BLAST, see:

Zhang, Zheng, Alejandro A. Schäffer, Webb Miller, Thomas L. Madden, David J. Lipman, Eugene V. Koonin, and Stephen F. Altschul (1998), "Protein sequence similarity searches using patterns as seeds", Nucleic Acids Res.26:3986-3990.

Prosite profile and pattern scans

Automated links to web pages (saved locally in the apps folder) are provided for Prosite profile and pattern scans. For more information about Prosite, see:

http://www.expasy.ch/prosite/

A profilescan compares a protein or nucleic acid sequence against a profile library.
A pattern scan scans a protein sequence for the occurrence of patterns stored in the Prosite database.

Both of these options may be found under Sequence->World Wide Web.

For a paper describing the Prosite database, see:

Hofmann, K., Bucher, P., Falquet, L., and Bairoch, A. (1999) The PROSITE database, its status in 1999. Nucleic Acids Res. 27:215-219.

nnPredict protein secondary structure prediction

Please note: The following brief information is taken directly from http://www.cmpharm.ucsf.edu/~nomi/nnpredict-instrucs.html

I am not qualified to discuss neural networks and parallel processing and BioEdit does not perform any protein structure prediction, but simply provides a link to this program through the World Wide Web.

"nnpredict is a program which uses a neural-network approach for predicting the secondary structure type for each residue in an amino acid sequence. nnpredict was written by Donald Kneller (Copyright (C) 1991 Regents of the University of California), and a WWW interface (the interface linked to by BioEdit) was written by Nomi Harris (nomi@cgl.ucsf.edu, nlharris@lbl.gov)."

For papers describing the algorithm and distributed processing methodology, see:

D. G. Kneller, F. E. Cohen and R. Langridge (1990) "Improvements in Protein Secondary Structure Prediction by an Enhanced Neural Network" J. Mol. Biol. (214) 171-182.

J. L. McClelland and D. E. Rumelhart. (1988) "Explorations in Parallel Distributed Processing" vol 3. pp 318-362. MIT Press, Cambridge MA.

Other links

Entrez and PubMed

These sites are maintained by the NCBI (National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov). PubMed contains the full Medline indexes freely available to the public.

Pedro’s BioMolecular Research Tools

This site contains a plethora of biotechnology and molecular biology links, especially to services available through server programs over the World Wide Web.

Constructing World Wide Web Bookmarks for BioEdit

BioEdit may store up to 500 World Wide Web bookmarks for which appear in the World Wide Web menu at startup. These bookmarks may be used with your favorite external Web browser as a convenient link to sequence analysis-related web sites from within a sequence editor.

The BioEdit web bookmarks are stored in a raw text file called "bookmark.txt". This file is found in the "apps" folder of the install directory. If the name of this file is changed, BioEdit will not recognize it. If this file is corrupted, BioEdit will allow you to automatically write a default file with some canned bookmarks.

The required format for the bookmarks file is very simple. Each entry consists of two lines of text, one for the description and one for the URL. The format is as follows:

```
name=<description>
address=<exact URL>
```

The following example shows the default bookmarks file that comes with BioEdit (or did at one time):

```
name=WebCutter Restriction Map Generator
address=http://www.firstmarket.com/cutter/cut2.html
name=BLAST at NCBI
address=http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-blast?Jform=1
name=PSI-BLAST at NCBI
address=http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-psi_blast
name=Pub Med Literature Search
address=http://www.ncbi.nlm.nih.gov/PubMed/medline.html
name=National Center for Biotechnology information
address=http://www.ncbi.nlm.nih.gov/
name=Pedro's BioMolecular Research Tools
address=http://www.fmi.ch/biology/research_tools.html
name=information about sequence logos (Tom Schneider)
address=http://www.bio.cam.ac.uk/seqlogo/
name=Sequence logo submission form
address=http://www.bio.cam.ac.uk/cgi-bin/seqlogo/logo.cgi
name=The Institute for Genomic Research (TIGR)
address=http://www.tigr.org/tigr_home/index.html
name=The RNase P Database
address=http://www.mbio.ncsu.edu/RNaseP/home.html
name=NCSU Microbiology Web Server
address=http://www.mbio.ncsu.edu/
```

That's all there is to it. If a bookmark entry is not formatted correctly, it will be ignored.
If you have made an entry and it does not appear in the World Wide Web menu after restarting BioEdit, check the entry to make sure the format matches exactly.

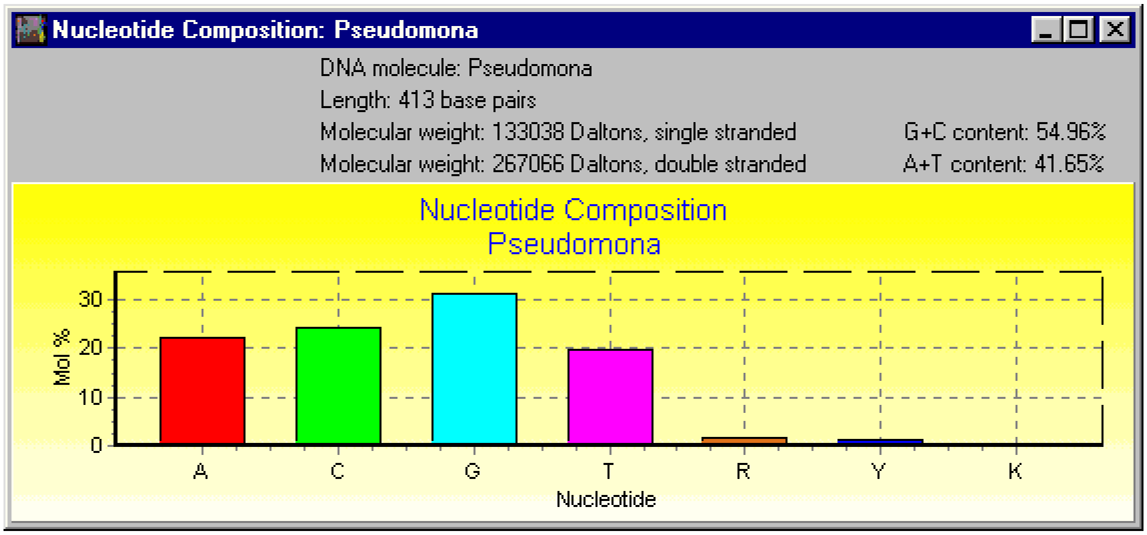
You may edit the bookmarks in any text editor. You may also edit them by choosing "View bookmarks" from the World Wide Web menu. The bookmarks will appear in a text window with the option to edit and save. At this time there is no graphical interface for bookmark manipulation.

Analyses Incorporated into BioEdit

Amino Acid and Nucleotide Composition

Amino acid or nucleotide composition summaries and plots may be obtained by choosing “Amino Acid Composition” from the “Protein” submenu of the “Sequence” menu, or “Nucleotide Composition” form the “Nucleic Acid” submenu of the “Sequence” menu, respectively. Bar plots show the Molar percent of each residue in the sequence. For nucleic acids, degenerate nucleotide designations are added to the plot if and as they are encountered. For example, a sequence that has only A, G, C and T will have four bars on the graph, but if there are R’s, Y’s , M’s, etc in the sequence, they will be added to the summary. For example, a nucleotide composition plot of the following sequence would look as follows

```
ATGAGCCAGGATTTTAGCCGTGAAAAACGTCTGCTGACCCCGCGTCATTTTAAAGCGGTGTTTGATAGCCCGACCGGC
AAAGTGCCGGGCAAAAACCTGCTGATTCTGGCGCGrTGAAAACGGCCTGGATCATCCGCGTCTGGGCCTGGTGyATTG
GCAAkAAAAAGCGTGAAACTGGCGGTGCrAGCGTAACCGTCTGAAACGTCTGATGCGTGATArGCTTTCGTCTrGAAC
CAGCAyGCTGCTGGyCGGGCCTGGATATTyGTGATTGTGGCGCGTAAAGGCCTGGGCGAAATTGAAAACCCGGAACTG
CArTCAGCATTTTGGCAAACTGTGGAAACGTCTGGCGCGTAGCCGTCCGyACCCCGGCGGTrGACCGCGAArCAGCGC
GGkGCGTGGATAGCCAGGATGCG
```



```
DNA molecule: Pseudomona
Length: 413 base pairs
Molecular weight: 133038 Daltons, single stranded        G+C content: 54.96%
Molecular weight: 267066 Daltons, double stranded        A+T content: 41.65%
```

Bar colors correspond to the residue colors specified in the color table.

A corresponding summary is generated and displayed in the text editor:

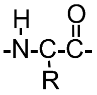
```
DNA molecule: Pseudomona
Length = 413 base pairs
Molecular Weight = 133038 Daltons, single stranded
Molecular Weight = 267066 Daltons, double stranded
G+C content = 54.96%
A+T content = 41.65%

Nucleotide  Number   Mol%
    A         91     22.03
    C         99     23.97
    G         128    30.99
    T        81       19.61
    R        7        1.69
    Y        5        1.21
    K        2        0.48
```

Amino Acid plots and summaries are similar, though residues other than the standard 20 amino acids are ignored.

Molecular weights:

The molecular weights of proteins are calculated as the sum of the internal molecular weights of each amino acid, or



where R = the side group, plus 2 hydrogens and an oxygen at the amino and carboxy termini, respectively.

Nucleotide weights are calculated as the sum of the monophosphate forms of each ribonucleotide or deoxyribonucleotide minus one water each. One water (18 Da) is added at the end to represent the 3' hydroxyl at the end of the chain and one more hydrogen at the 5' phosphate end. Nucleotide weights used are:

RNA	M.W.
Adenosine	328.2
Guanosine	344.2
Cytidine	304.2
Uridine	305.2
Average AUGC	320.5
DNA	
dAdenosine	312.2
dGuanosine	328.2
dCytidine	288.2
dThymidine	287.2
Average dATGC	304.0

These values were derived by adding all atoms in each nucleotide monophosphate minus one oxygen and two hydrogens using:

C = 12.011
O = 15.9994
N = 14.00674
P = 30.973762
H = 1.00794

Entropy plots

An entropy plot can give an idea of the amount of variability through a column in an alignment. It is a measure of the lack of "information content" at each position in the alignment. More accurately, it is a measure of the lack of predictability for an alignment position. If there are *x* sequences in an alignment (say *x* = 40 sequences) of DNA sequences, and at position *y* (say *y* = position 5) there is an 'A' in all sequences, we can assume we have a lot of information for position 5 and chances are if we had to guess at the base at position 5 of another homologous sequence, we would be correct to guess 'A'. We have maximum "information" for position 5, and the entropy is 0. Now, if there are four possibilities for each position (A, G, C or T) and each occurs at position 5 with a frequency of 0.25 (equally probable), then our information content (how well we could predict the position for a new incoming sequence) has been reduced to 0, and the entropy is at maximum variability.

Information is often measured in bits, which are basically either/or (yes/no, on/off) units, or a base-2 number system. If there are 4 possible residues at a given position, then two bits of information are required to determine the base at that position (e.g.., purine or pyrimidine? = 1 bit, if purine, 'A' or 'G'? = 1 more bit -- 2 bits total). For proteins (with 20 amino acids) at most 5 bits are required (e.g.. 1st 10? -- 1 bit, 1st 5 of block of 10? -- 1 more bit, 1st 3 of block of 5?, 1st 2 of 3?, 1st 1 or 2?: = 5 yes/no answers). If a position in an alignment can take any of four positions, but always turns up 'A', we have no uncertainty at that position and therefore can say we have maximum (two bits) of information.

Mathematically, the basis of information theory was defined by Claude Shannon:

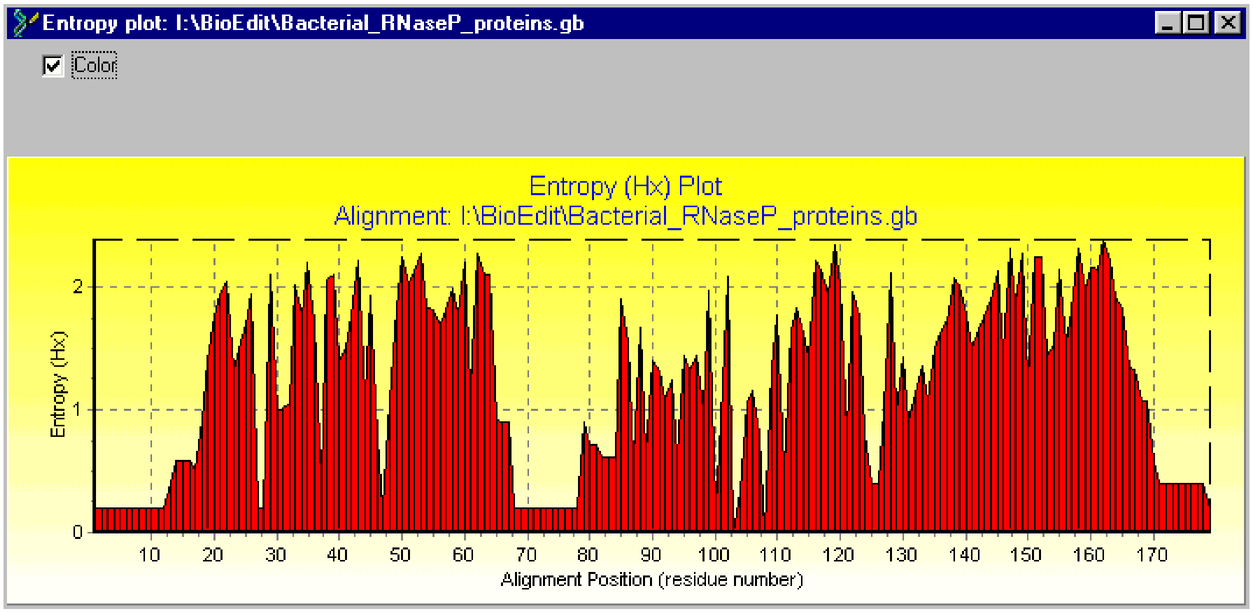
$H(l) = -\Sigma f(b,l)\log_{(\text{base }2)} f(b,l)$ (measured in bits)

where $H(l)$ = the uncertainty, also called ***entropy*** at position *l*, *b* represents a residue (out of the allowed choices for the sequence in question), and $f(b,l)$ is the frequency at which residue *b* is found at position *l*. The *information content* of a position *l*, then, is defined as a decrease in uncertainty or entropy at that position. As an alignment improves in quality, therefore, the entropy at each position (especially conserved regions) should decrease.

BioEdit plots the entropy at each position, rather than the information content, because, in order to determine information at a position, the total number of possible residues must be known. This will vary depending on whether one wishes to include gaps or degenerate nucleotide bases such as S, M, K, W, etc. in the analysis. For entropy plotting, the sequences are treated as a matrix of characters. Entropy at a column position is independent of the total information possible at a given position, and depends only upon the frequencies of characters that appear in that column. BioEdit uses the natural logarithm rather than log(base 2) for convenience, so the values are actually in nits rather than bits, but the data are the same relative to each other. Entropy is then calculated as $H(l) = -\Sigma f(b,l)\ln(f(b,l))$, which gives a measure of uncertainty at each position relative to other positions. Maximum total uncertainty will be defined by the maximum number of *different* characters found in a column. For example, if 20 amino acids and gaps are represented, but no user-defined characters are present, then the maximum uncertainty possible would be (21*(1/21)ln(1/21))=3.04 (if, say, there were 42 sequences in the alignment and each character was represented exactly twice at a given column position). This measure is not given in bits. Conversion to bits simply requires conversion to log base 2, however, and the entropy calculation could be made as: $H(l) = -\Sigma f(b,l)*(\ln(f(b,l)/\ln 2)$. This is not really necessary, however, since the entropy differences across the alignment still compare the same relative to each other.

To perform an entropy plot, highlight the titles of the sequences you want included in the analysis, then choose "Entropy (Hx) Plot" from the "Alignment" menu of an open alignment document. A graphical plot will be presented on one form and a numerical list of entropies by position will be displayed in a text editor. If a mask is used, only the mask positions will be analyzed, and if a numbering mask is used, the numbers will reflect the corresponding true positions in the numbering mask.

Below is an example of an entropy plot:


Entropy plot: I:\BioEdit\Bacterial_RNaseP_proteins.gb
☑ Color
Entropy (Hx) Plot
Alignment: I:\BioEdit\Bacterial_RNaseP_proteins.gb
Entropy (Hx)
0
1
2
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
Alignment Position (residue number)

Pierce, J.R. (1980). An Introduction to Information Theory: Symbols, Signals and Noise, Dover Publications, Inc., New York. second edition.

Schneider, T.D. and R.M. Stephens. (1990) Sequence Logos: A new Way to Display Consensus Sequences. Nucleic Acids Res. 18: 6097-6100.

Hydrophobicity Profiles

Mean Hydrophobicity profiles are generated using the general method of Kyte and Doolittle (1982). Kyte and Doolittle compiled a set of “hydropathy scores” for the 20 amino acids based upon a compilation of experimental data from the literature. A window of defined size is moved along a sequence, the hydropathy scores are summed along the window, and the average (the sum divided by the window size) is taken for each position in the sequence. The mean hydrophobicity value is plotted for the middle residue of the window.

Hydrophobic moment profiles plot the hydrophobic moment of segments of defined length along the sequence. For example, if the window size is 21 residues, the plotted value at a residue is the hydrophobic moment of the window of ten residues on either side of the current residue. Hydrophobic moment is calculated according to Eisenberg et. al. (1984):

$$\mu_H = \{[\Sigma H_n \sin(\delta n)]^{\wedge 2} + [\Sigma H_n \cos(\delta n)]\}^{\wedge (1/2)},$$

Where μ_H is the hydrophobic moment, H_n is the hydrophobicity score of residue H at position n, δ=100 degrees, n is position within the segment, and each hydrophobic moment is summed over a segment of the same defined window length.

Mean hydrophobic moment profiles plot the average hydrophobic moment for a segment of defined window length, using the same window width to calculate the hydrophobic moments. For example, for a window size of 21, the hydrophobic moments of 21 segments, each 21 residues long and each value representing the start residue of the corresponding segment, are summed and their average is taken and plotted for the center residue of the segment.

Previous versions of BioEdit simply plotted the mean hydrophobicity of a sequence segment at the first position of the segment. The result was that the mean hydrophobicity at the end of the plot, after the point *L-W*, where *L* is the sequence length and *W* is the window size, the mean value would become deceptively closer to 0. The current method is more akin to the method of Kyte and Doolittle, and may be more familiar.

Note: I do not have the expertise to make any claims about the predictive power of these profile plots. BioEdit makes no conclusions about hydrophobic and/or transmembrane segments of proteins, and interpretation of these plots is up to the judgment of the user.
For information and references about hydrophobicity analysis of proteins, see:

Cornette, J.L., K.B. Cease, H. Margalit, J.L. Spouge, J.A. Berzofsky and C. DeList. 1987. Hydrophobicity Scales and Computational Techniques for Detecting Amphipathic Structures in Proteins. *J. Mol. Biol. 195*: 659-685.

Eisenberg D. E. Schwarz, M. Komaromy and R.Wall. 1984. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. *J. Mol. Biol. 179(1)*:125-42.

Hopp, T.P. and K.R. Woods. 1981. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci. USA. 78(6)*: 3824-3828.

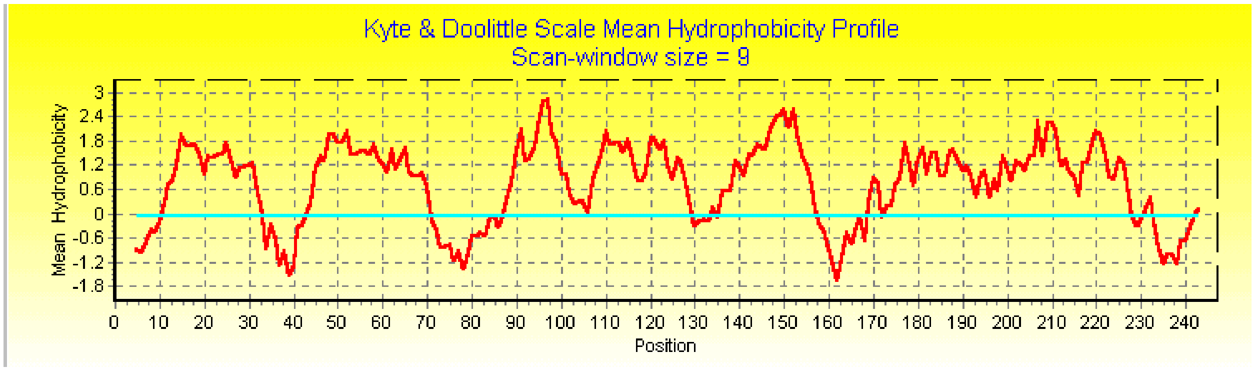
Kyte, J. and R.F. Doolittle. 1982. A Simple Method for Displaying the Hydrophobic Character of a Protein. *J. Mol. Biol. 157*: 105-142.

Parker, J.M.R., D. Guo and R.S. Hodges. 1986. New Hydrophilicity Scale Derived from High-Performance Liquid Chromatography Peptide Retention Data: Correlation of Predicted Surface Residues with Antigenicity and X-ray-Derived Accessible Sites. *Biochemistry 25*: 5425-5432.

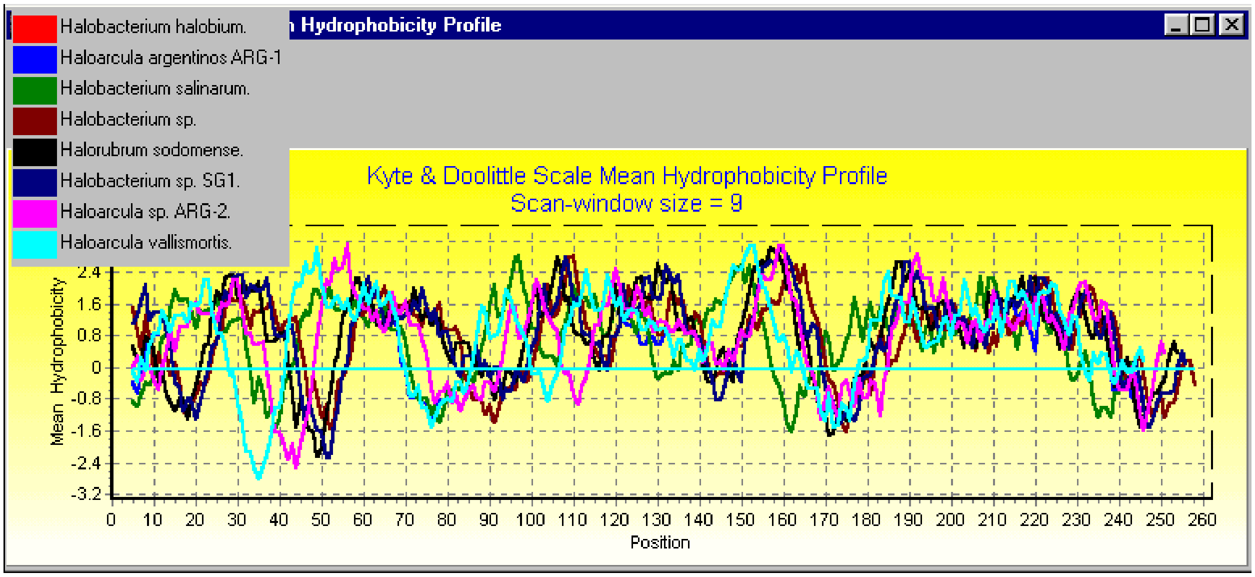
The following plots were generated from the sample file called "bacterio.gb", included in the main BioEdit folder. This is an alignment of Archaeal bacteriorhodopsin proteins. Bacteriorhodopsin is a membrane-bound, light energy-transducing proton pump with similarity to the rhodopsin. Bacteriorhodopsin is a membrane bound protein with several membrane-spanning regions. The following plots show:

1. Kyte and Doolittle mean hydrophobicity profile of *Halobacterium holbium* bacteriorhodopsin, window size = 9
2. Kyte and Doolittle mean hydrophobicity profile of eight unaligned bacteriorhodopsins, window size = 9
3. Kyte and Doolittle mean hydrophobicity profile of eight aligned bacteriorhodopsins, window size = 9. This demonstrates that superimposed hydrophobicity profiles may be used to help examine the quality of an alignment.
4. Hydrophobic moment profile for the 8 aligned sequences, window size =9
5. Mean hydrophobic moment profile for the 8 aligned sequences, window size = 9

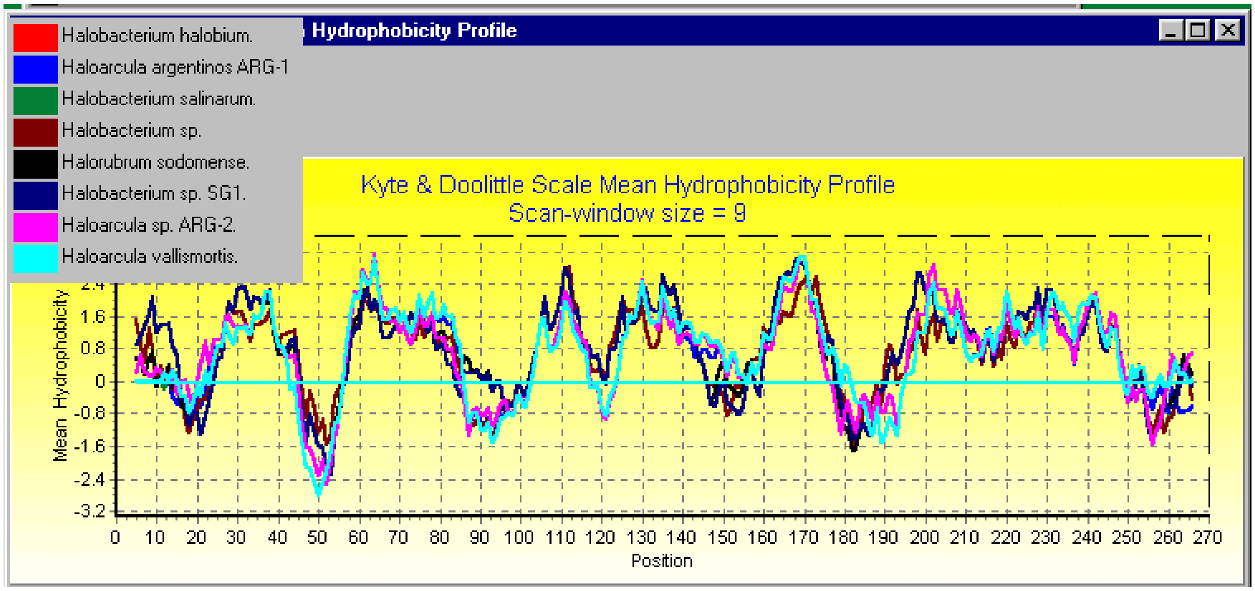
1.



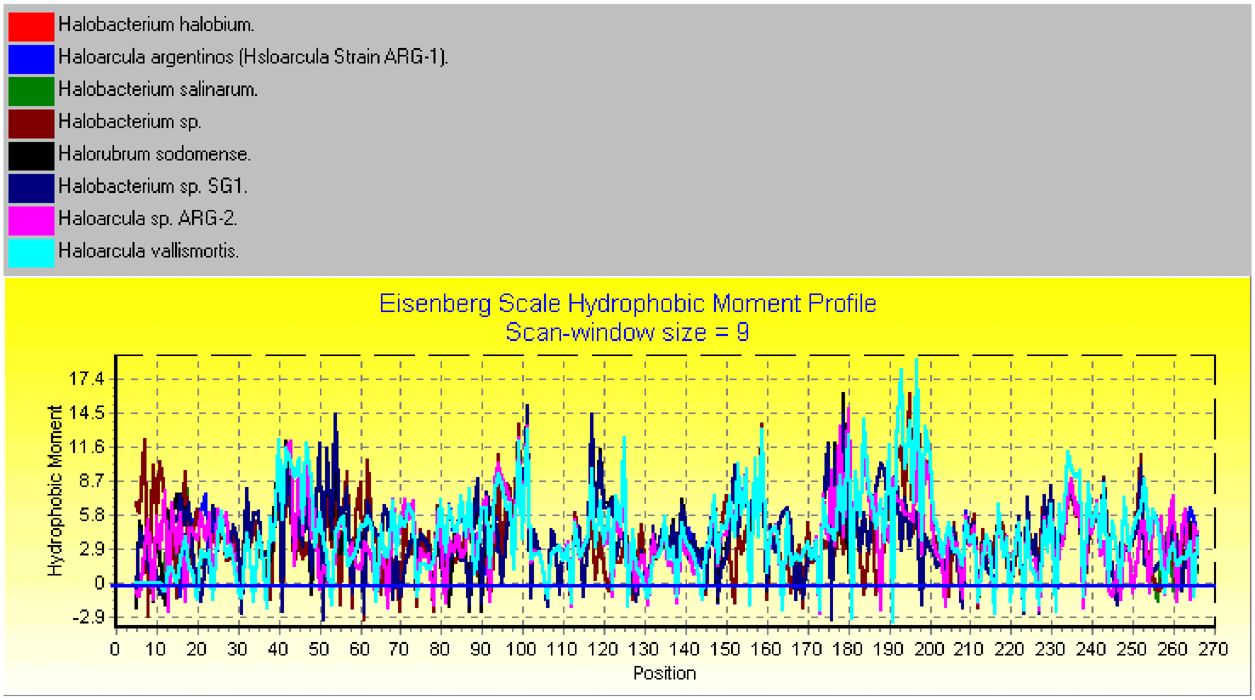
2.


🟥 Halobacterium halobium.
🟦 Haloarcula argentinos ARG-1
🟩 Halobacterium salinarum.
🟫 Halobacterium sp.
⬛ Halorubrum sodomense.
🟦 Halobacterium sp. SG1.
🟪 Haloarcula sp. ARG-2.
🟦 Haloarcula vallismortis.
Hydrophobicity Profile
Kyte & Doolittle Scale Mean Hydrophobicity Profile
Scan-window size = 9
Mean Hydrophobicity
2.4
1.6
0.8
0
-0.8
-1.6
-2.4
-3.2
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
Position

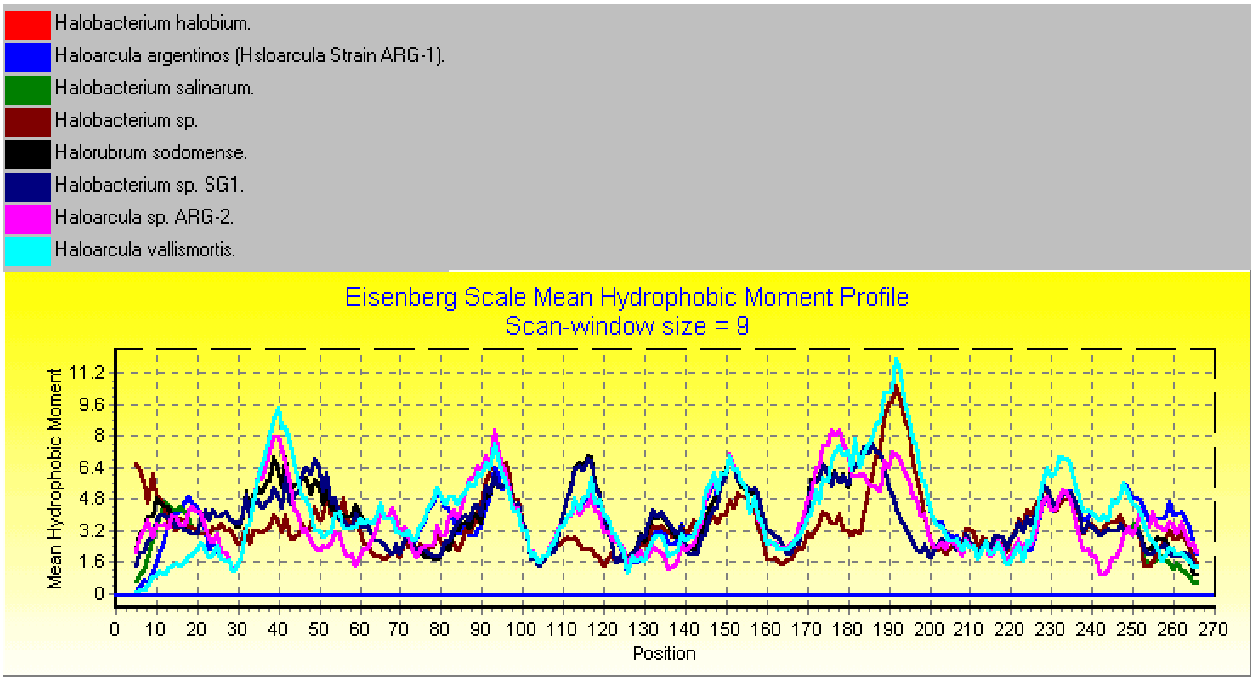
3.


🟥 Halobacterium halobium.
🟦 Haloarcula argentinos ARG-1
🟩 Halobacterium salinarum.
🟫 Halobacterium sp.
⬛ Halorubrum sodomense.
🟦 Halobacterium sp. SG1.
🟪 Haloarcula sp. ARG-2.
🟦 Haloarcula vallismortis.
Hydrophobicity Profile
Kyte & Doolittle Scale Mean Hydrophobicity Profile
Scan-window size = 9
Mean Hydrophobicity
2.4
1.6
0.8
0
-0.8
-1.6
-2.4
-3.2
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
Position

4.


🟥 Halobacterium halobium.
🟦 Haloarcula argentinos (Hsloarcula Strain ARG-1).
🟩 Halobacterium salinarum.
🟫 Halobacterium sp.
⬛ Halorubrum sodomense.
🟦 Halobacterium sp. SG1.
🟪 Haloarcula sp. ARG-2.
🟦 Haloarcula vallismortis.
Eisenberg Scale Hydrophobic Moment Profile
Scan-window size = 9
Hydrophobic Moment
17.4
14.5
11.6
8.7
5.8
2.9
0
-2.9
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270
Position

5.


🟥 Halobacterium halobium.
🟦 Haloarcula argentinos (Hsloarcula Strain ARG-1).
🟩 Halobacterium salinarum.
🟫 Halobacterium sp.
⬛ Halorubrum sodomense.
🟦 Halobacterium sp. SG1.
🟪 Haloarcula sp. ARG-2.
🟦 Haloarcula vallismortis.
Eisenberg Scale Mean Hydrophobic Moment Profile
Scan-window size = 9
Mean Hydrophobic Moment
11.2
9.6
8
6.4
4.8
3.2
1.6
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270
Position

Identity Matrix

An identity matrix shows the proportion of identical residues between all of the sequences in the alignment *as they are currently aligned*. The output is a 2-D matrix table which can either be tab-delimited or comma-delimited (*.csv). The output depends completely upon the quality of the alignment. The sequences are not automatically aligned before the procedure is run. BioEdit offers ClustalW as a means of computer-aided alignment.

To produce an identity matrix, first select the sequences you would like included in the matrix (any 2 or more sequences may be included, and you don't necessarily have to include the entire alignment). If no sequences are selected, the entire alignment will be selected automatically. After selecting the sequences to include, choose "Sequence Identity Matrix" from the "Alignment" menu.

Note: Sequence titles will be truncated to the first five characters.

Output: Following is an identity matrix generated from half of the sample file "RNaseP_prot.gb" included with the BioEdit install:
This is a small alignment of bacterial RNase P proteins.

```
Sequence Identity Matrix
Input Alignment File: C:\BioEdit\RNaseP_prot.gb

Seq->  E.col  P.mir  H.inf  P.put  B.aph  C.bur  S.bik  S.coe  M.lut  M.tub
E.col  1.000  0.773  0.593  0.374  0.426  0.305  0.242  0.242  0.198  0.231
P.mir   ---   1.000  0.563  0.396  0.400  0.279  0.210  0.210  0.191  0.214
H.inf   ---    ---   1.000  0.358  0.360  0.235  0.186  0.179  0.137  0.166
P.put   ---    ---    ---   1.000  0.282  0.276  0.176  0.204  0.165  0.164
B.aph   ---    ---    ---    ---   1.000  0.186  0.125  0.149  0.088  0.132
C.bur   ---    ---    ---    ---    ---   1.000  0.200  0.215  0.222  0.188
S.bik   ---    ---    ---    ---    ---    ---   1.000  0.876  0.419  0.338
S.coe   ---    ---    ---    ---    ---    ---    ---   1.000  0.427  0.346
M.lut   ---    ---    ---    ---    ---    ---    ---    ---   1.000  0.272
M.tub   ---    ---    ---    ---    ---    ---    ---    ---    ---   1.000
```

The score for each pair of sequences is generated as follows:

1. All positions are compared directly for each pair of sequences, one at a time.
2. All 'gap' or place-holding characters ('-', '~', '.', and '*') are treated as a gap.
3. Positions where both sequences have a gap do not contribute (they are not an identity, they simply don't exist).
4. Positions where there is a residue in one sequence and a gap in the other *do* count as a *mismatch*.
5. The reported number represent the ratio of identities to the length of the longer of the two sequences after positions where both sequences contain a gap are removed.

The above methodology should produce valid comparisons as long as the alignment is accurate. When the sequences in an alignment are properly aligned, gaps are simply added to the ends of each sequence until all lengths match the longest sequence.

Nucleic Acid Translation with Codon Usage

Nucleic acid sequences may be translated into predicted protein sequences with codon triplets separated by spaces. Choose “Translate” from the “Protein” submenu of the “Sequence” menu, then choose the frame in which to translate.

Example: The coding region for a hypothetical open reading frame from Methanobacterium is shown below:

>MTH671 coding region

```
ATGGTTGCAGTACCCGGCAGTGAGATACTGAGCGGTGCACTACACGTTGTCTCCCAGAGCCTCCTCATACCGGTTATA
GCAGGTCTACTGTTATTCATGGTATACGCCATAGTGACCCTCGGAGGGCTCATATCAGAGTACTCTGGAAGGATAAGG
ACTGATGTTAAGGAACTTGAATCGGCAATAAAATCAATTTCAAACCCAGGAACCCCTGAAAAGATAATTGAGGTCGTC
GATTCGATGGACATACCACAGAGCCAGAAGGCCGTGCTCACTGATATCGCAGGGACAGCTGAACTCGGACCAAAATCA
AGGGAGGCCCTCGCAAGGAAGTTGATAGAGAATGAGGAACTCAGGGCTGCCAAGAGCCTTGAGAAGACAGACATTGTA
ACCAGACTCGGCCCAACCCTTGGACTGATGGGGACACTCATACCCATGGGTCCAGGACTCGCAGCCCTCGGGGCAGGT
GACATCAATACACTGGCCCAGGCCATCATCATAGCCTTCGATACAACAGTTGTGGGACTTGCATCAGGGGGTATAGCA
TACATCATCTCCAAGGTCAGGAGAAGATGGTATGAGGAGTACCTCTCAAATCTTGAGACAATGGCCGAGGCAGTGCTG
GAGGTGATGGATAATGCCACTCAGACGCCGGCGAAGGCTCCTCTCGGATCAAAA
```

A frame 1 of this sequence is displayed as follows in the BioEdit text editor:

```
>MTH671 coding region

1      ATG GTT GCA GTA CCC GGC AGT GAG ATA CTG AGC GGT GCA CTA CAC   45
1      Met Val Ala Val Pro Gly Ser Glu Ile Leu Ser Gly Ala Leu His   15

46     GTT GTC TCC CAG AGC CTC CTC ATA CCG GTT ATA GCA GGT CTA CTG   90
16     Val Val Ser Gln Ser Leu Leu Ile Pro Val Ile Ala Gly Leu Leu   30

91     TTA TTC ATG GTA TAC GCC ATA GTG ACC CTC GGA GGG CTC ATA TCA   135
31     Leu Phe Met Val Tyr Ala Ile Val Thr Leu Gly Gly Leu Ile Ser   45

136    GAG TAC TCT GGA AGG ATA AGG ACT GAT GTT AAG GAA CTT GAA TCG   180
46     Glu Tyr Ser Gly Arg Ile Arg Thr Asp Val Lys Glu Leu Glu Ser   60

181    GCA ATA AAA TCA ATT TCA AAC CCA GGA ACC CCT GAA AAG ATA ATT   225
61     Ala Ile Lys Ser Ile Ser Asn Pro Gly Thr Pro Glu Lys Ile Ile   75

226    GAG GTC GTC GAT TCG ATG GAC ATA CCA CAG AGC CAG AAG GCC GTG   270
76     Glu Val Val Asp Ser Met Asp Ile Pro Gln Ser Gln Lys Ala Val   90

271    CTC ACT GAT ATC GCA GGG ACA GCT GAA CTC GGA CCA AAA TCA AGG   315
91     Leu Thr Asp Ile Ala Gly Thr Ala Glu Leu Gly Pro Lys Ser Arg   105

316    GAG GCC CTC GCA AGG AAG TTG ATA GAG AAT GAG GAA CTC AGG GCT   360
106    Glu Ala Leu Ala Arg Lys Leu Ile Glu Asn Glu Glu Leu Arg Ala   120

361    GCC AAG AGC CTT GAG AAG ACA GAC ATT GTA ACC AGA CTC GGC CCA   405
121    Ala Lys Ser Leu Glu Lys Thr Asp Ile Val Thr Arg Leu Gly Pro   135

406    ACC CTT GGA CTG ATG GGG ACA CTC ATA CCC ATG GGT CCA GGA CTC   450
136    Thr Leu Gly Leu Met Gly Thr Leu Ile Pro Met Gly Pro Gly Leu   150

451    GCA GCC CTC GGG GCA GGT GAC ATC AAT ACA CTG GCC CAG GCC ATC   495
151    Ala Ala Leu Gly Ala Gly Asp Ile Asn Thr Leu Ala Gln Ala Ile   165

496    ATC ATA GCC TTC GAT ACA ACA GTT GTG GGA CTT GCA TCA GGG GGT   540
166    Ile Ile Ala Phe Asp Thr Thr Val Val Gly Leu Ala Ser Gly Gly   180
```

```
541   ATA GCA TAC ATC ATC TCC AAG GTC AGG AGA AGA TGG TAT GAG GAG   585
181   Ile Ala Tyr Ile Ile Ser Lys Val Arg Arg Arg Trp Tyr Glu Glu   195

586   TAC CTC TCA AAT CTT GAG ACA ATG GCC GAG GCA GTG CTG GAG GTG   630
196   Tyr Leu Ser Asn Leu Glu Thr Met Ala Glu Ala Val Leu Glu Val   210

631   ATG GAT AAT GCC ACT CAG ACG CCG GCG AAG GCT CCT CTC GGA TCA   675
211   Met Asp Asn Ala Thr Gln Thr Pro Ala Lys Ala Pro Leu Gly Ser   225

676   AAA   678
226   Lys   226


Each codon is read as left nucleotide, top nucleotide, right nucleotide
Each entry is organized as follows:
   The number of occurrences of the codon in the sequence
   Preference of that codon in organism represented by the codon table
     (as a fraction of all codons coding for the same amino acid)
   Three-letter code for the amino acid coded for according to the codon table

   |A     C     G     T     |
----------------------------
 A |3     7     3     13    |A
   |0.76  0.12  0.04  0.07  |
   |Lys   Thr   Arg   Ile   |
----------------------------
 A |1     4     4     6     |C
   |0.61  0.43  0.27  0.46  |
   |Asn   Thr   Ser   Ile   |
----------------------------
 A |8     1     6     7     |G
   |0.24  0.23  0.03  1     |
   |Lys   Thr   Arg   Met   |
----------------------------
 A |4     3     1     3     |T
   |0.39  0.21  0.13  0.47  |
   |Asn   Thr   Ser   Ile   |
----------------------------
 C |0     5     0     2     |A
   |0.31  0.2   0.05  0.03  |
   |Gln   Pro   Arg   Leu   |
----------------------------
 C |1     2     0     14    |C
   |0.48  0.1   0.37  0.1   |
   |His   Pro   Arg   Leu   |
----------------------------
 C |5     2     0     5     |G
   |0.69  0.55  0.08  0.55  |
   |Gln   Pro   Arg   Leu   |
----------------------------
 C |0     2     0     5     |T
   |0.52  0.16  0.42  0.1   |
   |His   Pro   Arg   Leu   |
----------------------------
 G |5     11    8     3     |A
   |0.7   0.22  0.09  0.17  |
   |Glu   Ala   Gly   Val   |
----------------------------
 G |3     10    2     4     |C
   |0.41  0.25  0.4   0.2   |
   |Asp   Ala   Gly   Val   |
----------------------------
 G |12    1     5     5     |G
   |0.3   0.34  0.13  0.34  |
   |Glu   Ala   Gly   Val   |
----------------------------
 G |5     3     5     5     |T
   |0.59  0.19  0.38  0.29  |
   |Asp   Ala   Gly   Val   |
----------------------------
 T |0     7     0     1     |A
   |0.62  0.12  0.3   0.11  |
   |End   Ser   End   Leu   |
----------------------------
 T |4     2     0     2     |C
   |0.47  0.17  0.57  0.49  |
   |Tyr   Ser   Cys   Phe   |
-----------------------------
 T |0     2     1     1     |G
   |0.09  0.13  1     0.11  |
   |End   Ser   Trp   Leu   |
-----------------------------
 T |1     1     0     0     |T
   |0.53  0.19  0.43  0.51  |
   |Tyr   Ser   Cys   Phe   |
-----------------------------
```

The codon usage summary shows the number times that each codon appears in the sequence, as well as the frequency with which the organism (*E. coli* in this case) from which the codon table was compiled uses that codon for that amino acid. This type of summary may come in handy, for example, when planning to express a protein recombinantly.

You may also want to run, for example, only the selected region of a sequence, and you may want to use single-letter amino acid codes:

```
>Direct Submission

1      agg gaa ccg tca cct cct gat tgc aga ggg tgt gag gct cct ccc   45

46     tga gag tta aag gtg agt cca tga agg atg aag ata ctg cca cca   90
                                            M   K   I   L   P   P    6

91     aca ctg agg gtc ccc agg agg tac ata gcc ttt gag gtg atc agt   135
7       T   L   R   V   P   R   R   Y   I   A   F   E   V   I   S    21

136    gag agg gag ctc tca agg gag gaa ctt gtc tcc ctc ata tgg gat   180
22      E   R   E   L   S   R   E   E   L   V   S   L   I   W   D    36

181    agc tgc ctc aag ctg cat ggg gag tgt gaa aca tca aat ttc cgt   225
37      S   C   L   K   L   H   G   E   C   E   T   S   N   F   R    51

226    tta tgg ctc atg aag ctc tgg agg ttc gat ttt cca gac gcc gtc   270
52      L   W   L   M   K   L   W   R   F   D   F   P   D   A   V    66

271    agg gtg agg ggc ata ctc cag tgc cag agg ggc tat gag agg agg   315
67      R   V   R   G   I   L   Q   C   Q   R   G   Y   E   R   R    81

316    gtc atg atg gcc ctc aca tgc gcc cac cac cac agc ggg gtg agg   360
82      V   M   M   A   L   T   C   A   H   H   H   S   G   V   R    96

361    gtc gcc atc cac atc ctc ggc ctt tca ggg acg ata cgc tcg gca   405
97      V   A   I   H   I   L   G   L   S   G   T   I   R   S   A    111

406    aca caa aag ttt att aaa cct tcc aag aaa gat aaa tac tga tta   450
112     T   Q   K   F   I   K   P   S   K   K   D   K   Y

451    aaa tct tca tca cat gac tca tga tta cat aaa tta tcc atc aat   495

496    aaa   498
```

Positional Nucleotide Numerical Summary

This small routine simply lists the number of occurrences of each nucleotide at each position of a nucleic acid alignment. This was simply added because I needed it for something immediately and didn’t see a reason to remove it (which is also why it’s only for nucleotides right now -- maybe I’ll expand it later, but it’s not a priority right now). If a mask is used, only the mask positions are summarized, and if a numbering mask is used, each position is numbered according to the corresponding position of the numbering mask. Example output:

```
Summary of numbers of nucleotides at each position
Alignment: I:\BioEdit\Bac_Prot_genesclust.gb
Mask Sequence: Escherichi
Positions reflect sequence: Escherichi

Position     A      G     C      U      GAP

     1       3      1     1      0      8
     2       0      1     0      4      8
     3       0      4     0      1      8
     4       2      3     1      1      6
     5       0      0     0      7      6
     6       0      6     0      1      6
     7       6      1     1      0      5
     8       5      1     1      1      5
     9       4      2     2      0      5
     10      2      1     4      1      5
     11      3      1     0      4      5
     12      1      5     1      1      5
     13      6      4     1      1      1
     14      3      2     2      5      1
     15      0      6     3      3      1
     16      1      1     5      6      0
     17      0      0     0      13     0
     18      0      7     0      6      0
     19      8      0     5      0      0
     20      4      4     5      0      0
     21      4      5     4      0      0
     22      6      2     5      0      0
     23      4      5     3      1      0
     24      3      2     3      5      0
     25      3      8     1      1      0
     26      9      4     0      0      0
     27      8      0     3      2      0
     28      6      0     6      1      0
     29      6      4     0      3      0
     30      1      4     5      3      0
     31      2      1     10     0      0
     32      1      11    0      1      0
     33      1      1     1      10     0
     34      3      1     9      0      0
     35      0      0     0      13     0
     36      0      11    0      2      0
     37      2      0     11     0      0
     38      2      6     0      5      0
     39      2      5     0      6      0
     40      9      1     3      0      0
     41      4      3     6      0      0
     42      4      0     6      3      0
     43      5      0     8      0      0

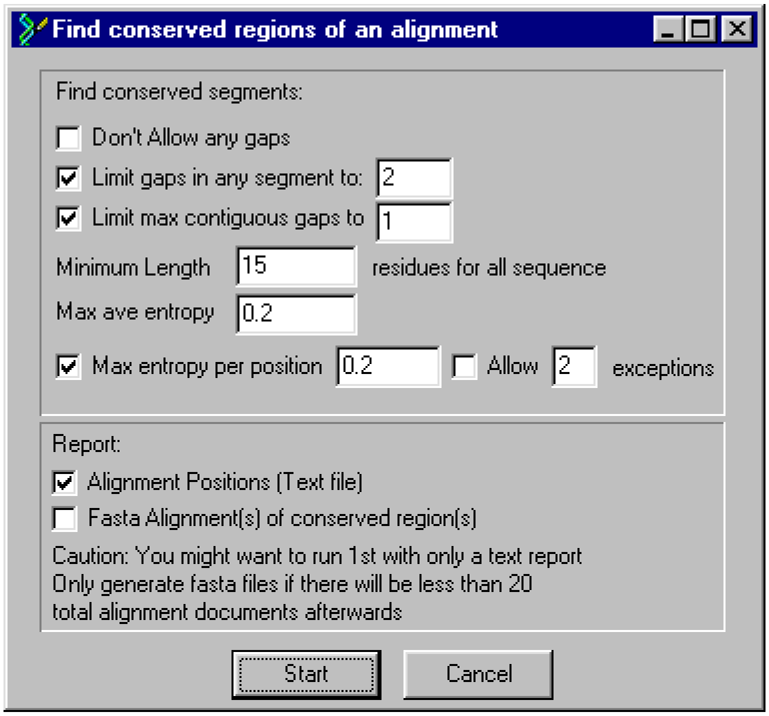
etc., etc., etc.
```

Search for conserved regions in an alignment

Sometimes it might be useful to locate regions of several sequences which are well conserved, even though there is a high degree of variation in most of the sequences. For example, one might want to create universal PCR primers that would likely work to amplify a sequence from an organism based upon a series of homologous sequences. BioEdit looks for stretches of low average "entropy" (defined as Hx = Σ(*f*bx*log(*f*(bx))), where *f*bx is the frequency of residue b at position x and the sum is taken over all possible residue types).

To search for conserved regions within an alignment (for example, to find possible targets for PCR primers), select the sequences you want included in the analysis, and choose Alignment->Find Conserved Regions.

The following dialog appears:



Find conserved regions of an alignment

Find conserved segments:

☐ Don't Allow any gaps

☑ Limit gaps in any segment to: 2

☑ Limit max contiguous gaps to 1

Minimum Length 15 residues for all sequence

Max ave entropy 0.2

☑ Max entropy per position 0.2 ☐ Allow 2 exceptions

Report:

☑ Alignment Positions (Text file)

☐ Fasta Alignment(s) of conserved region(s)

Caution: You might want to run 1st with only a text report
Only generate fasta files if there will be less than 20
total alignment documents afterwards

Don't allow gaps: No gaps in any sequence will be allowed for a reported region
Limit gaps in any segment to *x*: For a region to be reported as conserved, no sequence may have more than *x* gaps in that region.
Limit max contiguous gaps to *x*: For a region to be reported as conserved, no sequence may have more than *x* gaps in a row, regardless of how many total gaps are allowed.
Minimum length: This is the actual number of residues that must be present within the region in every sequence (not including gaps), regardless of the number of gaps allowed.
Max average entropy: The maximum average entropy (Hx/n, where n is the length of the segment) allowed.

Max entropy per position: A maximum entropy may be specified for every position which is greater or less than the maximum average entropy.
Allow *x* exceptions: If this is chosen, x exceptions to the per-position max entropy will be allowed in each reported segment.

Reports:

A text report or a series of alignments (Fasta report) may be chosen (or both). If a series of alignments is chosen, it is a good idea to first run the search with only a text report to make sure to choose parameters which only result in very few regions being detected, since BioEdit only allows 20 open alignment documents at a time.

Sample output for a text report: The following search was done on 75 16S ribosomal sequences from methanogenic Archaea. Compare the output for the first region to the example for information-based shading in the alignment window.

```
BioEdit version 4.7.1
Conserved region search
Alignment file: Q:\Ribosomal_RNA\some_methanos.bio
5/10/99 8:57:33 PM

Minimum segment length (actual for each sequence): 15
Maximum average entropy: 0.2
Maximum entropy per position: 0.2
Gaps limited to 2 per segment
Contiguous gaps limited to 1 in any segment

2 conserved regions found

Region 1: Position 755 to 774
Consensus:
755 AUUAGAUACCCGGGUAGUCC 774

Segment Length: 20
Average entropy (Hx): 0.0155
Position 755     : 0.0000
Position 756     : 0.0000
Position 757     : 0.0000
Position 758     : 0.0708
Position 759     : 0.0000
Position 760     : 0.0000
Position 761     : 0.0000
Position 762     : 0.0000
Position 763     : 0.0000
Position 764     : 0.0708
Position 765     : 0.0000
Position 766     : 0.1679
Position 767     : 0.0000
Position 768     : 0.0000
Position 769     : 0.0000
Position 770     : 0.0000
Position 771     : 0.0000
Position 772     : 0.0000
Position 773     : 0.0000
Position 774     : 0.0000


Region 2: Position 1206 to 1222
Consensus:
```

```
1206 ACACGCGGGCUACAAUG 1222

Segment Length: 17
Average entropy (Hx): 0.0182
Position 1206    : 0.0000
Position 1207    : 0.0000
Position 1208    : 0.0000
Position 1209    : 0.0000
Position 1210    : 0.0708
Position 1211    : 0.0708
Position 1212    : 0.0000
Position 1213    : 0.1679
Position 1214    : 0.0000
Position 1215    : 0.0000
Position 1216    : 0.0000
Position 1217    : 0.0000
Position 1218    : 0.0000
Position 1219    : 0.0000
Position 1220    : 0.0000
Position 1221    : 0.0000
Position 1222    : 0.0000
```

A less stringent search might find many regions, for example, for the same alignment:

```
BioEdit version 4.7.1
Conserved region search
Alignment file: Q:\Ribosomal_RNA\some_methanos.bio
5/10/99 9:34:06 PM

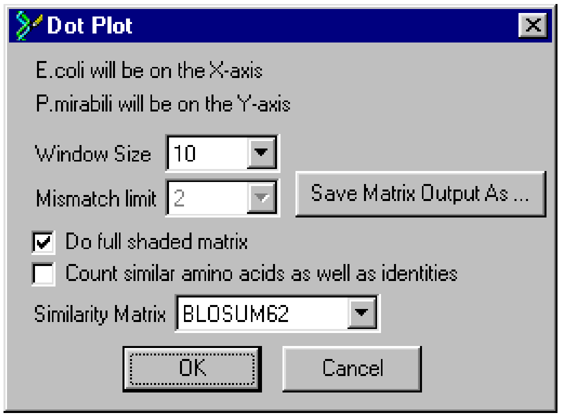
Minimum segment length (actual for each sequence): 10
Maximum average entropy: 0.4
Maximum entropy per position: 0.4 with 2 exceptions allowed
Gaps limited to 2 per segment
Contiguous gaps limited to 1 in any segment

36 conserved regions found ...

and so on ...
```

Dot Plot of two sequences

A dot plot compares two sequences at every position. The simplest form of dot plot places one sequence along the X axis and the other along the Y axis of a matrix and placing a dot at every intersecting cell where the current row in one sequence has the same residue as the current column in the other sequence. In BioEdit, a user defined window is taken, and matches are tabulated along the diagonal (an unbroken alignment of <window size> residues staring at position *x*, *y* in the matrix for every *x* and *y*). BioEdit allows a few options. When you choose to do a dot plot, first select the two sequences to compare, then choose "Sequence-> Dot Plot (pairwise comparison)". The following dialog will come up:



Dot Plot

E.coli will be on the X-axis

P.mirabili will be on the Y-axis

Window Size: 10

Mismatch limit: 2

☑ Do full shaded matrix

☐ Count similar amino acids as well as identities

Similarity Matrix: BLOSUM62

BioEdit plots a dot at the *x* and *y* center point of each scan window (for example, if the sequences were a 100% match, each was 100 bases long, and the window size was 20, there would be a solid line of dots down the center diagonal, but it would start at *x*, *y* = 10, 10, and end at *x*, *y* = 90. 90, because the center point of each full window is plotted).

The option "Do full shaded alignment" means that the number of matches down the window length will be tabulated and a dot will be plotted at the window's center point with an intensity of shading proportional to the ratio of matches to the window length, rather than the more traditional all or nothing plotting based upon a threshold of mismatches.

To only plot absolute black and white, uncheck the "Do full shaded alignment" option and specify a mismatch limit.

You may choose to count "similar" residues as matches as well by checking the "Count similar amino acids as well as identities" option. This option is only available when comparing sequences whose type is defined as "Protein".

You may choose to save the matrix data by pressing the "Save Matrix Output As ... " button and specifying a file name.

BioEdit produces a simple text-based matrix file that is brought up in the matrix plotter which plots a minimum of 1 pixel per data point, so this dot plot function is not suitable for large sequences (even moderately large -- the practical limit is really under 1500 to 2000 residues).

The results will come up in the BioEdit matrix plotter, which is mainly intended for mutual information plots, but is actually suitable for any reasonably small matrix.

Optimal Pairwise Sequence Alignment

BioEdit allows the option for very simple, optimal sequence alignments directly within an alignment document.

For alignment of sequences, a version of the general Smith and Waterman (1) algorithm is implemented. Actually, an algorithm similar to the Meyers and Miller (2) version of Gotoh's (3) modification of the Smith and Waterman algorithm (which itself is a derivation of the original Needlman-Wunsch (4) algorithm for optimal pairwise alignment) is used which keeps pointers through all of the paths through the alignment matrix, allowing traceback of the optimal alignment.

The basic alignment algorithm is this:

$$S_{i,j} = MAX \begin{cases} P_{i,j} \\ S_{i-1,j-1} + sub(a_i, b_j) \\ Q_{i,j} \end{cases}$$

$$P_{i,j} = MAX \begin{cases} S_{i-1,j} + w1 \\ P_{i-1,j} + v \end{cases}$$

$$Q_{i,j} = MAX \begin{cases} S_{i,j-1} + w1 \\ Q_{i,j-1} + v \end{cases}$$

In the above algorithm, *i* and *j* represent the rows and columns of a matrix *a* x *b,* where sequence *a* is written along the vertical and sequence *b* is written along the horizontal. sub(a_i, b_j) is the score (according to the scoring matrix) for pairing residue *i* in sequence *a* (a_i) with residue *j* in sequence *b* (b_j). *w1* is the cost, or penalty, of opening a gap. *v* is the cost of extending an already opened gap. $S_{i,\,j}$ is the total alignment score at position *i, j* in the matrix *S*, which holds the overall score at every possible alignment permutation. $P_{i,j}$ is the score in a matrix that holds a value for every possible alignment position that is *either* the overall score at position *j* of the last row plus a gap initiation penalty, or the value of position *j* of the last row of matrix *P* plus a gap extension penalty, *whichever is greater*. $Q_{i,j}$ is the score in a matrix that holds a value for every possible alignment position that is *either* the overall score at position *i* of the last column plus a gap initiation penalty, or the value of position *i* of the last column of matrix *Q* plus a gap extension penalty, *whichever is greater*. Matrices *P* and *Q* allow for a gap penalty system where there is a different penalty applied to extending a gap than there is for initiating one (it is fairly easy to imagine that if a deletion or insertion takes place in a sequence that it could consist of an entire region (perhaps a multiple of 3 for a protein-encoding DNA sequence), and the group of residues may only represent one evolutionary event, in which case a constant gap penalty is not likely to perform as well as an affine gap system). At every possible path through the main alignment matrix, the *P* and *Q* matrices are examined to see if it would yield a higher overall score at that point to open a gap or series of gaps than to try to align another pair of residues at the current *i* and *j* positions of the matrix.

An actual alignment is constructed by doing three basic things:

1. Calculating the *S*, *P* and *Q* matrices for sequences *a* and *b*.
2. Each time a value is filled in for matrix *S*, store a pointer to which cell in the matrix the score was derived from. If, at any position in the matrix, the next *S* is derived by pairing the next two residues, then the pointer for that position stores *i*-1, *j*-1. If the value came from $P_{i,j}$, then the pointer points to *i*-1, *j*. If it came from $Q_{i,j}$, then the pointer points to *i*, *j*-1.
3. Since all possible alignment paths have to end at the bottom right cell of the matrix, the optimal alignment can be constructed by tracing the pointers back that ultimately lead to the last cell.

The choice of matrix can have a large impact on an alignment. To align two sequences thought to be closely related, it is probably better to use a matrix reflecting less evolutionary divergence (such as a PAM matrix with a lower n number, e.g., PAM120 or PAM80), whereas more distantly related sequences may be better aligned with a more divergent matrix such as PAM250. For example, take the following (very) short sequences: TETSEFLY and TESTSEQ. We will align them with gap penalties of -8 to open and -2 to extend, and use the BLOSUM62 matrix (the default, which is used by default by BLAST) matrix. The results are different than if we use, say the PAM80 matrix:

sequence *a* = TETSEFLY
sequence *b* = TESTSEQ

If we line them up in our three matrices (plus one more for the pointers) and calculate according to the above algorithm, we get:

P matrix

		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0			-8	-16	-24	-32	-40	-48	-56
1	T		-10	-18	-26	-34	-42	-50	-58
2	E		-3	-11	-13	-15	-17	-19	-21
3	T		-5	2	-6	-8	-10	-12	-14
4	S		-7	0	3	-1	-7	-9	-11
5	E		-9	-2	1	4	3	-5	-7
6	F		-11	-4	-1	2	4	8	0
7	L		-13	-6	-3	0	2	6	5
8	Y		-15	-8	-5	-2	0	4	3

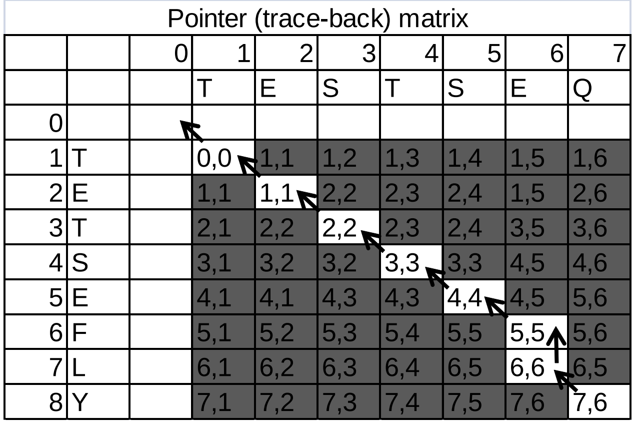
Q matrix

		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0									
1	T	-8	-10	-3	-5	-7	-9	-11	-13
2	E	-16	-18	-11	2	0	-2	-4	-6
3	T	-24	-26	-13	-6	3	1	-1	-3
4	S	-32	-34	-15	-8	-2	4	3	1
5	E	-40	-42	-17	-10	-7	-3	4	8
6	F	-48	-50	-19	-12	-9	-6	-4	1
7	L	-56	-58	-21	-14	-11	-8	-6	-2
8	Y	-64	-66	-23	-16	-13	-10	-8	-4

S matrix

		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0		0	-8	-16	-24	-32	-40	-48	-56
1	T	-8	5	-3	-5	-7	-9	-11	-13
2	E	-16	-3	10	2	0	-2	-4	-6
3	T	-24	-5	2	11	7	1	-1	-3
4	S	-32	-7	0	6	12	11	3	1
5	E	-40	-9	-2	1	5	12	16	8
6	F	-48	-11	-4	-1	2	4	9	13
7	L	-56	-13	-6	-3	0	2	6	7
8	Y	-64	-15	-8	-5	-2	0	4	5

Pointer (trace-back) matrix



		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0									
1	T		0,0	1,1	1,2	1,3	1,4	1,5	1,6
2	E		1,1	1,1	2,2	2,3	2,4	1,5	2,6
3	T		2,1	2,2	2,2	2,3	2,4	3,5	3,6
4	S		3,1	3,2	3,2	3,3	3,3	4,5	4,6
5	E		4,1	4,1	4,3	4,3	4,4	4,5	5,6
6	F		5,1	5,2	5,3	5,4	5,5	5,5	5,6
7	L		6,1	6,2	6,3	6,4	6,5	6,6	6,5
8	Y		7,1	7,2	7,3	7,4	7,5	7,6	7,6

Tracing back through the pointer numbers in the above matrix gives the following alignment:

```
TETSEFLY
TESTSE-Q
```

This is probably *not* the same as if you had just aligned these short sequences by hand. But, because of the gap penalties and the matrix, this alignment will tolerate 5 mismatched residues rather than accept an extra gap. When the PAM80 matrix is used, however, the following alignment is produced:

```
TE-TSEFLY
TESTSE--Q
```

Which might be closer to what you'd expect by looking at these sequences. The matrices calculated to produce this alignment are:

P matrix

		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0		0	-8	-16	-24	-32	-40	-48	-64
1	T		-10	-18	-26	-34	-42	-50	-66
2	E		-3	-11	-13	-15	-17	-19	-21
3	T		-5	3	-5	-7	-9	-11	-13
4	S		-7	1	5	0	-5	-7	-9
5	E		-9	-1	3	7	4	-3	-5
6	F		-11	-3	1	5	5	10	2
7	L		-13	-5	-1	3	3	8	2
8	Y		-15	-7	-3	1	1	6	0

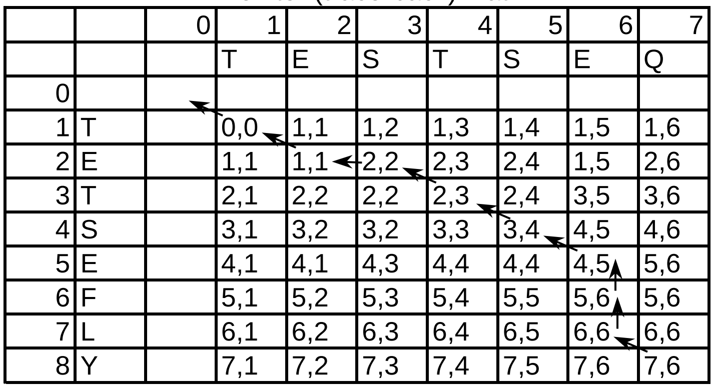
Q matrix

		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0		0							
1	T	-8	-10	-3	-5	-7	-9	-11	-13
2	E	-16	-18	-11	3	1	-1	-3	-5
3	T	-24	-26	-13	-5	5	3	1	-1
4	S	-32	-34	-15	-7	-1	7	5	3
5	E	-40	-42	-17	-9	-5	-1	5	10
6	F	-48	-50	-19	-11	-7	-3	-3	2
7	L	-56	-58	-21	-13	-9	-5	-5	0
8	Y	-64	-66	-23	-15	-11	-7	-7	-2

S matrix

		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0		0	-8	-16	-24	-32	-40	-48	-64
1	T	-8	5	-3	-5	-7	-9	-11	-13
2	E	-16	-3	11	3	1	-1	-3	-5
3	T	-24	-5	3	13	8	3	1	-1
4	S	-32	-7	1	7	15	12	5	3
5	E	-40	-9	-1	3	7	13	18	10
6	F	-48	-11	-3	1	5	5	10	10
7	L	-56	-13	-5	-1	3	3	8	7
8	Y	-64	-15	-7	-3	1	1	6	1

Pointer (trace-back) matrix



		0	1	2	3	4	5	6	7
			T	E	S	T	S	E	Q
0									
1	T		0,0	1,1	1,2	1,3	1,4	1,5	1,6
2	E		1,1	1,1	2,2	2,3	2,4	1,5	2,6
3	T		2,1	2,2	2,2	2,3	2,4	3,5	3,6
4	S		3,1	3,2	3,2	3,3	3,4	4,5	4,6
5	E		4,1	4,1	4,3	4,4	4,4	4,5	5,6
6	F		5,1	5,2	5,3	5,4	5,5	5,6	5,6
7	L		6,1	6,2	6,3	6,4	6,5	6,6	6,6
8	Y		7,1	7,2	7,3	7,4	7,5	7,6	7,6

Tracing these pointer back gives:

```
TE-TSEFLY
TESTSE--Q
```

It is possible to initialize the starting conditions of *i*=0 for all *j* or *j*=0 for all *i* (or both) with no initial penalty for adding and extending a gap, so that the end of one sequence can slide over the other without penalty until the alignment actually starts. This is what is done when the menu option "Sequence->Pairwise alignment->Align two sequence (allow ends to slide)" is chosen, as opposed to the option "Align two sequences (optimal GLOBAL alignment)", which penalizes gaps at the ends of sequences the same as internal gaps.

For comparison, the BLOSUM62 and PAM80 matrices are shown below. Notice that the scores for mismatched residues are generally much more negative in the PAM80 matrix than in the BLOSUM62 matrix.

BLOSUM62 scoring matrix

```
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0 -2 -1  0 -4
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3 -1  0 -1 -4
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3  3  0 -1 -4
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3  4  1 -1 -4
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1 -3 -3 -2 -4
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2  0  3 -1 -4
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3 -1 -2 -1 -4
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3  0  0 -1 -4
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3 -3 -3 -1 -4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1 -4 -3 -1 -4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2  0  1 -1 -4
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1 -3 -1 -1 -4
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1 -3 -3 -1 -4
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7 -1 -1 -4 -3 -2 -2 -1 -2 -4
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4  1 -3 -2 -2  0  0  0 -4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5 -2 -2  0 -1 -1  0 -4
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11  2 -3 -4 -3 -2 -4
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7 -1 -3 -2 -1 -4
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4 -3 -2 -1 -4
B -2 -1  3  4 -3  0  1 -1  0 -3 -4  0 -3 -3 -2  0 -1 -4 -3 -3  4  1 -1 -4
Z -1  0  0  1 -3  3  4 -2  0 -3 -3  1 -1 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
X  0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2  0  0 -2 -1 -1 -1 -1 -1 -4
* -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4  1
```

PAM80 scoring matrix

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A	4	-4	-1	-1	-4	-2	-1	0	-4	-2	-4	-4	-3	-5	0	1	1	-8	-5	0	-1	-1	-1	-11
R	-4	7	-2	-5	-5	0	-4	-6	0	-3	-5	2	-2	-6	-2	-1	-3	0	-7	-5	-3	-1	-3	-11
N	-1	-2	5	3	-6	-1	0	-1	2	-3	-5	0	-4	-5	-3	1	0	-5	-3	-4	4	0	-1	-11
D	-1	-5	3	6	-9	0	4	-1	-1	-4	-7	-2	-6	-9	-4	-1	-2	-10	-7	-5	5	2	-3	-11
C	-4	-5	-6	-9	9	-9	-9	-6	-5	-4	-9	-9	-8	-8	-5	-1	-4	-10	-2	-3	-7	-9	-5	-11
Q	-2	0	-1	0	-9	7	2	-4	2	-4	-3	-1	-2	-8	-1	-3	-3	-8	-7	-4	0	5	-2	-11
E	-1	-4	0	4	-9	2	6	-2	-2	-3	-6	-2	-4	-9	-3	-2	-3	-11	-6	-4	2	5	-2	-11
G	0	-6	-1	-1	-6	-4	-2	6	-5	-6	-7	-4	-5	-6	-3	0	-2	-10	-8	-3	-1	-2	-3	-11
H	-4	0	2	-1	-5	2	-2	-5	8	-5	-4	-3	-5	-3	-2	-3	-4	-4	-1	-4	0	1	-2	-11
I	-2	-3	-3	-4	-4	-4	-3	-6	-5	7	1	-4	1	0	-5	-4	-1	-8	-3	3	-4	-4	-2	-11
L	-4	-5	-5	-7	-9	-3	-6	-7	-4	1	6	-5	2	0	-4	-5	-4	-3	-4	0	-6	-4	-3	-11
K	-4	2	0	-2	-9	-1	-2	-4	-3	-4	-5	6	0	-9	-4	-2	-1	-7	-6	-5	-1	-1	-3	-11
M	-3	-2	-4	-6	-8	-2	-4	-5	-5	1	2	0	9	-2	-5	-3	-2	-7	-6	1	-5	-3	-2	-11
F	-5	-6	-5	-9	-8	-8	-9	-6	-3	0	0	-9	-2	8	-7	-4	-5	-2	4	-4	-7	-8	-5	-11
P	0	-2	-3	-4	-5	-1	-3	-3	-2	-5	-4	-4	-5	-7	7	0	-2	-9	-8	-3	-3	-2	-2	-11
S	1	-1	1	-1	-1	-3	-2	0	-3	-4	-5	-2	-3	-4	0	4	2	-3	-4	-3	0	-2	-1	-11
T	1	-3	0	-2	-4	-3	-3	-2	-4	-1	-4	-1	-2	-5	-2	2	5	-8	-4	-1	-1	-3	-1	-11
W	-8	0	-5	-10	-10	-8	-11	-10	-4	-8	-3	-7	-7	-2	-9	-3	-8	13	-2	-10	-7	-9	-7	-11
Y	-5	-7	-3	-7	-2	-7	-6	-8	-1	-3	-4	-6	-6	4	-8	-4	-4	-2	9	-5	-4	-6	-4	-11
V	0	-5	-4	-5	-3	-4	-4	-3	-4	3	0	-5	1	-4	-3	-3	-1	-10	-5	6	-4	-4	-2	-11
B	-1	-3	4	5	-7	0	2	-1	0	-4	-6	-1	-5	-7	-3	0	-1	-7	-4	-4	5	2	-2	-11
Z	-1	-1	0	2	-9	5	5	-2	1	-4	-4	-1	-3	-8	-2	-2	-3	-9	-6	-4	2	5	-2	-11
X	-1	-3	-1	-3	-5	-2	-2	-3	-2	-2	-3	-3	-2	-5	-2	-1	-1	-7	-4	-2	-2	-2	-3	-11
*	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	1

1. Smith, T.F. and Waterman, M.S. (1981) Identification of common molecular subsequences. *J. Mol. Biol.* **147**(1):195-7.

2. Myers, E.W. and Miller, W. (1988) Optimal alignments in linear space. *Comput. Appl. Biosci*. **4**(1):11-7.

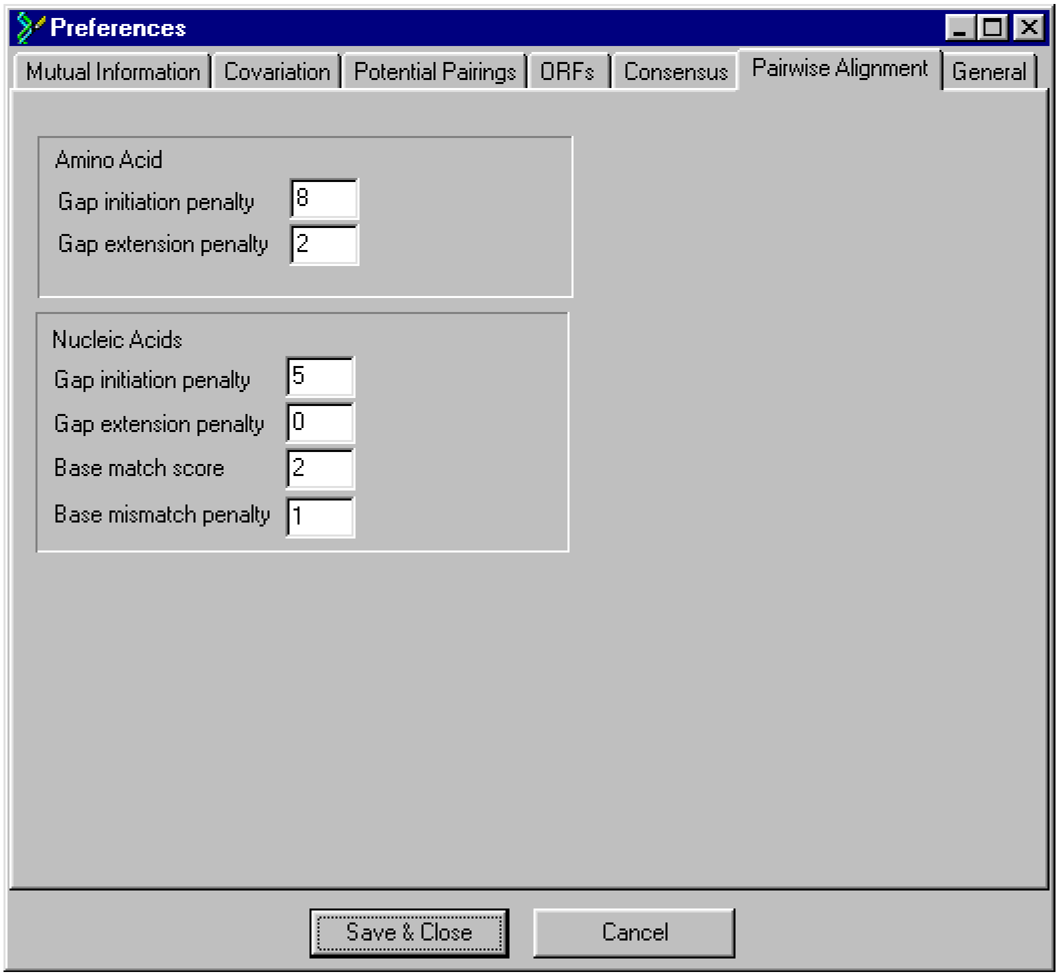
3. Gotoh, O. (1982) An improved algorithm for matching biological sequences. *J. Mol. Biol*. **162**(3):705-8.

4. Needleman, S.B. and Wunsch, C.D. (1970) A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol*. **48**(3):443-53.

Preferences for optimal pairwise alignment

To set gap penalty parameters for pairwise alignment, and match and mismatch scores for nucleic acid pairwise alignment, choose "Options->Preferences->Pairwise Alignment".

For information on pairwise alignments, see Optimal Pairwise Sequence Alignment



Amino Acid

Gap initiation penalty 8

Gap extension penalty 2

Nucleic Acids

Gap initiation penalty 5

Gap extension penalty 0

Base match score 2

Base mismatch penalty 1

Substitution Matrices used for pairwise alignment and alignment shading

When trying to construct the most likely alignment between two (or more) sequences assumed to be homologous (i.e., derived from the same ancestral sequence), criteria are needed to specify the level of "similarity" between two aligned residues in order to assess their contribution to the quality of the overall alignment. Although the level of similarity between two residues is not literally meaningful (they are either identical or not, and they either occupy a position in each respective sequence that is the same relative to the ancestral sequence, or they do not), we do not have the data for the ancestral sequence(s) nor the evolutionary steps that led to the current sequences, and we need a system that allows us to estimate the likelihood that one residue has been *substituted* for another through natural selection (some reflection of the frequency we can expect to see residue *a* substituted for residue *b*). A collection of similarity values that compares every combination of available residues is called a substitution matrix. For a general introduction to the generation of scoring matrices and basic sequence alignment, I would recommend reference 1 (below)

BioEdit provides a small collection of common substitution matrices for optimal pairwise alignment and for shading similar amino acids in the alignment document view and in the graphic alignment view.

The following matrices are provided and can be found in the BioEdit/apps folder in plain text format:

BLOSUM62: A commonly used matrix developed by Henikoff and Henikoff (2), which is suggested to be more sensitive than the older PAM matrices (3) for database searching (4) and is the default matrix used by the NCBI BLAST program.

The BLOSUM62 matrix supplied with BioEdit 5.0.0 was obtained from:

ftp://ncbi.nlm.nih.gov/repository/blocks/unix/blosum/BLOSUM/blosum62.bla

PAM40, PAM80, PAM120 and PAM250: Current PAM matrices, as generated by the PAM program -- see:

http://www.cmbi.kun.nl/bioinf/tools/pam.shtml

PAM is an acronym for "Point Accepted Mutation" (3). One PAM unit is the evolutionary "time", or divergence, that results in 1 amino acid substitution per 100 amino acids of protein on average (1%). Larger PAM values indicate greater evolutionary divergence. The PAM matrices were derived from alignments of closely related amino acid sequences, then extrapolated to reflect evolutionary times of *n* PAM units for a PAM*n* matrix (the PAM120 matrix gives an indication of the relative expected substitutions frequencies of all residue combinations in 120 PAM units of evolutionary distance). The number scores in these matrices are the "log odds" ratio of the frequency observed of each substitution (in a given amount of evolutionary "time") to the probability of finding the match randomly, or $\log(q^{n}_{a,b}/p_a,p_b)$, where $q_{a,b}$ is the observed frequency of a substitution in *n* units of evolution of residue *a* to residue *b*, and p_a and p_b are the individual probabilities of finding residue *a* and *b*, respectively. The PAM250 matrix, then, reflects the frequency we could expect a given amino acid to change to another relative to the random chance of finding the two amino acids when there have been a total of 250 substitutions over time per 100 residues.

DAYHOFF: The "DAYHOFF" matrix provided with BioEdit is an integer-rounded version of the original Dayhoff PAM-250 matrix (3) that I downloaded in it's current form off of the WWW. For the original PAM250 matrix, see (3) and/or refer to:

http://www.inf.ethz.ch/personal/hallett/drive/node160.html

This matrix is only included in case someone has an interest in using it for BLAST searching for any reason. It has largely been replaced by more recent PAM matrices generated with updated databases, however, and for database searching, the BLOSUM matrices are generally considered better (4).

IDENTIFY and **MATCH**: These matrices are simply for all-or nothing identity-based alignment or shading. For shading, they are identical. For alignment, the IDENTIFY matrix has a mismatch value of -10000 and a match score of 1 in all cases, which will select exclusively for stretches of exact matches. For Optimal alignment allowing the ends to slide, this will find a region of overlap between two identical amino acid sequences which are incomplete (one or both is missing residues on one end). If used with the local BLAST tool, this matrix will select for only exact local matches, with no internal mismatches. The MATCH matrix has a match score of 1 and a mismatch score of -1 in all cases, and can be used with BLAST to search for amino acid sequences based only upon identity, but not absolutely bound to no internal mismatches.

GONNET: Yet another PAM250 matrix, as recommended by (5).

1. Durbin, R, Eddy, S., Krogh, A. and Mitchison, G. (1998) Biological Sequence Analysis : Probabilistic Models of Proteins and Nucleic Acids. Cambridge : Cambridge University Press.

2. Henikoff, S. and Henikoff, J.G. (1992) Amino acid substitution matrices from protein blocks. *Proc Natl Acad Sci U S A* **89**(22): 10915-10919.

3. Dayhoff, M. O., Schwartz, R. M. and Orcutt, B. C. (1978) A model of evolutionary change in proteins. matrices for detecting distant relationships In M. O. Dayhoff, (ed.), Atlas of protein sequence and structure, volume 5, pp. 345-358 National biomedical research foundation Washington DC.

4. Henikoff, S. and Henikoff, J.G. (1993) Performance evaluation of amino acid substitution matrices. *Proteins* **17**(1): 49-61.

5. Gonnet, G.H., Cohen, M.A. and Benner S.A. (1992) Exhaustive matching of the entire protein sequence database. *Science* **256**(5062): 1443-5.

The scoring matrices supplied are shown below:

BLOSUM62:

```
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0 -2 -1  0 -4
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3 -1  0 -1 -4
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3  3  0 -1 -4
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3  4  1 -1 -4
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1 -3 -3 -2 -4
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2  0  3 -1 -4
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3 -1 -2 -1 -4
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3  0  0 -1 -4
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3 -3 -3 -1 -4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1 -4 -3 -1 -4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2  0  1 -1 -4
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1 -3 -1 -1 -4
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1 -3 -3 -1 -4
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7 -1 -1 -4 -3 -2 -2 -1 -2 -4
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4  1 -3 -2 -2  0  0  0 -4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5 -2 -2  0 -1 -1  0 -4
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11  2 -3 -4 -3 -2 -4
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7 -1 -3 -2 -1 -4
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4 -3 -2 -1 -4
B -2 -1  3  4 -3  0  1 -1  0 -3 -4  0 -3 -3 -2  0 -1 -4 -3 -3  4  1 -1 -4
Z -1  0  0  1 -3  3  4 -2  0 -3 -3  1 -1 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
X  0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2  0  0 -2 -1 -1 -1 -1 -1 -4
* -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4 -4  1
```

DAYHOFF (an older PAM250)

```
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  2 -2  0  0 -2  0  0  1 -1 -1 -2 -1 -1 -4  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 -4 -1  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 12 -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 -1  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  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 -2 -1 -8
F -4 -4 -4 -6 -4 -5 -5 -5 -2  1  2 -5  0  9 -5 -3 -3  0  7 -1 -4 -5 -2 -8
P  1  0 -1 -1 -3  0 -1 -1  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  0 -8
T  1 -1  0  0 -2 -1  0  0 -1  0 -2  0 -1 -3  0  1  3 -5 -3  0  0 -1  0 -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 10 -2 -3 -4 -2 -8
V  0 -2 -2 -2 -2 -2 -2 -1 -2  4  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  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  1
```

PAM250

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
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	12	-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	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	-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	0	-8
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3	-5	-3	0	0	-1	0	-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	10	-2	-3	-4	-2	-8
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	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	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	1

PAM120

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A	3	-3	-1	0	-3	-1	0	1	-3	-1	-3	-2	-2	-4	1	1	1	-7	-4	0	0	-1	-1	-8
R	-3	6	-1	-3	-4	1	-3	-4	1	-2	-4	2	-1	-5	-1	-1	-2	1	-5	-3	-2	-1	-2	-8
N	-1	-1	4	2	-5	0	1	0	2	-2	-4	1	-3	-4	-2	1	0	-4	-2	-3	3	0	-1	-8
D	0	-3	2	5	-7	1	3	0	0	-3	-5	-1	-4	-7	-3	0	-1	-8	-5	-3	4	3	-2	-8
C	-3	-4	-5	-7	9	-7	-7	-4	-4	-3	-7	-7	-6	-6	-4	0	-3	-8	-1	-3	-6	-7	-4	-8
Q	-1	1	0	1	-7	6	2	-3	3	-3	-2	0	-1	-6	0	-2	-2	-6	-5	-3	0	4	-1	-8
E	0	-3	1	3	-7	2	5	-1	-1	-3	-4	-1	-3	-7	-2	-1	-2	-8	-5	-3	3	4	-1	-8
G	1	-4	0	0	-4	-3	-1	5	-4	-4	-5	-3	-4	-5	-2	1	-1	-8	-6	-2	0	-2	-2	-8
H	-3	1	2	0	-4	3	-1	-4	7	-4	-3	-2	-4	-3	-1	-2	-3	-3	-1	-3	1	1	-2	-8
I	-1	-2	-2	-3	-3	-3	-3	-4	-4	6	1	-3	1	0	-3	-2	0	-6	-2	3	-3	-3	-1	-8
L	-3	-4	-4	-5	-7	-2	-4	-5	-3	1	5	-4	3	0	-3	-4	-3	-3	-2	1	-4	-3	-2	-8
K	-2	2	1	-1	-7	0	-1	-3	-2	-3	-4	5	0	-7	-2	-1	-1	-5	-5	-4	0	-1	-2	-8
M	-2	-1	-3	-4	-6	-1	-3	-4	-4	1	3	0	8	-1	-3	-2	-1	-6	-4	1	-4	-2	-2	-8
F	-4	-5	-4	-7	-6	-6	-7	-5	-3	0	0	-7	-1	8	-5	-3	-4	-1	4	-3	-5	-6	-3	-8
P	1	-1	-2	-3	-4	0	-2	-2	-1	-3	-3	-2	-3	-5	6	1	-1	-7	-6	-2	-2	-1	-2	-8
S	1	-1	1	0	0	-2	-1	1	-2	-2	-4	-1	-2	-3	1	3	2	-2	-3	-2	0	-1	-1	-8
T	1	-2	0	-1	-3	-2	-2	-1	-3	0	-3	-1	-1	-4	-1	2	4	-6	-3	0	0	-2	-1	-8
W	-7	1	-4	-8	-8	-6	-8	-8	-3	-6	-3	-5	-6	-1	-7	-2	-6	12	-2	-8	-6	-7	-5	-8
Y	-4	-5	-2	-5	-1	-5	-5	-6	-1	-2	-2	-5	-4	4	-6	-3	-3	-2	8	-3	-3	-5	-3	-8
V	0	-3	-3	-3	-3	-3	-3	-2	-3	3	1	-4	1	-3	-2	-2	0	-8	-3	5	-3	-3	-1	-8
B	0	-2	3	4	-6	0	3	0	1	-3	-4	0	-4	-5	-2	0	0	-6	-3	-3	4	2	-1	-8
Z	-1	-1	0	3	-7	4	4	-2	1	-3	-3	-1	-2	-6	-1	-1	-2	-7	-5	-3	2	4	-1	-8
X	-1	-2	-1	-2	-4	-1	-1	-2	-2	-1	-2	-2	-2	-3	-2	-1	-1	-5	-3	-1	-1	-1	-2	-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

PAM80

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A	4	-4	-1	-1	-4	-2	-1	0	-4	-2	-4	-4	-3	-5	0	1	1	-8	-5	0	-1	-1	-1	-11
R	-4	7	-2	-5	-5	0	-4	-6	0	-3	-5	2	-2	-6	-2	-1	-3	0	-7	-5	-3	-1	-3	-11
N	-1	-2	5	3	-6	-1	0	-1	2	-3	-5	0	-4	-5	-3	1	0	-5	-3	-4	4	0	-1	-11
D	-1	-5	3	6	-9	0	4	-1	-1	-4	-7	-2	-6	-9	-4	-1	-2	-10	-7	-5	5	2	-3	-11
C	-4	-5	-6	-9	9	-9	-9	-6	-5	-4	-9	-9	-8	-8	-5	-1	-4	-10	-2	-3	-7	-9	-5	-11
Q	-2	0	-1	0	-9	7	2	-4	2	-4	-3	-1	-2	-8	-1	-3	-3	-8	-7	-4	0	5	-2	-11
E	-1	-4	0	4	-9	2	6	-2	-2	-3	-6	-2	-4	-9	-3	-2	-3	-11	-6	-4	2	5	-2	-11
G	0	-6	-1	-1	-6	-4	-2	6	-5	-6	-7	-4	-5	-6	-3	0	-2	-10	-8	-3	-1	-2	-3	-11
H	-4	0	2	-1	-5	2	-2	-5	8	-5	-4	-3	-5	-3	-2	-3	-4	-4	-1	-4	0	1	-2	-11
I	-2	-3	-3	-4	-4	-4	-3	-6	-5	7	1	-4	1	0	-5	-4	-1	-8	-3	3	-4	-4	-2	-11
L	-4	-5	-5	-7	-9	-3	-6	-7	-4	1	6	-5	2	0	-4	-5	-4	-3	-4	0	-6	-4	-3	-11
K	-4	2	0	-2	-9	-1	-2	-4	-3	-4	-5	6	0	-9	-4	-2	-1	-7	-6	-5	-1	-1	-3	-11
M	-3	-2	-4	-6	-8	-2	-4	-5	-5	1	2	0	9	-2	-5	-3	-2	-7	-6	1	-5	-3	-2	-11
F	-5	-6	-5	-9	-8	-8	-9	-6	-3	0	0	-9	-2	8	-7	-4	-5	-2	4	-4	-7	-8	-5	-11
P	0	-2	-3	-4	-5	-1	-3	-3	-2	-5	-4	-4	-5	-7	7	0	-2	-9	-8	-3	-3	-2	-2	-11
S	1	-1	1	-1	-1	-3	-2	0	-3	-4	-5	-2	-3	-4	0	4	2	-3	-4	-3	0	-2	-1	-11
T	1	-3	0	-2	-4	-3	-3	-2	-4	-1	-4	-1	-2	-5	-2	2	5	-8	-4	-1	-1	-3	-1	-11
W	-8	0	-5	-10	-10	-8	-11	-10	-4	-8	-3	-7	-7	-2	-9	-3	-8	13	-2	-10	-7	-9	-7	-11
Y	-5	-7	-3	-7	-2	-7	-6	-8	-1	-3	-4	-6	-6	4	-8	-4	-4	-2	9	-5	-4	-6	-4	-11
V	0	-5	-4	-5	-3	-4	-4	-3	-4	3	0	-5	1	-4	-3	-3	-1	-10	-5	6	-4	-4	-2	-11
B	-1	-3	4	5	-7	0	2	-1	0	-4	-6	-1	-5	-7	-3	0	-1	-7	-4	-4	5	2	-2	-11
Z	-1	-1	0	2	-9	5	5	-2	1	-4	-4	-1	-3	-8	-2	-2	-3	-9	-6	-4	2	5	-2	-11
X	-1	-3	-1	-3	-5	-2	-2	-3	-2	-2	-3	-3	-2	-5	-2	-1	-1	-7	-4	-2	-2	-2	-3	-11
*	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	-11	1

PAM40

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A	6	-6	-3	-3	-6	-3	-2	-1	-6	-4	-5	-6	-4	-7	-1	0	0	-12	-7	-2	-3	-2	-3	-15
R	-6	8	-5	-9	-7	-1	-8	-8	-1	-5	-8	1	-3	-8	-3	-2	-5	-1	-9	-7	-6	-3	-5	-15
N	-3	-5	7	2	-9	-3	-1	-2	1	-4	-6	0	-7	-8	-5	0	-1	-7	-4	-7	6	-2	-3	-15
D	-3	-9	2	7	-12	-2	3	-3	-3	-6	-11	-4	-9	-13	-7	-3	-4	-13	-10	-7	6	2	-5	-15
C	-6	-7	-9	-12	9	-12	-12	-8	-7	-5	-13	-12	-12	-11	-7	-2	-7	-14	-3	-5	-11	-12	-8	-15
Q	-3	-1	-3	-2	-12	8	2	-6	1	-7	-4	-2	-3	-11	-2	-4	-5	-11	-10	-6	-2	6	-4	-15
E	-2	-8	-1	3	-12	2	7	-3	-4	-5	-8	-4	-6	-12	-5	-4	-5	-15	-8	-6	2	6	-4	-15
G	-1	-8	-2	-3	-8	-6	-3	6	-8	-9	-9	-6	-7	-8	-5	-1	-5	-13	-12	-5	-2	-4	-4	-15
H	-6	-1	1	-3	-7	1	-4	-8	9	-8	-5	-5	-9	-5	-3	-5	-6	-6	-3	-6	-1	0	-4	-15
I	-4	-5	-4	-6	-5	-7	-5	-9	-8	8	-1	-5	0	-2	-7	-6	-2	-12	-5	2	-5	-5	-4	-15
L	-5	-8	-6	-11	-13	-4	-8	-9	-5	-1	7	-7	1	-2	-6	-7	-6	-5	-6	-2	-8	-6	-5	-15
K	-6	1	0	-4	-12	-2	-4	-6	-5	-5	-7	6	-1	-12	-6	-3	-2	-10	-8	-8	-2	-3	-4	-15
M	-4	-3	-7	-9	-12	-3	-6	-7	-9	0	1	-1	11	-3	-7	-5	-3	-11	-10	-1	-8	-4	-4	-15
F	-7	-8	-8	-13	-11	-11	-12	-8	-5	-2	-2	-12	-3	9	-9	-6	-8	-4	2	-7	-9	-12	-7	-15
P	-1	-3	-5	-7	-7	-2	-5	-5	-3	-7	-6	-6	-7	-9	8	-1	-3	-12	-12	-5	-6	-3	-4	-15
S	0	-2	0	-3	-2	-4	-4	-1	-5	-6	-7	-3	-5	-6	-1	6	1	-4	-6	-5	-1	-4	-2	-15
T	0	-5	-1	-4	-7	-5	-5	-5	-6	-2	-6	-2	-3	-8	-3	1	7	-11	-6	-2	-2	-5	-3	-15
W	-12	-1	-7	-13	-14	-11	-15	-13	-6	-12	-5	-10	-11	-4	-12	-4	-11	13	-4	-14	-9	-13	-9	-15
Y	-7	-9	-4	-10	-3	-10	-8	-12	-3	-5	-6	-8	-10	2	-12	-6	-6	-4	10	-6	-6	-8	-7	-15
V	-2	-7	-7	-7	-5	-6	-6	-5	-6	2	-2	-8	-1	-7	-5	-5	-2	-14	-6	7	-7	-6	-4	-15
B	-3	-6	6	6	-11	-2	2	-2	-1	-5	-8	-2	-8	-9	-6	-1	-2	-9	-6	-7	6	1	-4	-15
Z	-2	-3	-2	2	-12	6	6	-4	0	-5	-6	-3	-4	-12	-3	-4	-5	-13	-8	-6	1	6	-4	-15
X	-3	-5	-3	-5	-8	-4	-4	-4	-4	-4	-5	-4	-4	-7	-4	-2	-3	-9	-7	-4	-4	-4	-4	-15
*	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	-15	1

GONNET

```
C   S   T   P   A   G   N   D   E   Q   H   R   K   M   I   L   V   F   Y   W   X   *
C  12   0   0  -3   0  -2  -2  -3  -3  -2  -1  -2  -3  -1  -1  -2   0  -1   0  -1  -3  -8
S   0   2   2   0   1   0   1   0   0   0   0   0   0  -1  -2  -2  -1  -3  -2  -3   0  -8
T   0   2   2   0   1  -1   0   0   0   0   0   0   0  -1  -1  -1   0  -2  -2  -4   0  -8
P  -3   0   0   8   0  -2  -1  -1   0   0  -1  -1  -1  -2  -3  -2  -2  -4  -3  -5  -1  -8
A   0   1   1   0   2   0   0   0   0   0  -1  -1   0  -1  -1  -1   0  -2  -2  -4   0  -8
G  -2   0  -1  -2   0   7   0   0  -1  -1  -1  -1  -1  -4  -4  -4  -3  -5  -4  -4  -1  -8
N  -2   1   0  -1   0   0   4   2   1   1   1   0   1  -2  -3  -3  -2  -3  -1  -4   0  -8
D  -3   0   0  -1   0   0   2   5   3   1   0   0   0  -3  -4  -4  -3  -4  -3  -5  -1  -8
E  -3   0   0   0   0  -1   1   3   4   2   0   0   1  -2  -3  -3  -2  -4  -3  -4  -1  -8
Q  -2   0   0   0   0  -1   1   1   2   3   1   2   2  -1  -2  -2  -2  -3  -2  -3  -1  -8
H  -1   0   0  -1  -1  -1   1   0   0   1   6   1   1  -1  -2  -2  -2   0   2  -1  -1  -8
R  -2   0   0  -1  -1  -1   0   0   0   2   1   5   3  -2  -2  -2  -2  -3  -2  -2  -1  -8
K  -3   0   0  -1   0  -1   1   0   1   2   1   3   3  -1  -2  -2  -2  -3  -2  -4  -1  -8
M  -1  -1  -1  -2  -1  -4  -2  -3  -2  -1  -1  -2  -1   4   2   3   2   2   0  -1  -1  -8
I  -1  -2  -1  -3  -1  -4  -3  -4  -3  -2  -2  -2  -2   2   4   3   3   1  -1  -2  -1  -8
L  -2  -2  -1  -2  -1  -4  -3  -4  -3  -2  -2  -2  -2   3   3   4   2   2   0  -1  -1  -8
V   0  -1   0  -2   0  -3  -2  -3  -2  -2  -2  -2  -2   2   3   2   3   0  -1  -3  -1  -8
F  -1  -3  -2  -4  -2  -5  -3  -4  -4  -3   0  -3  -3   2   1   2   0   7   5   4  -2  -8
Y   0  -2  -2  -3  -2  -4  -1  -3  -3  -2   2  -2  -2   0  -1   0  -1   5   8   4  -2  -8
W  -1  -3  -4  -5  -4  -4  -4  -5  -4  -3  -1  -2  -4  -1  -2  -1  -3   4   4  14  -4  -8
X  -3   0   0  -1   0  -1   0  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -2  -2  -4  -1  -8
*  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8  -8   1
```

MATCH

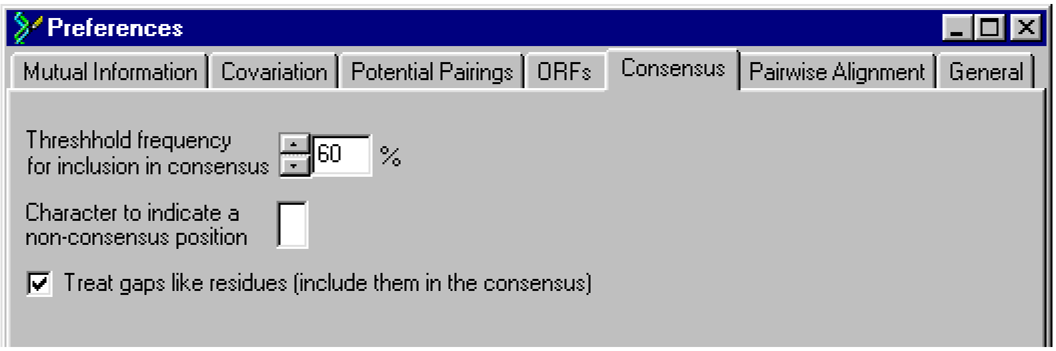
```
A   R   N   B   D   C   Q   Z   E   G   H   I   L   K   M   F   P   S   T   W   Y   V   X   *
A   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
R  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
N  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
B  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
D  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
C  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
Q  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
Z  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
E  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
G  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
H  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
I  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
L  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
K  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1
M  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1  -1
F  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1  -1
P  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1  -1
S  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1  -1
T  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1  -1
W  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1  -1
Y  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1  -1
V  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   1  -1  -1
X  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   0  -1
*  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1  -1   0
```

IDENTIFY

Same as MATCH, with -10000 in place of all of the 1's.

Consensus sequences

BioEdit allows the generation of a simple consensus sequence with the following parameters (to change the settings, choose "Options->Preferences->Consensus"):



Threshhold frequency for inclusion in consensus 60 %

Character to indicate a non-consensus position

☑ Treat gaps like residues (include them in the consensus)

You may choose to include or exclude gaps in the consensus sequence. If gaps are included, they will show up in the consensus if they are the dominant character. If gaps are not included, they will not show up in the consnsus, but will be calculated into the percentages. However, if they are not chosen as valid residue characters (see "Valid residue characters vs non-residue characters"), they will not participate in the calculations either. Keep in mind that all characters are treated separately for the handling of valid residues vs non-residue characters, so if all gap characters are to be recognized (-, ~ and .), they *all* must be present in both the amino acid and nucleic acid lists of valid residues.

RNA comparative analysis

The basis of phylogenetic comparative analysis

Reproduced with permission from “Covariation”, the Macintosh hypercard program by Dr. James W. Brown

This program can be found from the RNase P database:
http://jwbrown.mbio.ncsu.edu/RNaseP

The structures of RNAs are primarily defined by the interactions between nucleotide bases - in the simplest case, by Watson-Crick base-pairing between base pairs in a helix. The phylogenetic comparative method for analyzing RNA structure is based on the premise that the important secondary and tertiary structure in an RNA molecule remains the same despite changes in the nucleotide sequence of the RNA during evolution; any change in the sequence that might disrupt the structure is compensated for by change elsewhere in the sequence that allows the maintenance of the active structure. The homologous RNAs of different organisms will therefore contain "compensating base changes", or covariations. The structure of an RNA can therefore be elucidated by examination of homologous RNA sequences from a variety of organisms in order to identify such compensating base changes. For example, a given sequence, e.g. GAAGA, will have the potential to base pair with any UCUUC sequence within the RNA - such a sequence will most likely occur several times in that RNA. In order to identify which UCUUC sequence the GAAGA actually base pairs with (if any), the homologous nucleotides in the RNA from different organisms are examined in an attempt to identify compensating base changes:

```
                   *           x               x            *
organism #1 -----GAAGA---------UCUUC--------UCUUC---------UCUUC-------
organism #2 -----GAUGA---------UCUUC--------UCUGC---------UCAUC-------
organism #2 -----GAUGA---------GCUUC--------UCUAC---------UCAUC-------
organism #2 -----GACGA---------UCUUC--------UCUGC---------UCGUC-------
```

In the above example, only the last UCUUC (UCUUC in organism #1, that is) sequence changes to maintain the ability to base pair with the GAAGA sequence. Such compensating base changes at two positions in a potential helix are considered "proof" of the presence of that helix. Failure of two sequence to maintain complementarity suggests that the pairing do not occur.
The key to a phylogenetic comparative analysis of RNA structure is the alignment of the sequences - homologous nucleotides must be properly aligned. Homology is used here in its strictest sense - "homologous" nucleotides are defined as those which share a common ancestor. It is best therefore to begin by aligning closely related sequences, which can be aligned reliably on the basis of sequence similarity without the need for numerous alignment gaps. A handful of covariations between complementary sequences can usually be readily identified from these alignments, which starts the process of building of the secondary structure model. Starting with the beginning secondary structure model, more divergent sequences can be added to the alignment. This process is repeated by the sequential addition of new sequences & covariation analysis until both the alignment and secondary structure model emerge. A complete description of this process can be found in the references (see "More Information").

Once a complete secondary structure model is available, covariation analysis can be used to identify interactions between nucleotides that are not in helices (higher-order structure), non-canonical interactions, etc. Such interactions are identified because the associated nucleotides will vary in concert (i.e. covary), even if the do not form a canonical base pair or are part of a longer helix.

Using Masks

Masks are used to indicate a subsection of an alignment to be included in an analysis, at the exclusion of everything else. For example, if you have an alignment of long RNA sequences and you want to do a comparative analysis on only a small region to decipher a localized secondary structure, you might want to exclude from the analysis the parts of the sequences that you do not want data for. By specifying a mask prior to running covariation, potential pairings or mutual information analyses, you tell the program to report data only for specified positions. Sometimes one wishes to analyze an RNA secondary structure element in a structure for which a standardized numbering system based on the structure from one organism exists (for example the RNase P RNA from E. coli is often used to number nearly universal positions for all bacterial RNase P RNAs). For this purpose, a sequence may be set as a numbering mask, and positions of bases in a comparative analysis will be numbered to correspond to the numbering mask. Often the numbering mask and sequence mask are the same.

Conventions used in BioEdit for masks:
For any mask, the three characters '-', '~' and '.' (all gap indicators) designate that a position is *not to be included* in an analysis.
Any other character specifies that the position be included.
For masks created in BioEdit, a '*' generally indicates inclusion, a '-' indicates exclusion.

A mask might look like this:

```
-----**********-----**********-----
```

This specifies that the first 5 are excluded, the next 10 included, etc.

A sequence and numbering mask may be used simultaneously in an analysis, but neither one is a requirement. If the numbering is set to mask numbering in an analysis preferences set, then there must be a numbering mask specified.

To set a sequence as a mask or numbering mask, choose the "Set as Sequence Mask" or "Set as Numbering Mask" option under the "Sequence" menu.

To create a new mask, select the "Create New Mask" option under the "Sequence" menu. The new mask will be created as a series of asterisks (e.g. "***")

To toggle mask positions on or off, select the region to be toggled with the mouse, then choose "Toggle Mask" under the "Sequence" menu.

Covariation

Covariation refers to the situation where two residues in a sequence vary in concert. Strictly speaking, this means that whenever position ‘x’ varies from the norm in an alignment, position ‘y’ also varies, and that the variation is consistent (for example, if ‘x’ changes to ‘A’ and ‘y’ to ‘T’, then every time ‘x’ is an ‘A’ ‘y’ must be a ‘T’). Covariation between residues suggests that there might be an essential interaction between them and that nature has selected for compensatory mutations when mutations in important structural residues have occurred (see **The basis of phylogenetic comparative analysis**).

Covariation example:

Let’s say we have an alignment of sequences that represent a particular RNA with a conserved secondary structure from several different organisms. We want to use information contained in the alignment to deduce something about the secondary structure of this RNA. As an example, take this short segment of an alignment:

```
             ....|....| ....|....| ....|....
                      10         20
sample 1     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 2     CCGGAUACUA UCUUGGCGAA AGUAUCUGG
sample 3     CGGGAUACGA UCGACGCGUA CGUAUCCCG
sample 4     CGCGGUACCA UCCACCCCUA GGUACCGCG
sample 5     CCGGAUACGA UCGUCCCGUU CGUAUCCGG
sample 6     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 7     CCGGACACGA UCGUCGGGUA CGUAUCCGG
sample 8     CCAGAUACGA UCGAAACUUU CGUAUCUGG
sample 9     CCGGUUACCA UCGUCGGGUA GGUAACCGG
sample 9     CCGGAUACGA UCGACAGGAA CGUAUCCGG
sample 10    CCGGAUACGA UCGUCCCGUA CGUAUCCGG
sample 11    CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 12    CCUGAUACUA UCGUCGCCUA AGUAUCGGG
sample 13    CGGGGUACGA UCGAGGCCUA CGUACCCCG
sample 14    CCCGCUACGA UCGAGGCCUU CGUAGCGGG
sample 15    CCGGAUACGA UCGAGGCCUU CGUAUCCGG
```

```
Covariation analysis
Input file: I:\BioEdit\help\samples.gb
Position numbering is relative to the alignment numbering.
No mask was used.

1     CCCCCCCCCCCCCCCC
------------------------

Position 2:
------------------------
2     CCGGCCCCCCCCCGCC
28    GGCCGGGGGGGGGCGG    All potential Watson Crick or G-U pairs
------------------------
3     GGGCGGGAGGGGUGCG
------------------------
4     GGGGGGGGGGGGGGGG
------------------------
```

```
Position 5:
------------------------
5     AAAGAAAAUAAAAGCA
25    UUUCUUUUAUUUUCGU    All potential Watson Crick or G-U pairs
------------------------
6     UUUUUUCUUUUUUUUU
------------------------
7     AAAAAAAAAAAAAAAA
------------------------
8     CCCCCCCCCCCCCCCC
------------------------

Position 9:
------------------------
9     GUGCGGGGCGGGUGGG
21    CACGCCCCGCCCACCC    All potential Watson Crick or G-U pairs
------------------------
10    AAAAAAAAAAAAAAAA
------------------------
11    UUUUUUUUUUUUUUUU
------------------------
12    CCCCCCCCCCCCCCCC
------------------------
13    GUGCGGGGGGGGGGGG
------------------------
14    UUAAUUUAUAUUUAAA
------------------------
15    CGCCCCCACCCCCGGG
------------------------
16    GGGCCGGAGACGGGGG
------------------------
17    GCCCCGGCGGCGCCCC
------------------------
18    GGGCGGGUGGGGCCCC
------------------------
19    UAUUUUUUUAUUUUUU
------------------------
20    AAAAUAAUAAAAAAUU
------------------------

Position 21:
------------------------
21    CACGCCCCGCCCACCC
9     GUGCGGGGCGGGUGGG    All potential Watson Crick or G-U pairs
------------------------
22    GGGGGGGGGGGGGGGG
------------------------
23    UUUUUUUUUUUUUUUU
------------------------
24    AAAAAAAAAAAAAAAA
------------------------

Position 25:
------------------------
25    UUUCUUUUAUUUUCGU
5     AAAGAAAAUAAAAGCA    All potential Watson Crick or G-U pairs
------------------------
26    CCCCCCCCCCCCCCCC
------------------------
27    CUCGCCCUCCCCGCGC
------------------------
Position 28:
------------------------
```

```
28    GGCCGGGGGGGGGCGG
2     CCGGCCCCCCCCCGCC    All potential Watson Crick or G-U pairs
------------------------
29    GGGGGGGGGGGGGGGG
------------------------
```

There are 3 pairs of positions that “covary” in this alignment section: 2/28, 5/25 and 9/21. When two bases covary, there is a strong possibility that they interact. When a mutation occurs in a base that makes an important contact with another nucleotide (usually a base pair), selection pressure may dictate that only instances where the other base shows a compensatory change survive. The covariations in the three pairs of bases above suggest that theses bases may interact. The fact that they all involve bases that can form canonical base pairings (Watson-Crick, or G-U for RNA), suggests that they may base-pair. Residues 2 and 5 are the same distance apart as 5 and 25, and 5 and 25 are the same distance apart as 9 and 21. By looking at the alignment, we can see that these intervening bases can also form base-pairs, suggesting that the two ends of this alignment may be joined in a helix, as shown below for the sequence “sample 1":

```
                      U C
                    A     G
-- C C G G A T A C G       U
-- G G C C T A T G C       C
                    A     G
                     U G G
```

The other bases along the helix are invariant. Comparative analysis at these positions does not provide direct evidence of interaction. However, combined with analysis of potential pairings, the positions of these residues suggests that they may be involved in helix base-pairing.

Brown, J.W. 1991. Phylogenetic comparative analysis on Macintosh computers. *Comput. Appl. Biosci. 7(3)*:391-393.

Gutell, R.R. 1985. Comparative anatomy of 16-S-like ribosomal RNA. *Prog. Nucl. Acids Res. Mol. Biol. 32*:155-216.

Using Covariation in BioEdit

BioEdit provides two basic output formats for covariation data: list format or table format. Click on either of the above for a description and example of each format.
The output is raw text for both. Table formats may be tab delimited or comma delimited. Tab delimited files are best if the table will be viewed in a text editor. Comma delimited files (*.csv) allow easy importing into a spreadsheet such as Microsoft Excel (most also read tab delimited files). Files may be written in PC or Macintosh format.

To perform a covariation analysis from a BioEdit alignment document:

1. Set the preferences you want (file format, output type -- you may choose to output both a list and table if you want).

2. If you want to analyze only a portion of the alignment, create a mask (or set an existing sequence to be a mask). If you would like to analyze only a portion of the alignment, but would like the numbering of alignment positions to match the number of a standard sequence (must be included in the alignment), set that sequence as the numbering mask).

3. Select all the sequences you want included in the analysis. Only selected sequences will be analyzed. If there is a mask specified that is not an actual sequence, you will want to exclude it from the analysis. If no sequences are selected, all sequences in the document will be automatically selected.

4. Run Covariation from the "RNA" menu. You will be prompted for the name(s) of the output file(s). If you choose to generate a list, it will be opened for you in the BioEdit text editor.

Considerations for each format:

List files can be quite long. each column is printed out as a string and for each two columns which covary, the two columns are printed one on top of the other. If only the positions are desired, this may be specified in the preferences. Also, the option to show or exclude output of columns for invariant positions is offered.

Table format: A table is often nice to look at in a spreadsheet on-screen, but is often not very convenient for printing out, especially when the analysis is fairly large.

Table output

Covariation tables are formatted as a 2-dimensional matrix of alignment positions (each position compared to every other position). When two positions covary, a ‘5' is placed at the matrix intersection of the two positions. If both of the positions are invariant, a ‘1' is placed in that position. When they are not both invariant and do not covary, a 0 is placed at their intersection.

As an example, take the following short alignment:

```
                 ....|....| ....|....| ....|....
                      10         20
sample 1     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 2     CCGGAUACUA UCUUGGCGAA AGUAUCCGG
sample 3     CGGGAUACGA UCGACGCGUA CGUAUCCCG
sample 4     CGGGGUACCA UCCACCCCUA GGUACCCCG
sample 5     CCGGAUACGA UCGUCCCGUU CGUAUCCGG
sample 6     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 7     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 8     CCGGAUACGA UCGAAACUUU CGUAUCCGG
sample 9     CCGGUUACCA UCGUCGGGUA GGUAACCGG
sample 9     CCGGAUACGA UCGACAGGAA CGUAUCCGG
sample 10    CCGGAUACGA UCGUCCCGUA CGUAUCCGG
sample 11    CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 12    CCGGAUACUA UCGUCGCCUA AGUAUCCGG
sample 13    CGGGGUACGA UCGAGGCCUA CGUACCCCG
sample 14    CCGGCUACGA UCGAGGCCUU CGUAGCCGG
sample 15    CCGGAUACGA UCGAGGCCUU CGUAUCCGG
```

Table output of covariation data would look like this (larger tables will appear wrapped in a word processor, but may be viewed unwrapped in an editor such as WordPad):

```
Covariation analysis
Input file: D:\BioEdit\help\samples.gb
Matrix Output
5 = Covariation, 1 = Invariant
Position numbering is relative to the alignment numbering.
No mask was used.

   1  2  3  4  5  6  7  8  9  10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
1  0  0  1  1  0  1  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
2  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  5  0
3  1  0  0  1  0  1  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
4  1  0  1  0  0  1  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
5  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  5  0  0  0  0
6  1  0  1  1  0  0  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
7  1  0  1  1  0  1  0  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
8  1  0  1  1  0  1  1  0  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
9  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  5  0  0  0  0  0  0  0  0
10 1  0  1  1  0  1  1  1  0  0  1  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
11 1  0  1  1  0  1  1  1  0  1  0  1  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
12 1  0  1  1  0  1  1  1  0  1  1  0  0  0  0  0  0  0  0  0  0  1  1  1  0  1  1  0  1
13 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
14 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
15 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
16 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
17 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
18 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
19 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
20 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
21 0  0  0  0  0  0  0  0  5  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
22 1  0  1  1  0  1  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  0  1  1  0  1  1  0  1
23 1  0  1  1  0  1  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  0  1  0  1  1  0  1
24 1  0  1  1  0  1  1  1  0  1  1  1  0  0  0  0  0  0  0  0  0  1  1  0  0  1  1  0  1
25 0  0  0  0  5  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0
```

```
26 1   0   1   1   0   1   1   1   0   1   1   1   0   0   0   0   0   0   0   0   0   1   1   1   0   0   1   0   1
27 1   0   1   1   0   1   1   1   0   1   1   1   0   0   0   0   0   0   0   0   0   1   1   1   0   1   0   0   1
28 0   5   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0
29 1   0   1   1   0   1   1   1   0   1   1   1   0   0   0   0   0   0   0   0   0   1   1   1   0   1   1   0   0
```

Where two positions covary, a 5 is placed at their intersection. When there is no covariation, there is a 0. A 1' indicated a pair of columns where both are invariant. Sometimes it is useful to look at these positions along with the covariation data to see if invariant positions that fall in line with covarying pairs are able to form base pairs. This type of information can also be gathered in an analysis of potential pairings. A comma-delimited file may also be produced which allows easy opening in a spreadsheet program such as Microsoft Excel or Quattro Pro.

List output

An example of covariation list output is shown in the main section of covariation. Covariation lists may be written with the following options:

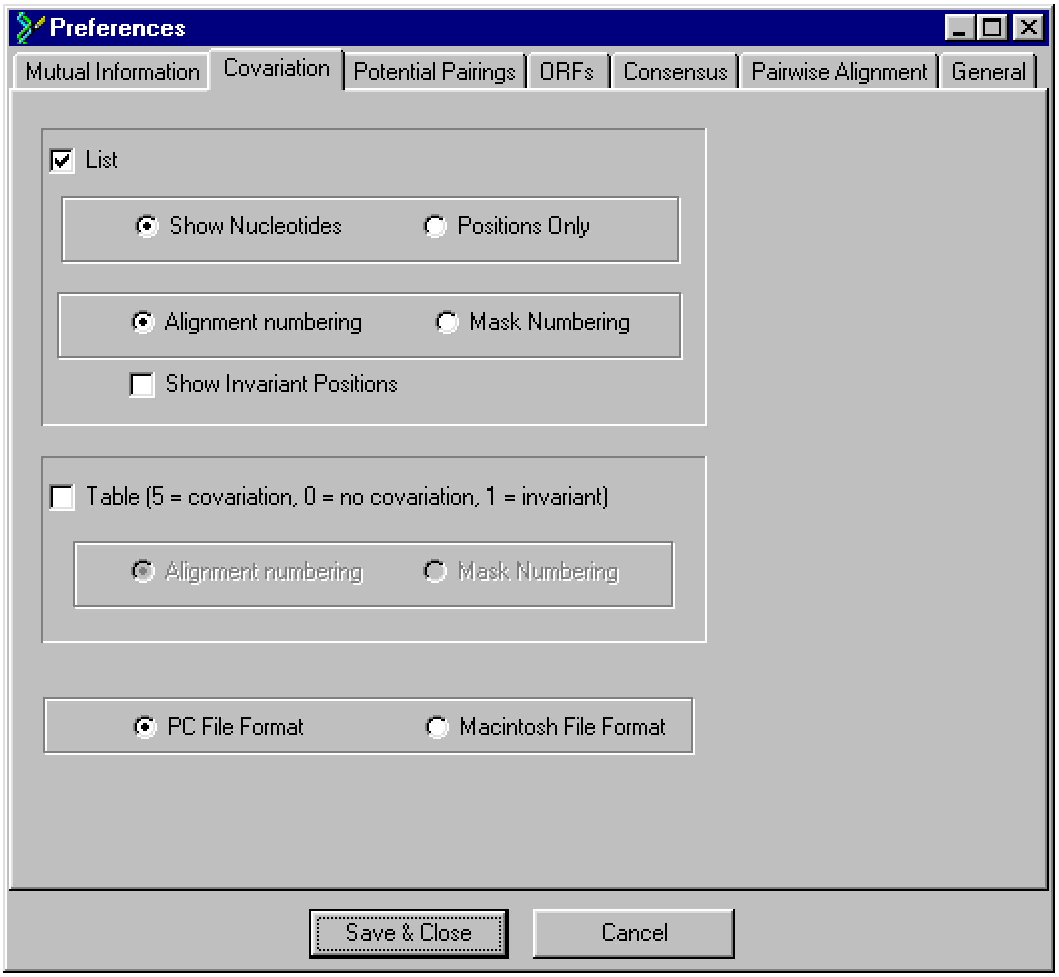
-- Show nucleotides or show positions only:
Show nucleotides: Reports the each column of the alignment (position) as a string of nucleotide bases.
Show positions only: Only shows the positions of pairs of positions that covary. This is useful if you want a small file and will be looking at a printout of the alignment or viewing it on-screen. Position-only output of the same analysis shown in the example would be:

```
2, 28    All potential Watson Crick or G-U pairs
5, 25    All potential Watson Crick or G-U pairs
9, 21    All potential Watson Crick or G-U pairs
21, 9    All potential Watson Crick or G-U pairs
25, 5    All potential Watson Crick or G-U pairs
28, 2    All potential Watson Crick or G-U pairs
```

Often this is much easier to look at.

Covariation analysis preferences

The covariation preferences dialog may be brought up by choosing “Preferences” from the “Options” menu:



☑ List

● Show Nucleotides ○ Positions Only

● Alignment numbering ○ Mask Numbering

□ Show Invariant Positions

□ Table (5 = covariation, 0 = no covariation, 1 = invariant)

● Alignment numbering ○ Mask Numbering

● PC File Format ○ Macintosh File Format

Both list and table formats may be chosen at the same time. Mask numbering will cause the reported position numbers to match the true position numbers of the selected numbering mask. Reporting nucleotides compared to positions only is explained in covariation lists.

The Covariation Algorithm

The covariation algorithm is quite simple. Two positions are said to covary if they both show at least some variation through the alignment (are not invariant) and follow an identical pattern (vary in perfect concert). The algorithm works like this:

1. The alignment is divided up into vertical columns (an alignment is really a 2-D matrix of characters with rows and columns of characters).

2. Each column is converted to a string of numbers in the following manner:

a. The first residue in each column is assigned the number 1.
b. If the next residue is the same as the first, it is assigned number 1, otherwise it is assigned 2.
c. As each characters in each column is examined one at a time, if it is unique (the first occurrence of that character in that column), it is assigned the next untaken integer, otherwise it is given the same number as the previous occurrence of that character.
d. When this is done, columns that represent invariant positions will be strings of all 1's. Two positions that covary (vary in exact concert) will have an identical pattern of numbers.

Example: Take the following four columns:

```
A C G G
A C G G
A C G G
A A U G
A G C C
A C G C
A C G C
A U A C
A C G G
A A U G
```

The number string representations for these would be:

```
1.  1111111111
2.  1112311412
3.  1112311412
4.  1111222211
```

Position 1 is invariant and is represented by all 1's. Positions 2 and 3 covary and result in the same string of numbers. Position 4 does not covary with 1, 2 or 3. This algorithm is easily implemented by comparing strings for an exact match. It is the simple and quick, but it does not allow for any exceptions in the pattern (for example, an A-T pair might change to a G-C in one sequence and a G-U in another -- this would be missed by covariation). On the other hand, it does not depend on guessing at which interactions are likely and may pick out necessary tertiary interactions in large sequence sets.

Brown, J.W. 1991. Phylogenetic comparative analysis on Macintosh computers. *Comput. Appl. Biosci. 7(3)*:391-393.

Gutell, R.R. 1985. Comparative anatomy of 16-S-like ribosomal RNA. *Prog. Nucl. Acids Res. Mol. Biol. 32*:155-216.

Potential Pairings

When two nucleotides in an RNA molecule have a necessary base-pairing interaction, sometimes a compensatory base change to more than one particular nucleotide is sufficient to complement a mutation (for example, an A-U pair may mutate to a G-C in one sequence and to a G-U in another). This type of interaction will be missed by a covariation analysis because the variation will not follow an identical pattern at each position. An analysis of positions that have the ability to form pairs conforming to a set of allowed choices (set in the preferences dialog) will allow the identification of these positions.

Potential pairings, as implemented in BioEdit, does not require that the positions show any variation, and so invariant positions which can form base pairs will also be reported. The option to filter out invariant-invariant pairings is offered in the preferences setup screen.

Potential pairings example

Using the same sample alignment as used in the covariation example:

```
             ....|....| ....|....| ....|....
                      10         20
sample 1     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 2     CCGGAUACUA UCUUGGCGAA AGUAUCUGG
sample 3     CGGGAUACGA UCGACGCGUA CGUAUCCCG
sample 4     CGCGGUACCA UCCACCCCUA GGUACCGCG
sample 5     CCGGAUACGA UCGUCCCGUU CGUAUCCGG
sample 6     CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 7     CCGGACACGA UCGUCGGGUA CGUAUCCGG
sample 8     CCAGAUACGA UCGAAACUUU CGUAUCUGG
sample 9     CCGGUUACCA UCGUCGGGUA GGUAACCGG
sample 9     CCGGAUACGA UCGACAGGAA CGUAUCCGG
sample 10    CCGGAUACGA UCGUCCCGUA CGUAUCCGG
sample 11    CCGGAUACGA UCGUCGGGUA CGUAUCCGG
sample 12    CCUGAUACUA UCGUCGCCUA AGUAUCGGG
sample 13    CGGGGUACGA UCGAGGCCUA CGUACCCCG
sample 14    CCCGCUACGA UCGAGGCCUU CGUAGCGGG
sample 15    CCGGAUACGA UCGAGGCCUU CGUAUCCGG
```

An analysis of potential pairings allowing for A-U, G-C and G-U base pairs with 1 mismatch would yield the following (list format, filtered to ignore potential pairings between two invariant positions). Examination of this data compared to the sample output for covariation reveals a possible base-pair between positions 3 and 27 which is not picked up by the covariation algorithm. Potential pairing data may also be written as a numerical table (2-D matrix) of either frequency of allowed pairings or raw number of allowed pairings between each pair of positions.

```
Potential Pairings List
Input File: I:\BioEdit\help\samples.gb
Allowed Mispairings = 1
16 total sequences, 29 nucleotides per sequence.
Axes reflect numbering of the entire alignment.
No Mask was used.
Hits on invariant pairs have been filtered out.


------------------------
```

```
1    CCCCCCCCCCCCCCCC
------------------------
Position: 2
------------------------
2    CCGGCCCCCCCCCGCC
28   GGCCGGGGGGGGGCGG   0 mis-matches
------------------------

Position: 3
------------------------
3    GGGCGGGAGGGGUGCG
27   CUCGCCCUCCCCGCGC   0 mis-matches
------------------------

Position: 4
------------------------
4    GGGGGGGGGGGGGGGG
6    UUUUUUCUUUUUUUUU   0 mis-matches
------------------------

Position: 5
------------------------
5    AAAGAAAAUAAAAGCA
25   UUUCUUUUAUUUUCGU   0 mis-matches
------------------------

Position: 6
------------------------
6    UUUUUUCUUUUUUUUU
4    GGGGGGGGGGGGGGGG   0 mis-matches
------------------------
6    UUUUUUCUUUUUUUUU
7    AAAAAAAAAAAAAAAA   1 mis-matches
------------------------
6    UUUUUUCUUUUUUUUU
10   AAAAAAAAAAAAAAAA   1 mis-matches
------------------------
6    UUUUUUCUUUUUUUUU
22   GGGGGGGGGGGGGGGG   0 mis-matches
------------------------
6    UUUUUUCUUUUUUUUU
24   AAAAAAAAAAAAAAAA   1 mis-matches
------------------------
6    UUUUUUCUUUUUUUUU
29   GGGGGGGGGGGGGGGG   0 mis-matches
------------------------

Position: 7
------------------------
7    AAAAAAAAAAAAAAAA
6    UUUUUUCUUUUUUUUU   1 mis-matches
------------------------

8    CCCCCCCCCCCCCCCC
------------------------
Position: 9
------------------------
9    GUGCGGGGCGGGUGGG
21   CACGCCCCGCCCACCC   0 mis-matches
------------------------

Position: 10
------------------------
10   AAAAAAAAAAAAAAAA
6    UUUUUUCUUUUUUUUU   1 mis-matches
------------------------

11   UUUUUUUUUUUUUUUU
------------------------
12   CCCCCCCCCCCCCCCC
------------------------
13   GUGCGGGGGGGGGGGG
------------------------
```

```
14   UUAAUUUAUAUUUAAA
------------------------
15   CGCCCCCACCCCCGGG
------------------------
16   GGGCCGGAGACGGGGG
------------------------
17   GCCCCGGCGGCGCCCC
------------------------
18   GGGCGGGUGGGGCCCC
------------------------
19   UAUUUUUUUAUUUUUU
------------------------
20   AAAAUAAUAAAAAAUU
------------------------
Position: 21
------------------------
21   CACGCCCCGCCCACCC
9    GUGCGGGGCGGGUGGG   0 mis-matches
------------------------

Position: 22
------------------------
22   GGGGGGGGGGGGGGGG
6    UUUUUUCUUUUUUUUU   0 mis-matches
------------------------

23   UUUUUUUUUUUUUUUU
------------------------
Position: 24
------------------------
24   AAAAAAAAAAAAAAAA
6    UUUUUUCUUUUUUUUU   1 mis-matches
------------------------

Position: 25
------------------------
25   UUUCUUUUAUUUUCGU
5    AAAGAAAAUAAAAGCA   0 mis-matches
------------------------

26   CCCCCCCCCCCCCCCC
------------------------
Position: 27
------------------------
27   CUCGCCCUCCCCGCGC
3    GGGCGGGAGGGGUGCG   0 mis-matches
------------------------

Position: 28
------------------------
28   GGCCGGGGGGGGGCGG
2    CCGGCCCCCCCCCGCC   0 mis-matches
------------------------

Position: 29
------------------------
29   GGGGGGGGGGGGGGGG
6    UUUUUUCUUUUUUUUU   0 mis-matches
------------------------
```

Brown, J.W. 1991. Phylogenetic comparative analysis on Macintosh computers.   *Comput. Appl. Biosci.* *7(3)*:391-393.

Using Potential Pairings in BioEdit

BioEdit provides two basic output formats for potential pairings data: lists or table format. Click on either of the above for a description and example of each format.
The output is raw text for both. Table formats may be tab delimited or comma delimited. Tab delimited files are best if the table will be viewed in a text editor. Comma delimited files (*.csv) allow easy importing into a spreadsheet such as Microsoft Excel (most also read tab delimited files). Files may be written in PC or Macintosh format.

To perform a potential pairings analysis from a BioEdit alignment document:

1. Set the preferences you want (file format, output type -- you may choose to output both a list and table if you want).

2. Before staring, you will also want to decide which pairings are to be allowed. By default, allowed pairings are initially set to A-T, C-G and G-U, but whenever preferences are saved from the preferences dialog, these become the new defaults.

3. If you want to analyze only a portion of the alignment, create a mask (or set an existing sequence to be a mask). If you would like to analyze only a portion of the alignment, but would like the numbering of alignment positions to match the number of a standard sequence (must be included in the alignment), set that sequence as the numbering mask).

4. Select all the sequences you want included in the analysis. Only selected sequences will be analyzed. If there is a mask specified that is not an actual sequence, you will want to exclude it from the analysis. If no sequences are selected, all sequences in the document will be automatically selected.

5. Run “Potential Pairings” from the “RNA” menu of an open alignment document. You will be prompted for the name(s) of the output file(s). If you choose to generate a list, it will be opened for you in the BioEdit text editor.

Considerations for each format:

List files can be quite long. Invariant positions are shown and can often produce many redundant matches that most likely don’t mean anything. For this reason, pairings between two invariant positions may be filtered out.

Table format: A table is often nice to look at in a spreadsheet on-screen, but is often not very convenient for printing out, especially when the analysis is fairly large.

List output

An example of list output for a potential pairings is shown in the main section of potential pairings. If you do not wish to show all the nucleotides, and do not want positions displayed that have no potential pairings matches to other positions, choose the “positions only” option in the preferences dialog. An example of list output with the positions only option chosen for the sample alignment shown in the main section of potential pairings is shown below. The option is also offered to filter out matches between two invariant positions.

```
------------------------
Position: 1
------------------------
4     0 mis-matches
22    0 mis-matches
29    0 mis-matches

Position: 2
------------------------
28    0 mis-matches

Position: 3
------------------------
27    0 mis-matches

Position: 4
------------------------
1     0 mis-matches
6     0 mis-matches
8     0 mis-matches
11    0 mis-matches
12    0 mis-matches
23    0 mis-matches
26    0 mis-matches

Position: 5
------------------------
25    0 mis-matches

Position: 6
------------------------
4     0 mis-matches
7     1 mis-matches
10    1 mis-matches
22    0 mis-matches
24    1 mis-matches
29    0 mis-matches

Position: 7
------------------------
6     1 mis-matches
11    0 mis-matches
23    0 mis-matches

Position: 8
------------------------
4     0 mis-matches
22    0 mis-matches
29    0 mis-matches

Position: 9
------------------------
```

```
21    0 mis-matches

Position: 10
------------------------
6     1 mis-matches
11    0 mis-matches
23    0 mis-matches

Position: 11
------------------------
4     0 mis-matches
7     0 mis-matches
10    0 mis-matches
22    0 mis-matches
24    0 mis-matches
29    0 mis-matches

Position: 12
------------------------
4     0 mis-matches
22    0 mis-matches
29    0 mis-matches

Position: 21
------------------------
9     0 mis-matches

Position: 22
------------------------
1     0 mis-matches
6     0 mis-matches
8     0 mis-matches
11    0 mis-matches
12    0 mis-matches
23    0 mis-matches
26    0 mis-matches

Position: 23
------------------------
4     0 mis-matches
7     0 mis-matches
10    0 mis-matches
22    0 mis-matches
24    0 mis-matches
29    0 mis-matches

Position: 24
------------------------
6     1 mis-matches
11    0 mis-matches
23    0 mis-matches

Position: 25
------------------------
5     0 mis-matches

Position: 26
------------------------
4     0 mis-matches
22    0 mis-matches
29    0 mis-matches

Position: 27
------------------------
```

```
3     0 mis-matches

Position: 28
------------------------
2     0 mis-matches

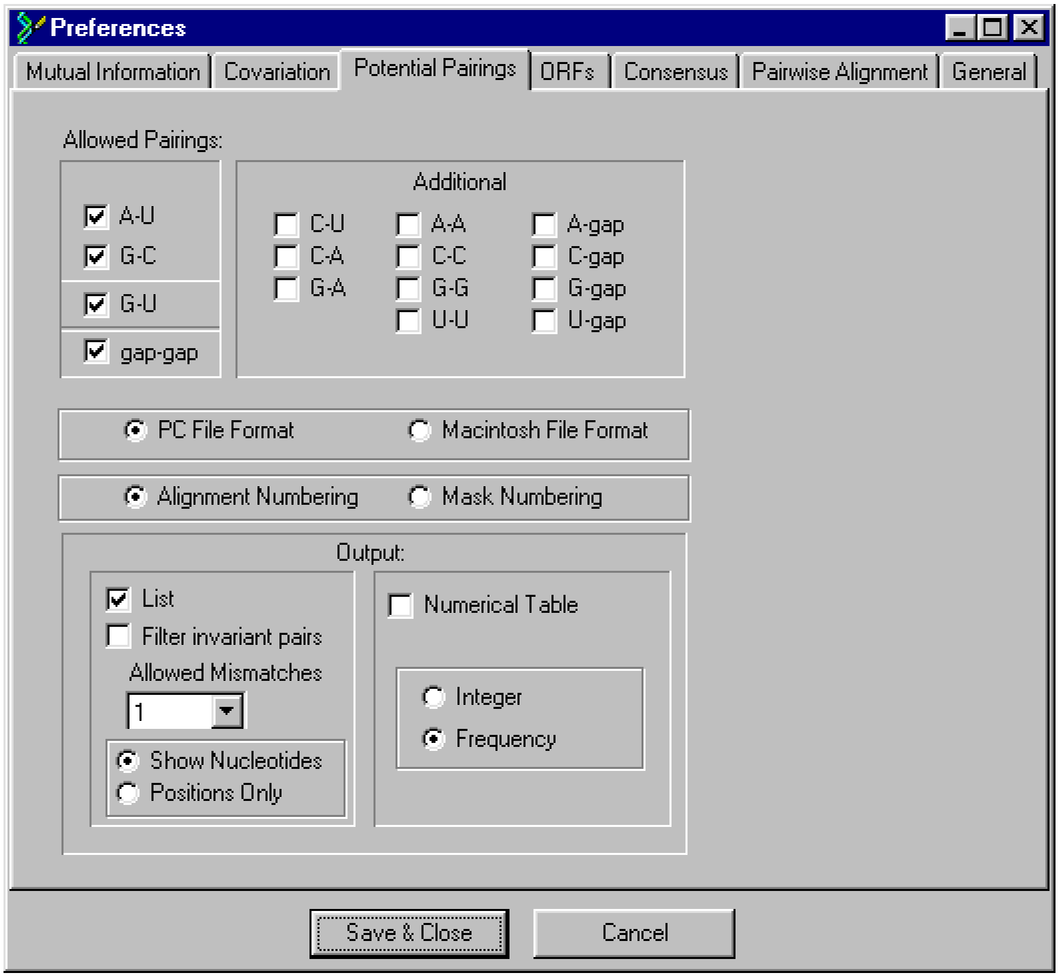
Position: 29
------------------------
1     0 mis-matches
6     0 mis-matches
8     0 mis-matches
11    0 mis-matches
12    0 mis-matches
23    0 mis-matches
26    0 mis-matches
```

Table ouput

Potential pairings data can also be output as a numerical 2-D matrix, with the intersection of each two positions containing either the number of sequences in which they can form an allowed pair, or the frequency with which they form an allowed pair. A table of potential pairings data will be formatted in the same manner as a covariation table.

Potential pairings analysis preferences

To bring up the potential pairings preferences, choose “Preferences” from the “Options” menu and click on the tab for potential pairings:



Allowed Pairings:

☑ A-U
☑ G-C
☑ G-U
☑ gap-gap

Additional

☐ C-U ☐ A-A ☐ A-gap
☐ C-A ☐ C-C ☐ C-gap
☐ G-A ☐ G-G ☐ G-gap
☐ U-U ☐ U-gap

● PC File Format ○ Macintosh File Format

● Alignment Numbering ○ Mask Numbering

Output:

☑ List
☐ Filter invariant pairs
Allowed Mismatches: 1
● Show Nucleotides
○ Positions Only

☐ Numerical Table
○ Integer
● Frequency

For a particular type of base pair to be allowed, it must be checked in the preferences dialog prior to running the analysis. The general default allowed pairings are the canonical A-T, G-C and G-U base pairs commonly seen in RNA helices. It is also recommended to allow gap-gap pairings, since gaps represent the absence of a position in a sequence that is homologous to the other sequences in the alignment. Just because this position does not exist in some sequences does not mean that it does not form a defined structure in those sequences that have it. If gap-gap matches are not allowed, these positions will be seen as *mis*matches rather than the absence of residues. Like covariation, position numbering may be according to the numbering of the entire alignment, or to a specified numbering mask. When setting any preferences, pressing Save Preferences will cause *all* preferences in the preferences dialog (all four sheets) to be saved as the default.

If the Numerical table option is checked, a 2-D matrix will be saved with either the raw number of potential pairings for each pair of positions (Integer choice) or the frequency (matches/total sequences) of potential pairings for each position.

The Potential Pairings Algorithm

The basic algorithm for potential pairings analysis is very straightforward and is essentially a brute-force examination of every position compared to every other position in an alignment against a set of allowed pairings.

Computationally, BioEdit approaches this numerically by assigning each nucleotide in each sequence an integer value as follows:

```
A   = 2
G   = 3
C   = 5
U   = 9
GAP = 14
```

The sum of any two pairs of these numbers is unique (including each one paired to itself):

A+A = 4
A+C = 7
A+G = 5
A+U = 11
A+GAP =16
C+C = 10
C+G = 8
C+U = 14
C+GAP = 19
G+G = 6
G+U = 12
G+GAP = 17
U+U = 18
U+GAP = 23
GAP+GAP = 28

An array of 28 values which can take the value 1 or 0 is thus set up such that each of the above sums represents the index to an array position holding a value which states that the pair is allowed (1) or not allowed (0). As the residues are scanned for each pair of column positions in the alignment, the value present at the array index of the sum of the two position residues for each sequence is added to a total sum for that pair of positions (1 is added if the pair is a match, otherwise 0 is added). If the total for a pair of positions is greater than or equal to the required number (depends upon mismatches allowed), the potential pairing is reported in the output (for table output, the total or total/number of sequences is reported for all positions).

Mutual Information Analysis

Before using mutual information as a structure probe, you probably want to read:
Gutell, R.R., A. Power, G.Z. Hertz, E.J. Putz and G.D. Stormo. 1992. Identifying constraints on the higher-order structure of RNA: continued development and application of comparative sequence analysis methods. *Nucleic Acids Res. 20(21)*:5785-5795.

General Overview of Mutual Information

Mutual information, as applied to phylogenetic comparative analysis, is a measure of the amount of information shared by two positions in a set of properly aligned sequences. This measurement, symbolized as *M(x,y)* (the mutual information shared by positions *x* and *y*), gives an idea of how strongly the identities of two positions are correlated, which is often a sign of direct interaction such as base-pairing. Two other measurements that BioEdit will calculate, R1 and R2, give a measure of the contribution made by positions *x* and *y* to *M(x,y)*. These measurements are described at the end of mathematical overview of mutual information.

General Overview -- What is mutual information?

Mutual information analysis is an extension of the idea that information content may be assessed as a general decrease in uncertainty about a particular situation. Given no prior knowledge about a particular situation (such as the identity of a nucleotide in an RNA sequence), uncertainty is at a maximum and one possesses no information about that situation. If the identity of the nucleotide is discovered, uncertainty is removed and information about that nucleotide is at a maximum. Now consider a large set of sequences, all of which contain a homologous nucleotide at this position. Knowing the nucleotide's identity in the first sequence does not necessary offer much information about the same position in the next sequence or a randomly chosen sequence. However, if the identity of this residue is known in many sequences, and in nearly all of these sequences it is a particular base (say 'C'), and is never a particular other residue (say 'G'), uncertainty drops way down about the possible identity of this base in an as yet unexamined sequence. There is now considerable "information" accumulated about this base which can be used to predict the probability of its identity being a particular base if a new sequence is examined. This is the basis of the sequence logo (1) or the BioEdit implementation of an entropy plot.

Mutual information extends this basic principal to examine the information content shared between a pair of positions in an alignment. This is related to, and depends upon, the information content of two individual positions, but refers specifically to the information that the two positions possess together. More generally, it is a measure of the decrease in uncertainty about the extent to which two things influence each other, or interact with each other. The use of mutual information to probe RNA structure was developed by Robin Gutell (2). This measure is ideally suited to phylogenetic comparative analysis because a high degree of mutual influence between two positions may be a strong indication that these two residues directly interact.

As an example, examine the following small piece of an alignment:

```
  1 2 3 4
```

```
A C G U
A C G U
A G C U
A U A U
A U A U
A A U U
A A U U
A G C U
```

There are 8 total sequences. Positions 1 and 4 are invariant. The information content at each of these two bases is maximum (we could feel pretty certain about the identity of the next sequence if we had to guess). Bases 2 and 3 are both evenly divided between A, G, C and U. The information content of each of these positions would be zero, since we would have no idea which of four bases to choose if we had to guess them for a new sequence. The *shared information* between any of these bases, however, is different. The shared information (mutual information) refers to our decrease in uncertainty about how much the identity of one base influences the identity of another. Although we have a lot of information about the identities of bases 1 and 4, we have no way of determining if and how much they influence each other (because they never change, and so there is no chance to test this). The mutual information shared between them is thus zero. On the other hand, although bases 2 and 3 each individually carry essentially no information, *together* they share a certain amount of information about how they influence each other. If asked to guess the identity of position 2 in a new sequence, I couldn't. But, if I was told that position 3 was a 'C', I would have a strong feeling now that position 2 would be a 'G'. This guess can be made based upon the mutual information that these two positions share (they are seen to follow the same pattern of pairs). This mutual information suggests that these bases may interact (their particular identities further suggest that they probably base-pair together).

Mutual information analysis of a sequence alignment gives a mathematical measure of covariation between pairs of positions in the alignment. It differs from covariation analysis in that it gives a quantitative measure of the *extent* of covariation between two positions. For a more in-depth explanation of mutual information, see Mathematical overview of mutual information.

Brown, J.W. 1991. Phylogenetic comparative analysis on Macintosh computers. *Comput. Appl. Biosci. 7(3)*:391-393.

Gutell, R.R., A. Power, G.Z. Hertz, E.J. Putz and G.D. Stormo. 1992. Identifying constraints on the higher-order structure of RNA: continued development and application of comparative sequence analysis methods. *Nucleic Acids Res. 20(21)*:5785-5795.

Mathematical Overview of Mutual Information

Mutual information refers to the amount of information shared by the interaction of two things, or the decease in uncertainty in one thing based upon knowledge about another. In phylogenetic comparative analysis, this refers to the degree to which two positions in an alignment *are not independent* of each other. The concept of mutual information was applied to RNA structure analysis in 1992 by Robin Gutell.

If two positions, x and y, show a strong interdependence, then mutual information, or M(x,y), will be relatively high. If the identities of x and y appear to be uncorrelated, M(x,y) will be low or zero. Mutual information may be defined (in nits) as:

$M(x,y) = \Sigma(fb_xb_y)\ln(fb_xb_y/fb_xfb_y)$ for all bases b_x and b_y (or in bits if the log base 2 is used) where b_x and b_y refer to the identities of each possible base at positions *x* and *y* (A, G, C, U or GAP in BioEdit -- ambiguous bases are ignored), fb_x and fb_y are the frequencies of each base at each position, and fb_xb_y is the frequency of each possible pair of bases at *x* and *y*. When there is no variation in one or both positions, mutual information is zero, and no interdependence can be shown (although this does not prove that there is none, it just can't be shown if the bases never vary). For example, if *x* is always 'A', fb_xfb_y is 0 for all combinations except when b_x is 'A'. When b_x = 'A', $fb_xfb_y = fb_y$, and $fb_xb_y=fb_y$, so $\ln(fb_xb_y/fb_xfb_y)=0$.. For these two bases, $fb_xb_y/fb_xfb_y = 1$ and $\ln(fb_xb_y/fb_xfb_y)=0$. M(x,y) therefore will be zero when either base is invariant. When both positions show maximum variation, $fb_x = fb_y = 1/n$ for all *b*, where *n* is the number of possible bases to choose from (5 in BioEdit, treating gaps as a base). Now, for all b_x and b_y, $0<=fb_xb_y<=1/n$ (because the frequency of a combination can't possibly exceed the frequency of either of its contributing bases). When all bases are completely independent of each other, $fb_xb_y = 1/n\wedge^{2}$ for all combinations b_xb_y. For all b_xb_y, then, $(fb_xb_y)\log_2(fb_xb_y/fb_xfb_y) = 1/n\wedge^{2}(\ln((1/n\wedge^{2})/1/n\wedge^{2})) = 1/n\wedge^{2}(\ln(1)) = 0$. $M(x,y) = 0$ when the bases at *x* and *y* vary independently of each other. When the two bases are completely correlated, $fb_xb_y = 1/n$ for each of the possible pairs, and 0 for all others. Therefore, $(fb_xb_y)\ln(fb_xb_y/fb_xfb_y)=0$ for all combinations of b_x and b_y except for exactly *n* times, for which $(fb_xb_y)\ln(fb_xb_y/fb_xfb_y) = 1/n(\ln((1/n)/1/n\wedge^{2})) = 1/n(\ln(n)) = (\ln(n))/n$. Therefore, the maximum value of M(x,y) occurs when the two positions are completely correlated and is equal to $M(x,y)(_{\max)} = n(\ln(n))/n = \ln(n)$. If $n = 5$ (A, G, C, U or GAP, as implemented in BioEdit), then $M(x,y)(_{\max}) = 1n(5) =$ ca. 1.609.

The way BioEdit actually calculates *M(x,y)* is based upon the method used by Gutell et al. $M(x,y)=\Sigma(fb_xb_y)\ln(fb_xb_y/fb_xfb_y)$ is equivalent to:

$$M(x,y) = H(x) + H(y) - H(x,y),$$

where H(x) and H(y) are the *entropy* at positions x and y, respectively (see Entropy plots). The entropy terms *H(x)* and *H(y)* are calculated as:

$$H(x) = -\Sigma fb_x\ln(fb_x),$$

and

$$H(x,y) = -\Sigma fb_xb_y\ln(fb_xb_y).$$

Examination of these formulas will reveal that they will yield the same result as those shown above. BioEdit uses the natural logarithm (ln) for convenience. If log base 2 is used instead, information will be in bits, but the data are the same relative to each other.

Because *M(x,y)* measures the mutual interdependence of two positions, and depends equally on frequencies of bases at each positions, it is a symmetric calculation (*M(x,y) = M(y, x)*). In some cases where there is an interdependence between two bases, but some other factor constrains one of those positions, the small amount of variation in one position causes M(x,y) to be so small that the covariation between the two positions is missed. In these cases, two other terms, *R1(x),* and *R2(x)* can sometimes bring this to light. *R1* and *R2* are ratios of mutual information to entropy at the x and y positions, respectively.

R1(x) = M(x,y)/*H(x)*

R2(x) = M(x,y)/*H(y)* (and, incidentally, *R2(x) = R1(y)*)

If position x shows little variation, but the variation it does show correlates with the variation seen in position y, *R1(x)* will be relatively large. *R1(x)* and *R2(x)* will often not be equal.

Gutell, R.R., A. Power, G.Z. Hertz, E.J. Putz and G.D. Stormo. 1992. Identifying constraints on the higher-order structure of RNA: continued development and application of comparative sequence analysis methods. *Nucleic Acids Res. 20(21)*:5785-5795.

Using Mutual Information in BioEdit

BioEdit calculates Mutual Information as M(x,y) = H(x) + H(y) - H(x,y) (see General Overview of Mutual Information and Mathematical Overview of Mutual Information). This information can give a good indication of mutual interdependence of two bases in an evolutionarily conserved molecular structure which can be used to help build and refine secondary and tertiary structures of RNA molecules through phylogenetic comparative analysis.

If you're not familiar with mutual information analysis, you probably want to read:

Gutell, R.R., A. Power, G.Z. Hertz, E.J. Putz and G.D. Stormo. 1992. Identifying constraints on the higher-order structure of RNA: continued development and application of comparative sequence analysis methods. *Nucleic Acids Res. 20(21)*:5785-5795.

Before doing a mutual information analysis, like any comparative sequence analysis, a high quality alignment is absolutely necessary. If bases are not lined up in their homologous positions, the resulting data and structures they lead to will necessarily be incorrect.

Also, be sure to choose the output type(s) you would like before running the analysis (see Setting Mutual Information Preferences).

BioEdit allows the following options for mutual information output:

1. Tabular output (matrix):
    a. M(x,y) -- full table or only above the diagonal (M(x,y) is symmetric).
    b. R1(x) -- full table only
    c. R2(x) -- full table only

Any or all of the above may be chosen at the same time. If more than one are chosen together, a single table will be generated with the second value right below the first in each column. For example, if M(x,y) and R1(x) are chosen together, R1(x) values will be printed directly below M(x,y) values.
The option to set M(x,y) to 0 when x=y is offered (the real value is M(x,y) = H(x) when x=y). Setting M(x,y) to 0 when x=y will suppress the diagonal on a plot of the M(x,y) matrix.
Output may be comma delimited or tab delimited. For text editor viewing, tab delimited is recommended. For small tables, the BioEdit Rich Text Editor works well on "no word wrap" mode.
An external application such as Excel may be linked to if you wish. As long as it can read the file format and will accept a file as a command-line parameter, it will open your table after it is generated.

2. List outputs:
    a. Pbest: A Pbest list reports all scores within a user-specified percentage of the highest M(x,y), R1(x) or R2(x) value. All three values are reported, but the cut-off for reporting and sorting are according to the measure specified by the preferences. The Pbest output may be a percentage of the highest score for each individual position taken separately, or for the highest score in the entire analysis. P may be specified as 0 to 50% (whole numbers only).

b. Nbest: An Nbest list reports the N highest scores either for each position separately, or for the entire analysis (chosen by the user). Like Pbest, M(x,y), R1(x) and R2(x) are reported, but the score threshold and sorting are according to the value specified in the preferences.

For all types of analysis, the numbering of positions may be according to the numbering as seen in the alignment window (alignment numbering), or to the true positions of the mask (only included residues are incremented).

File output is in raw text which may be in PC or Macintosh format (only carriage returns are different. For example, if one has access to a Macintosh and would like to use a program such as SpyGlass Transform to view data, then M(x,y) matrix files should be output in Macintosh format (otherwise the files need to be converted by a word processor).

To do a mutual information analysis from a BioEdit alignment document:

1. Have the alignment open in an alignment document window.

2. If you would like to use a mask, create one or specify an existing sequence to be the mask.

3. Select the preferences you would like for output (choose Analysis preferences" under the "Options" menu, then click the "Mutual Information" tab).

4. Select all of the sequences you would like to be included in the analysis (sequences are selected by selecting their *titles*). ***Only sequences whose titles are selected will be included in the analysis***. If no sequences at all are selected, *all sequences* will be automatically selected for you. If you are using a mask that is not a sequence and specifies positions only, make sure to exclude that from the analysis. An easy way to select all but one sequence is to use "Select all Sequences" from the "Edit" menu, then deselect one sequence by Ctrl+left-mouse-click.

5. To run the analysis, choose "Mutual Information" from the "RNA" menu. You will be prompted for a file name for each output chosen. List outputs will be opened in the text editor, but matrix outputs will not. If you would like to link matrix outputs to a spreadsheet or other external program, this may be done by setting this option in the preferences.

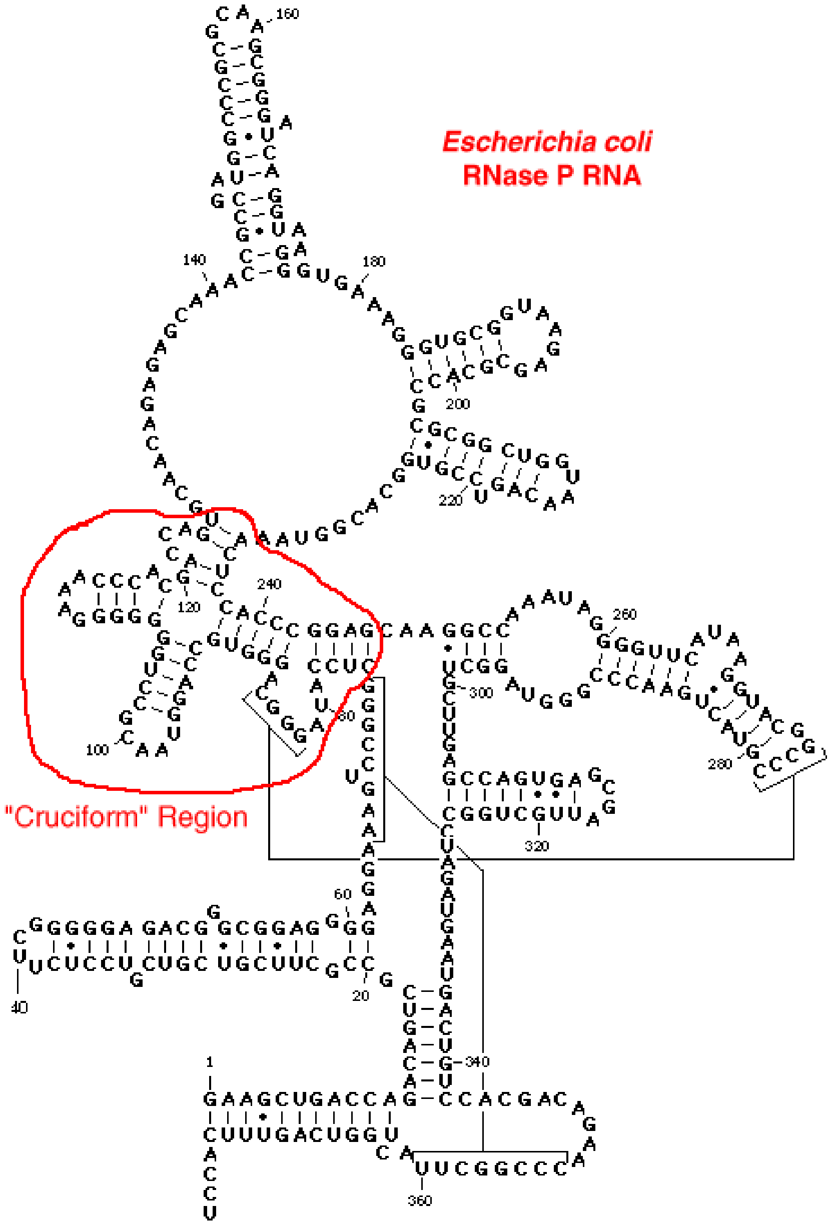
If you have output data as a matrix, you might want to view the data with the BioEdit matrix plotter.

Mutual Information Example

In this example, mutual information analysis is performed on a segment of a sequence alignment of bacterial RNase P RNA sequences. (click on alignment to view the alignment). The chosen output is a full table of *M(x,y)* values and an Nbest list of the 5 highest scores for each position. (see Setting mutual information preferences). Both the sequence and numbering masks are set as *E. coli*. The numbering is according to the *E. coli* mask sequence. This part of the alignment contains a highly structured region referred to as the “cruciform region” of RNase P RNA (see *E. coli* sample RNase P RNA structure). The matrix text is too large to view within this help, but a graphical plot of the table is a convenient way to look at it. With the BioEdit matrix plotter, the data may be dynamically examined numerically as well as graphically.

Sample RNA structure

Below is the current model of the secondary structure of the RNase P RNA from *E. coli*. The "cruciform" region analyzed in the mutual information example and shown in the sample matrix plot output is circled. This image, as well as the full collection of currently known bacterial and archaeal RNase P structures and sequences can be found at the RNase P database:
Brown, J.W. 1998. The Ribonuclease P Database. *Nucleic Acids Res. 26*:351-352.
http://jwbrown.mbio.ncsu.edu/RNaseP



J.W. Brown (1998) THe Ribonuclease P Database. Nucleic Acids Res. 26:351-352.
http://jwbrown.mbio.ncsu.edu/RNaseP

Sample Alignment for Mutual Information

Below is a subsection of an alignment of RNase P RNAs from bacteria. There are 138 sequences, which is more than plenty for a meaningful analysis. This alignment contains what is referred to as the "cruciform region" (see the sample RNA structure). The region of the alignment where "~~~" appears in every sequence denotes a section RNase P RNA of poorly conserved sequence and length that has been removed from the alignment.

Brown, J.W. 1998. The Ribonuclease P Database. *Nucleic Acids Res. 26*:351-352. http://jwbrown.mbio.ncsu.edu/RNaseP.

```
Alignment: I:\BioEdit\bacterial_analysis\minimized_cruciform.gb

                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....
                           10         20         30         40         50         60         70
Escherichia-col   CUCCAUAGGG CAGGGUGCCA GGUAACGCCU GGGGGGGAAA CCCACGACCA GU~~~GCGUA AACUCCACCC GGAG
Rhodospirillum-   CUCCACGGAA CACGGUGCCG GGUAACGCCC GGCGGGGCGA CCUAGGGAAA GU~~~GCGUA AACCCCACCG GGAG
Agrobacterium-t   CUCCACGAAA UACGGUGCCG GAUAACGUCC GGCGGGGCGA CCCAGGGAAA GU~~~GCGUA AACCCCACCG GGAG
Alcaligenes-eut   CUCCACAGGG CAGGGUGUUG GCUAACAGCC AUCCACGCAA GUGCGGAAUA GG~~~GCGUA ACCUCCACCU GGAG
Pseudomonas-tes   cUGCAUAGGG CGGCGUAGCA GCUAACAGCU GUCCACGUGA GUGAGGAUCA GA~~~GCGCA AUCUCUACGC GCAG
Thiobacillus.th   cUCCACAGAG CAGGAUGCCG GCUAACGGCC GGACGCGCGA GCGAGGAAUA GG~~~GCGUA ACCUCCAUCC GGAG
N-meningitidis    CUCCGCAgGG UAGAAUGCCG GUUAACGGCC GGGCGCGUAA GCGACGGAAA GU~~~GGCCA AACCCCAUUC GGAG
N-gonnorhoeae     CUCCGCAGGG UAGAAUGCCG GUUAACGGCC GGGCGCGUAA GCGACGGAAA GU~~~GGCCA AACCCCAUUC GGAG
Thiobacillus-fe   CUCCAUAGGG CAAGGCGCCG GUUAACGGCC GGGGGGGUGA CCUACGGAAA GU~~~GCGAA AACCCCGCCU GGAG
Salmonella-typh   CUCCAUAGGG CAGGGUGCCA GGUAACGCCU GGGGGGGAAA CCCACGACCA GU~~~GCGUA AACUCCACCC GGAG
Klebsiella-pneu   CUCCAUAGGG CAAGGUGCCA GGUAACGCCU GGGGGGUCAC CCCACGACCA GU~~~GCGUA AACUCCACCC GGAG
Erwinia-agglome   CUCCAUAGGG CAAGGUGCCA GGUAACGCCU GGGGGGUCAC CCCACGACCA GU~~~GCGUA AACUCCACCC GGAG
Serratia-marces   CUCCAUAGGG CAGGGUGCCA GGUAACGCCU GGGAGGGCAA CCUACGACUA GU~~~GCGUA AACUCCACCC GGAG
H.influenza       CUACACAGGG CAGAGUGCCG GAUAACGUCC GGGCGGGUGA CCGACGACCA GU~~~GCGUA AACUCCACUC GUAG
Vibrio-cholera    CUCCAUAGAG CAGGGUGCCA GGUAACGCCU GGGGGGGCAA CCUACGACAA GU~~~GCGUA AACUCCACCC GGAG
Pseudomonas-flu   CUCCAUAGGG CGAAGUGCCA GGUAAUGCCU GGGGGGGUGA CCUACGGAAA GU~~~GCGUA AACCCCACUU GGAG
Chromatium-vino   CUCCAUAGGG CAGGGUGCCA GGUAACGCCU GGGGGGGAGA UCCACGGAAA GU~~~GCGUA AACCCCACCC GGAG
Desulfovibrio-d   CUCCAAAGGG CAGAACGCUG GAUAACAUCC AGGGAGGCAA CUC-CGGACA GC~~~GCGCA UACCCCGUUC GGAG
Myxococcus-xant   cUCCAGAGGG CAGGGUGCUG GCUAACGGCC AGUCGAGCGA UCGCAGGAAA GU~~~GCGUA AACCCCGCCU GGAG
H-pylori          CUACAUUAGA CAAAAUUCCA UCUAACGGAU GGCUAGGCAA CUAAGGGAAA GU~~~GCGCA AACCCAAUUU GAAG
Streptomyces-bi   CUCCACAGAG CAGGGUGGUG GCUAACGGCC ACCCGGGUGA CCGCGGGACA GU~~~GCGUA AACCCCACUC GGAG
Streptomyces-li   CUCCACAGGG CAGGGUGAUG GCUAaCGgCC ACCCGGGUGA CCGCGGGACA GU~~~GCGUA AACCCCACCC GGAG
Micrococcus-lut   cACCGCAGAG CAGGAUGGUG GACAACAUCC ACCCGGGCGA CCGCGGGCCA GU~~~GCGUA AACCCCAUCC GGUG
Eubacterium-the   cUCCGCAGGG CAGGAUGCUG GGUAAUACCC AGUGGGGCGA CCUAAGGAUA GU~~~GCGUA AACCCCAUCU GGAG
Clostridium-ace   CUCCAUAGGG CAGGGUGCCG GGUAACUCCC GGUCAAGCGA UUGAAGGAAA GU~~~GCGUA AACCCCAUCU GGAG
Clostridium-spo   cUCCAUAGGG CAGGGUGCUG GGUAAUACCC AGUGGAGCGA UUUAAGGAAA GU~~~GCGUG AACCCCAUCU GGAG
Mycobacterium_l   CUUCACAGAG CAGGGUGAUU GCUAACAGCA AUCCGAGUGA UCGCGGGAUA GU~~~GCGUA AACCCCACCC GAAG
Mycobacterium_t   CUUCACAGAG CAGGGUGAUU GCUAACGGCA AUCCGAGUGA UCGCGGGAAA GU~~~GCGCA AACCCCACCC GAAG
Bacillus-subtil   CUCCAG---- -UUCGUGCCA GCAGUCAGCU GGGCAGUUAG CUGACGGCAA GU~~~GCGUA AACCCCUCGA GGAG
Bacillus-brevis   CUCCAG---- -UUCGUACCG GC-GCAAGCC GGGCAGGCAA CUGACGGCAA GU~~~GCGUA AACCCUGCGA GGAG
Bacillus-stearo   CUCCAG---- -UUCGUGCCA GCAUCCAGCU GGGCAGUUCG CUGACGGCAA GU~~~GCGUA AACCCCACGA GGAG
Bacillus-megate   CUCCAG---- -UUCGUGCCA GUAAAAAGCU GGGCAG-UAU CUGACGGCAA GU~~~GCGUA AACCCCACGA GGAG
Staphylococcus-   cUCCAG---- -UUCGUGCUG AUAACAAAUC AGGCA-UAAU -UGACGGCAA GU~~~GCGUA AACCCCUCGA GGAG
Streptococcus-f   cUCCAG---- -UUCGUGCUA GCAAUCAGCU AGGUAC-UUU GUAACGGCAA GU~~~GCGUA AACCCCUCGA GGAG
Streptococcus-p   CUACAG---- -AUUGUGCUG GCACACAGCC AGGGAUCAUA AUUACGGCAA GU~~~GCGUA AACCCCUCAA GUAG
Streptococcus-f   cUCCAG---- -UUCGUGCUA GCAACAAGCU AGGUGC-AUU GUAACGGCAA GU~~~GCGUA AACCCCUCGA GGAG
Lactobacillus-a   cCCCAG---- -UUCGUGCUA GCCAAUAGCU AGGGGCGUAA GCUACGGCAA GU~~~GCGUA AACCCCGCGA GGAG
Acholeplasma_la   cUACAG---- -UUUGUGCUA GGAAUCACCU AGGUAUUAUA AUAACGGCAA GU~~~GCGUA AACCCCUCAA GUAG
Mycoplasma-ferm   CUACAG---- -AUCAUGCUG GCCAAUAGCC AGGC---UUA --GACGACUA GU~~~GCGUA AACUCCAUGA GUAG
Mycoplasma-floc   CUACAG---- -UUCAUGUUG GUUAAUAACC AAGC---UUA --GACGACAA GU~~~GCGUA AACUCCAUGA GUAG
Mycoplasma-hyop   CUACAG---- -UUCAUGUUG AUUAAUAAUC AAGC--UUUA --GACGACCA GU~~~GCGUA AACUCCAUGA GUAG
Mycoplasma.geni   CUUCAG---- -UUUGUG-UA AUAGCGAGAU UAGGAUGAUA AUAACGACAA GU~~~GCGUA AACUCCACAA GAAG
Mycoplasma-capr   CUUC-G---- -UUUAUGCUA AUAAAUAUUU AGGCAGUUAA AUAACAACAA GU~~~GCGUA AACCCCAUAA GAA-
Mycoplasma-pneu   CUUCAG---- -UUUGUG-UA AUAACAAGAU UAGGACUAAU --GACGUCAA GU~~~ACGUA AACUCCACAA GAAG
Clostridium-inn   cUCCAG---- -UUUGUGCUG AUAACGAAUC AGGCAGGUAA CUGACGGCAA GU~~~GCGUA AACCCCGCAA GGAG
Heliobacillus.m   cuCCAG---- -UUCGUGCCG UUCGUAAGGC GGGCAGUUUU CUGACGGCAA GU~~~GGCUA AACCCCACGA GGAG
Cyanophora.para   CUCUUAAGGU UAGAAUGCUG GGUAAUUCCC AGUACGGAUA CGUGAGGAUA GU~~~GCGUA AACCCCGUUC AGAG
Anacystis-nidul   CUCCAAAGAC CAGACUGCUG GGUAACGCCC AGUGCGGUGA CGUGAGGAGA GU~~~GCGUA AACCCCGGUU GGAG
Anabaena-PCC712   CUCCGAAGAC CAGACUGCUG GAUAACGUCC AGUGCGGCGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
Calothrix-PCC76   CUCCGAAGAC CAAACUGCUG GAUAACGUCC AGUGCGGCGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
Synechocystis-P   CUUCCAAGGC CAAACUGCUG GGUAACGCCC AGUGCGGCGA CGUGAGGACA GU~~~GCGUA AACCCCGGUU GAAG
Pseudoanabaena-   CUUCAAAGAU CAGGCUGCUG GAUAACGCCC AGUGCGGCAA CGUGAGGAUA GU~~~GCGUA AACCCCAGUC GAAG
Synechocystis.P   cuCCAAAGAU CAAACUGCUG GGUAACGCCC AGUGCGGUGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUG GGAG
Anabaena.ATCC29   cuCCGAAGAC CAGACUGCUG GAUAACUUCC AGUGCGGCGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
Nostoc.PCC6705    cuCCGAAGAC CAGACUGCUG GAUAACGUCC AGUGCGGCGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
Nostoc.PCC7107    cuCCGAAGAC CAAACUGCUG GAUAACGUCC AGUGCGGCGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
Fischerella.UTE   cuUCGAAGAC CAAACUGCUG GGUAACGCCC AGUGCGGCGA CGCGAGGAUA GU~~~GCGUA AACCCCGGUU GAAG
Dermocarpa.PCC7   cUCCGAAGAC CAAACUGCUG GGUAACGCCC AGUACAGCGA UGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
```

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Nostoc.PCC7413      cuCCGAAGAC CAGACUGCUG GAUAACGUCC AGUGCGGCGA CGUGAGGAUA GU~~~GCGUA AACCCCGGUU GGAG
Oscillatoria.PC     cuCCAAAGGC CAAGCUGCUG GGUAACGCCC AGUGCGGCGA CGCGAGGAUA GU~~~GCGUA AACCCCGGCU GGAG
Syenchococcus.P     cUUCGAAGAC CAAGCUGCUG GGUAACGCCC AGUGCGGUGA CGUGAGGAAA GU~~~GCGUA AACCCCGGCU GAAG
Synechococcus.P     cUCCCAUGGC CAGGCUGCUG GGUAACGCCC AGUGCGGUGA CGUGAGGAGA GU~~~GCGUA AACCCCGGCC GGAG
Synechococcus.P     cACACAGGCU GGUUGUGGGU AAUUCCCAGU GCGCGCAGCG GAGGAUAGUG CC~~~ACGGU AAACCCCGCU GAGG
Synechococcus.P     CCAAAGCCAG ACUUGUGGGU AACGCCCAGU GCGGGUACCG GAGGAGAGUG CC~~~AC-GU AAACCCCGGU UGGA
Chlorobium-limi     CUUCACAGGG CAGGG-GCCG UCACCUGGGC GGGGGCGCAA GUCACAGAGA GU~~~GCUGA AACCUC-CCC GAAG
Chlorobium-tepi     CUUCACAGGG CAGGG-GCCG UCACGUUGAC GGGGGCGCAA GUCACAGAGA GU~~~GCUGA AACCUC-CCC GAAG
Bacteroides-the     CAACACAGAG CAUCCUACUU CCUAACAGAA AGCUGUGCGA GUA-GAG-UA AC~~~GUGUA CGUCUUAGGA GUUG
Flavobacterium-     cAACACAGAG CAACUCACUU CCUAACGGGA AGGCUCUCAG GAGACAGCAA GU~~~GCGUA AACCUUGAGU GUUG
Planctomyces-ma     cUCCACAGGG CACGGUGGUG GGUAACGCCC ACCGUCGCGA GACAGGGAAA GU~~~GCGUA AACCCCACCG GGUG
Pirellula-stayl     cUCCACAGGA CAGGGUGGUC GAUAACGUCG ACCGGUGUGA AUCAGGGACA GU~~~GCGUA AACCCCGCCC GGAG
Chlamydia-trach     cUUUAUAAGA AAAGAUGCUG GAGAAAUUCC AGGGGCGUAA GCUACGGAAA GU~~~GCGUA AACCCCAUCU GAAG
Chlamydia-psitt     cUUCAUAAGA AAAGAUACUG GAGAAACUCC AGGGGCGUAA GCUACGGAAA GU~~~GCGCA AACCCUAUCU GAAG
Borrelia-hermsi     cUCCAA-AGA GAUAAUGCUA GGUAAUGCCU AGGAGU-UAA ACU-UAGAGA GU~~~GUGUA AACCUCAUUA GGAG
Borrelia-burgdo     CUCCAA-AGA AAUAAUGCUA GGUAAUGCCU AGGGGU-UUA ACC-UAGAAA GU~~~GUGUA AACCUCAUUA GGAG
Leptospira-borg     cACCAG-AAA CACGGGACCG GGUAAUCCCC GGAGUUGAAA AAUAUGGAAA GU~~~GCGUA AACCCUCCCG GGUG
Leptospira-weil     cACCAG-AAA CACGGGACCG GGUAAUUCCC GGAAUUGAAA AAUAUGGAAA GU~~~GCGUA AACCCUCCCG GGUG
Deincoccus-radi     CACCGCAGGG CAGGAUGCCA GCUAACGGCU GGUCGGGCCG CCGAAGGACA GU~~~GCGUC AACCCCAUCC GGUG
Thermus-aquatic     CACCAUAGGG CAGGGUGCCA GCUAACGGCU GGGCGGGCAA CCGACGGAAA GU~~~GCGCA AACCCCACCC GGUG
Thermus-thermop     CACCAUAGGG CAGGGUGCCA GGUAACGCCU GGGCGGGUAA CCGACGGAAA GU~~~GCGCA AACCCCACCC GGUG
Thermomicrobium      CUGCACAGAG CGGGG-GCCU GGGUCAACCA GGGCAGACCG CUGACAGUGA GC~~~AAGCA AUCCUC-CCU GCAG
Chloroflexus-au     cUCCAUAGAG CAGGGUGGUG GGUAACGCCC ACCCGGGUGA CCGCGGGAAA GU~~~GCGCA AACCCCACCU GGAG
Herpetosiphon-a     cUCCAUAGGG CGAAGUGCCA GGUAAUGCCU GGGAGGGUGA CCUACGGAAA GU~~~GCGCA AACCCCACCU GGAG
Thermoleophilum     cACCGCAGGG CAGGGUGGUC GGGAAA-CCG ACCCGGGAAA CCGCGGGAAA GU~~~GCGCA AACCCCACCC GGUG
Thermotoga-mari     CUCU--GGAG CGGGGUGCCG GGUAACGCCC GGGAGGGUGA CCU-CGGACA GG~~~GCGCA ACCCCCACCU GGAG
Thermotoga-neap     CUCU--GGAG CGGGGUGCCG GGUAACGCCC GGGAGGGUGA CCU-CGGACA GG~~~GCGCA ACCCCCACCU GGAG
vB11                cUCCACAGAG CAGGAUGCCG GCUAACGGCC GGACGCGCGA GCGAGGAAUA GG~~~GCGUA ACCUCCAUCC GGAG
vHge8-3             cUCCGCAGGG CAGGGUGCCG GGUAACUCCC GGUGAAGUGA UUCAAGGAAA GU~~~GCGUA AACCCCACUC GGAG
Mxa1                cUGCACAGAG CGGGAUGACG GCUAACGGCC GUACGCGUAA GCGAGGAAUA GG~~~GCGUA ACCUCCAUCU GCAG
ESH7-4              cUCCACAGGG CAGGAUGCUG GCUAACGGCC AGGCGUGCGA GCGACGGAAA GU~~~GGCUA AACCCCAUCC GGAG
ESH7-9              cUUCAACGGG CAAGGUGCCA GGUAACGCCU GGGCGGGUGA CCGACGGAAA GU~~~GCGUA AACCCCACCG GAAG
ESH7-16             cUACAUAGGG CAGCGUGCCA GCUAACGGCU GGGCAGGUAA UUGACGACCA GU~~~GCGUA AACUCCACGC GUAG
ESH17b-7            cUCCAUAGGG CGGAGUGCCA GGUAAUGCCU GGGGGGGUGA CCUACGGAAA GU~~~GCGUA AACCCCACUC GGAG
ESH20b-4            cUCCAUAGGG CAGAGCGCCA GGUAACACCU GGGAGGGUGA CCUACGGAAA GU~~~GCGUA AACCCCACUU GGAG
ESH20b-1            cUUCACAGGG CAGGAUGCCA GAUAACGUCU GGCGGAGCGA UCCAGGGAAA GU~~~GCGUA AACCCCAUCC GAAG
ESH21b-4            cUCCAUAGGG CGAAGUGCCA GGUAAUGCCU GGGAGGGUGA CCUACGGAAA GU~~~GCGUA AACCCCACUU GGAG
ESH26-4             cUGCACAGAG CGGGAUGACG GCUAACGGCC GUACGCGCAA GCGAGGAAUA GG~~~GCGUA ACCUCCAUCA GCAG
ESH30-3             cAACACAGAG CAUCCUACUU CUUAACGGGA AGCUAUGCGA GUA-GAGU-A AU~~~GUGUA CGUCUUAGGA GUUG
ESH46a-1            cUCCACAGAG CAGAAUGCCG GCUAACGGCC GGGCGCGCAA GCGACGGAAA GU~~~GGGUA AACCCCAUUC GGAG
ESH167E             cAGUACAGAG CAACCCACCG GUGAACAGCC GGCCACAAUU GUGAGAGAAA GU~~~GCGUA AACCUUGGGU GCUG
ESH167F             cUCCAUAGAG CAGGGUGAUG GCUAACGACC AUCCACGUGA GUGCGGAAUA GG~~~GCGUA ACCGCCACCC GGAG
ESH183A             cACCACAGGG CUGGU-GCUG GGUAAUGCCC AGUGCGGUGA CGUGAGGAUA GU~~~GCGUA AACCCC-ACU GGUG
ESH183D             cUCCAUAGGG CAGGGUGUUG GCUAAUAGCC AUCCACGCAA GUGCGGAAUA GG~~~GCGUA ACCUCCACCU GGAG
ESH210B             cUCUACAGAG CAAAGUGGUG GAUAACUUCC ACCCGGGCGA CCGCGGGAUA GU~~~GCGCA AACCCCACUU UGAG
ESH212C             cuCCAUCGAC AACGGUGCCG GAUAACACCC GGCGGGGCGA CCCAGGGAAA GU~~~GCGCA AACCCCACCG GGAG
PS#1                cUCCACGAAA CACGGUGCCG GAUAAUGCCC GGCGGGGUGA CCCAGGGAAA GU~~~GCGCA AACCCCACCG GGAG
PS#2                cUCCACGGAA GAUGGUGCCG GGUAACGCCC GGCGGGGCGA CCCAGGGACA GC~~~GCGCA AGCCCCGCCA GGAG
PS#4                cACCAAAGGG CUGGU-GCUG GGUAACGCCC AGUGCGGUGA CGUGAGGAUA GU~~~GCGUA AACCCC-GCU GGUG
PS#6                cUCCACGGAA CGCGGUGCCG GGUAACGCCC GGCGGGGCGA CCCAGGGAAA GU~~~GCGCA AACCCCACCG GGAG
PS#8                cAACACAGAG CAGGAUACUU CUUAACGAGA AGCAGUGCGA GCU-GAG-UA GU~~~GUGUA CGCCUUAUGG GUUG
PS#22               cCCCACAGAG CAGGAUGCCG GCUAACGGCC GGGCGCGCGA GCGACAGACA GU~~~GCGCA AACCUCAUCC GGGG
PS#24               cUCCACAUAA CACGGUGCCG GGUAACGCCC GGCGGUCGUG GCAAGGGACA GU~~~GCGCA AACCCCACCG GGAG
PS#26               cUCCACGAAA CACGGUGCCG GAUAAUGUCC GGCGAGGUGA CUUAGGGAAA GU~~~GCGCA AACCCCACCC GGAG
PS#27               cUCCACGGAA CGCGGUGCCG GGUAACACCC GGCGGGGCGA CCCAGGGACA GU~~~GCGCA AACCCCACCG GGAG
PS#31               cUCCACGAAA CAGGGUGGCG GGUAACGCCC GCCGGCUUCG GCAAGGGAAA GU~~~GCGCA AACCCCACCC GGAG
PS#33               cACCACAGGG CAGGAUGC-G GCUAACGGCC -GGCGCGUGA GCGACGGAAA GU~~~GCGCA AACCCCAUCC GGUG
LGA#1               cAGCACAGAG CAAUGCACCG GUGAAUAGCC GGGUUC-UUU GAAACAGACA GU~~~GCGUA AACCUUGCAU GCUG
LGA#2               cCCCACAGAG CAGGAUGCCG GCUAACGGCC GGGCGCGCAA GCGACAGACA GU~~~GCGC- AACCUCAUCC GGGG
LGA#6               cUCCACAGGA CAGAGUGGUC GGUAACGCCG ACCGGCGAAA GCUCGGGACA GG~~~GCGCA ACCCCCACUC GGAG
LGA#8               cUCCACAGGA CAGAGUGGUC GCUAACGGCG ACCGGCGCAA GCUCGGGAAA GU~~~GCGCA AACCCCACUC GGAG
LGB#5               cUCCACAGGG cAAGAUGGUU GCUAGCGGCA ACUGUCUAGU GAUAAGGAAA GU~~~GCGUA AACCCCAUCU GGAG
LGA#10              cUCCU-UGGA CAAACUGCCA GGUAGCACCU GGGCACAUGA GUGACGGAAA GU~~~GCGUA AACCCCAGUU GGAG
LGB#21              cAUCGACGGG CAGGAUGGUC UCUAACGGAG ACUGGGGUAA CCUAAGGAAA GU~~~GCGCA AACCCCAUCC GAUG
LGB#23              cUCCAUGAAG CAGGGUGCCG GGUAAUGCCC GGCCGGGAAA CCGAGGGAAA GC~~~GCGCA AGCCCCACCC GGAG
LGB#27              cAACACAGGG CAGCGUACUU CCUAACGGGA AGGCCCUUAG GGGACAGAAA GU~~~ACGCA AACCUUACGC GUUG
LGB#32              cUCCU-UGGA CAAACUGCCA GGUAACACCU GGGCACAUGA GUGACGGAAA GU~~~GCGUA AACCCCAGUU GGAG
LGB#41              cUCCACGGAA CACGGUGCCG GGUAACGCCC GGCGGCCUCG GUUAGGGAAA GU~~~GCGUA AACCCCACCG GGUG
LGW#17              cUCCAUAGGG CAGGAUGCCA GUUAACGGCU GGGUGCGCAA GCAACGGACA GU~~~GCGUG AACCCCAUCC GGAG
LGW#18              cUCCGCAGGG CAGUGUGGUU CCUAACGGGA ACCGGGGUAA CCCAGGGAAA GU~~~GCGUA AACCCCACAC GGAG
LGW#23              cUCCAUAGGG CAAGGUGCCA GGUAACGCCU GGGGGGGCGA CCCACGGACA GU~~~GCGCA AACCCCACCU GGAG
LGW#46              cACCAUAGGA CAGGGUGGUG GGUAACGCCC ACCGGCGUAA GUUAGGGAAA GU~~~GCGUA AACCCCGCCC GGUG
LGW#113             cUCCACGGAA CACGGUGCCG GGUAACGCCC GGCGGCCUCG GUUAGGGAAA GU~~~GCGUA AACCCCACCG GGUG
LGW#116             cUCCGCAGGA CAGGGUGGUC GGUAACGCCG ACCGGCGCAA GCUCGGGAAA GU~~~GCGCA AACCCCACCC GGAG
EM14b-9             cUCAGAGGUG CGCGUAUCCG UUGAUGAAGC GGGGCGGAGA CGC-CGGAAA GU~~~GCGUA AACCCAUACG CGAG
EM14b-11            cUCCAAAGGG CAGGGCGCCG GGUAAUACCC GGGGUG-AAA CACACGGUAA GG~~~GCGUA ACUCCCGCCC GGAG
PF#101              cUCCAUAGGG CAGGGCGCUG UCGGAA-GGC GGGAGUAGA- ACUUCGGAAA GU~~~GCGCA AACCCCGCCC GGAG
BH#145              cUCCAUAGGG CGAAGUGCCA GGUAAUGCCU GGGAGGGUGA CCUACGGAAA GU~~~GCGUA AACCCCACUU GGAG
P#126               cUCCAUAGGG CGAAGUGCCA GGUAAUGCCU GGGAGGGUGA CCUACGGAAA GU~~~GCGUA AACCCCACUU GGAG
P#131               cUCCAUAGGG CAGGGUGCCA GGUAACGCCU GGGAAGGCGA CUUACGACAA GU~~~GCGUA AACUCCACCC GGAG
```

N-best sample output

Below is the N-best list generated from our sample alignment using *E. coli* as the mask. Positions for which mutual information is 0 for all pairs (such as invariant positions) are not reported, because the highest score would be zero (for example, position 1 is invariant).

Brown, J.W. 1991. Phylogenetic comparative analysis on Macintosh computers. *Comput. Appl. Biosci. 7(3)*:391-393.

```
Mutual information analysis, BioEdit v1.0 M(xy) values
7/20/98 5:39:51 PM
Input from I:\BioEdit\samples\bac_cruciform.gb
N-best:  N = 5

Data are reported as the 5 best scores for each position.
Position numbering is relative to the numbering of the mask.
Mask Sequence: Escherichia-coli.

X      Y             M(xy)         R1(xy)        R2(xy)

2      64            0.41919       0.74220       0.77571
2      57            0.09703       0.26234       0.17955
2      17            0.09703       0.26234       0.17955
2      46            0.07606       0.19712       0.14075
2      58            0.07188       0.07427       0.13302

3      63            0.74543       0.88974       0.88562
3      64            0.41919       0.74220       0.77571
3      60            0.19326       0.17130       0.22960
3      14            0.15305       0.13573       0.18184
3      48            0.14794       0.18927       0.17576

4      63            0.74543       0.88974       0.88562
4      62            0.09973       0.61816       0.35290
4      27            0.08872       0.09120       0.31397
4      9             0.08024       0.07587       0.28395
4      48            0.07747       0.09912       0.27415

5      63            0.74543       0.88974       0.88562
5      6             0.29015       0.20469       0.40527
5      10            0.20639       0.16705       0.28828
5      45            0.16331       0.14267       0.22810
5      42            0.15679       0.12421       0.21899

6      63            0.74543       0.88974       0.88562
6      10            0.55651       0.45044       0.39259
6      7             0.46708       0.46467       0.32950
6      42            0.41007       0.32486       0.28929
6      45            0.40599       0.35468       0.28641

7      63            0.74543       0.88974       0.88562
7      10            0.50095       0.40546       0.49836
7      13            0.48571       0.40235       0.48321
7      6             0.46708       0.32950       0.46467
7      8             0.46486       0.61807       0.46246

8      63            0.74543       0.88974       0.88562
8      10            0.52448       0.42451       0.69735
8      7             0.46486       0.46246       0.61807
8      11            0.45904       0.60117       0.61035
8      9             0.40559       0.38349       0.53928

9      63            0.74543       0.88974       0.88562
```

```
9      10            0.45687      0.36979      0.43198
9      7             0.44283      0.44054      0.41870
9      11            0.41858      0.54817      0.39577
9      8             0.40559      0.53928      0.38349

10     63            0.74543      0.88974      0.88562
10     6             0.55651      0.39259      0.45044
10     8             0.52448      0.69735      0.42451
10     7             0.50095      0.49836      0.40546
10     11            0.45784      0.59959      0.37058

11     63            0.74543      0.88974      0.88562
11     8             0.45904      0.61035      0.60117
11     10            0.45784      0.37058      0.59959
11     9             0.41858      0.39577      0.54817
11     13            0.40752      0.33758      0.53369

12     63            0.74543      0.88974      0.88562
12     8             0.32859      0.43689      0.42700
12     9             0.31537      0.29819      0.40983
12     11            0.30674      0.40170      0.39860
12     10            0.30231      0.24469      0.39285

13     63            0.74543      0.88974      0.88562
13     61            0.73672      0.57245      0.61029
13     7             0.48571      0.48321      0.40235
13     10            0.42854      0.34686      0.35499
13     11            0.40752      0.53369      0.33758

14     60            0.91933      0.81491      0.81527
14     63            0.74543      0.88974      0.88562
14     13            0.35197      0.29157      0.31213
14     10            0.34776      0.28148      0.30840
14     6             0.30004      0.21166      0.26608

15     60            0.91933      0.81491      0.81527
15     59            0.88391      0.85251      0.86956
15     42            0.35116      0.27819      0.34546
15     35            0.33429      0.28780      0.32887
15     44            0.27533      0.27510      0.27086

16     60            0.91933      0.81491      0.81527
16     58            0.29422      0.30400      0.59885
16     42            0.12736      0.10089      0.25923
16     35            0.11830      0.10185      0.24079
16     15            0.10383      0.10215      0.21134

17     60            0.91933      0.81491      0.81527
17     57            0.36986      1.00000      1.00000
17     21            0.12789      0.21581      0.34579

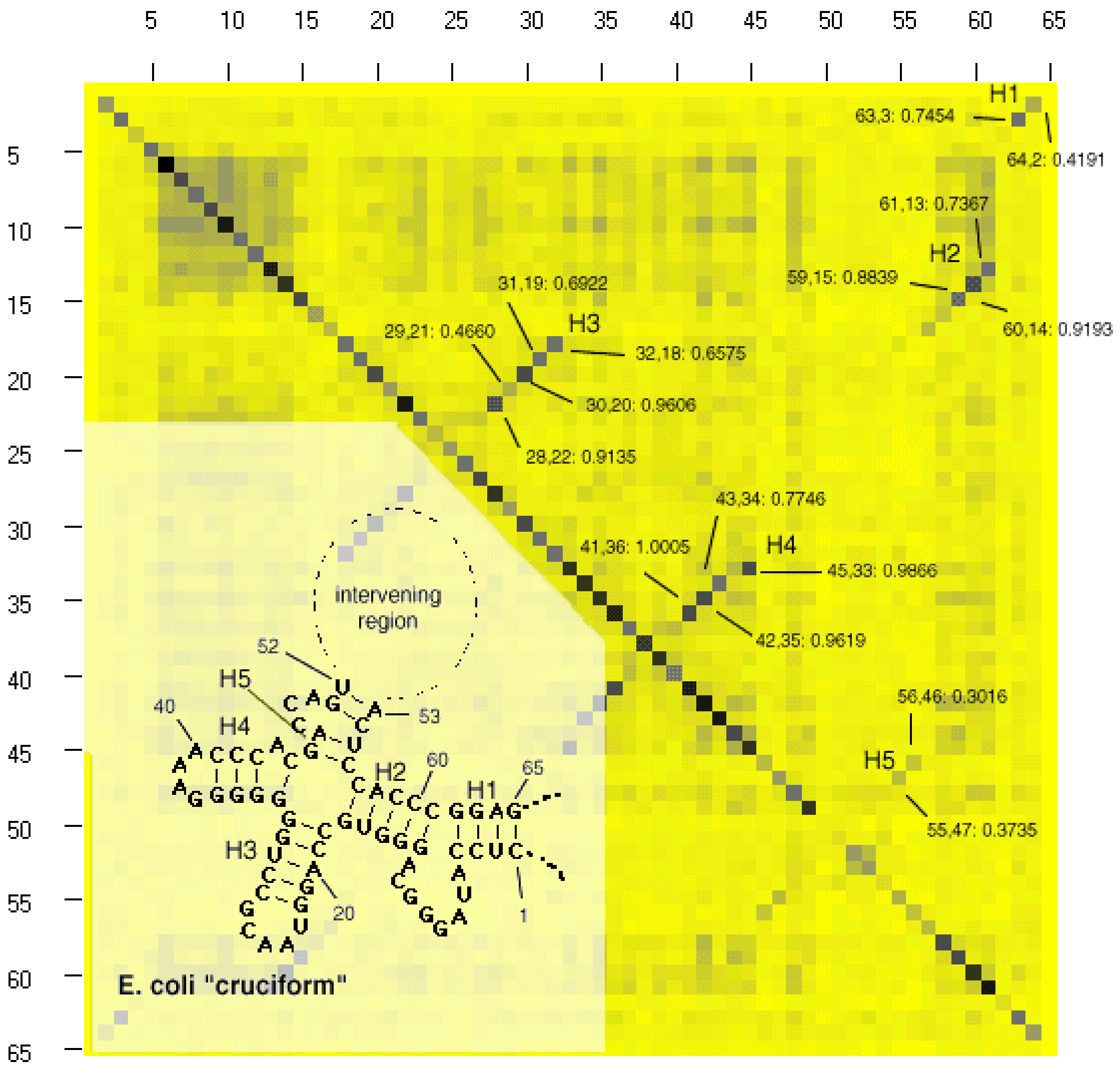
18     60            0.91933      0.81491      0.81527
18     32            0.65757      0.90669      0.86769
18     45            0.19840      0.17332      0.26180
18     44            0.18460      0.18444      0.24359
18     20            0.18288      0.17954      0.24132

19     60            0.91933      0.81491      0.81527
19     31            0.69220      0.87127      0.86915
19     44            0.27079      0.27056      0.34002
19     42            0.16344      0.12948      0.20523
19     6             0.14507      0.10234      0.18216

etc., etc., etc.
```

Mutual Information Plot Example

Below is shown a matrix plot of mutual information data from the sample alignment:

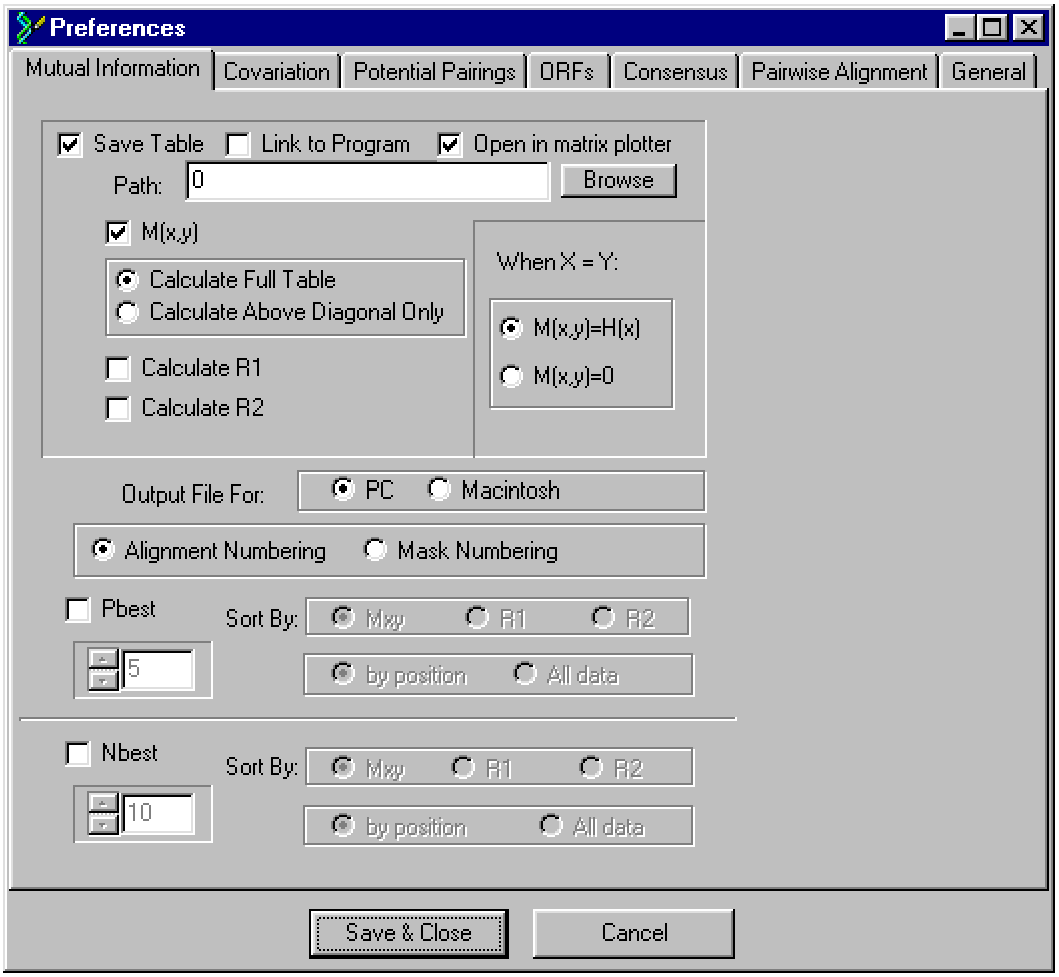


High-information pairs are diagrammed. Currently, BioEdit will produce this matrix plot with the axes, but will not add the additional annotation. Later releases may include automatic annotation of high-scores. The representative structure of this region from *E. coli* is shown in the lower left of the plot. This region represents a portion of the RNase P RNA from *E. coli*. As seen on the plot, regions of high information running perpendicular to the diagonal represent pairs of positions with highly correlated identities (they appear to influence each other), suggesting that they base-pair. Diagonal runs of high information strongly suggest the presence of base-paired helices. If you look at the partial *E. coli* RNase P structure below, you will notice localized diagonal regions of high information that correspond directly to positions along the helices (labeled H1-H5 in both the structure and the data). For an overview of viewing the data interactively with the matrix data plotter and the line area graphs, see "Using the Matrix Plotter for Mutual Information Data" and "1-D plots of matrix data rows and columns".

Brown, J.W. 1998. The Ribonuclease P Database. *Nucleic Acids Res. 26*:351-352
http://jwbrown.mbio.ncsu.edu/RNaseP

Setting Mutual Information Preferences

The mutual information preferences dialog may be brought up by choosing “Preferences” from the “Options” menu and clicking the “mutual information” tab.



☑ Save Table ☐ Link to Program ☑ Open in matrix plotter

Path: 0

☑ M(x,y)

● Calculate Full Table
○ Calculate Above Diagonal Only

☐ Calculate R1
☐ Calculate R2

When X = Y:
● M(x,y)=H(x)
○ M(x,y)=0

Output File For: ● PC ○ Macintosh

● Alignment Numbering ○ Mask Numbering

☐ Pbest 5 Sort By: ● Mxy ○ R1 ○ R2
● by position ○ All data

☐ Nbest 10 Sort By: ● Mxy ○ R1 ○ R2
● by position ○ All data

The options available are:

1. Save table: check this if you want a matrix of any or all of M(x,y), R1(x), or R2(x) values. If this option is checked, the following options are available:

a. Link to external program: A spreadsheet such as Excel or Quattro Pro may be specified on your computer to automatically open your saved table file after the analysis is run. This option is only available for the table. Lists will be opened in the BioEdit text editor.
b. M(x,y), R1(x) and R2(x) checkboxes: Choose any or all of these, depending on what you want. The data are not stored as separate tables if you choose more than one, so if you plan to use the matrix plotter or an external program such as SpyGlass Transform to view the table, you must make a separate table for each value. If you choose to put more than one value into the same table, the data for each position will be grouped vertically in the same order as shown on the preferences dialog. If you only want one half of the matrix (since *M(x,y)* is symmetrical), you may choose to calculate above the diagonal only. If you want to use the matrix plotter, you must have a full table. The R1 and R2 options will cause a full table to automatically be generated.
c. When x = y: When x = y, M(x,y) =H(x). If you are going to view this data in a plotting program and want the diagonal out of the picture, values along the diagonal may be set to zero.

2. File format may be PC or Macintosh. This will affect the output of all mutual information analyses.

3. Alignment Numbering vs. mask numbering: You may want the numbering of positions in the output to reflect the numbers as seen in the alignment window, or to the sequential positions of the mask. For example, if you are basing a structural analysis on the positions of a standard sequence which was used as the mask, you may want the numbering to reflect positions in that sequence rather than the gapped-out positions in the whole alignment. This is simply offered for convenience in analyzing the data.

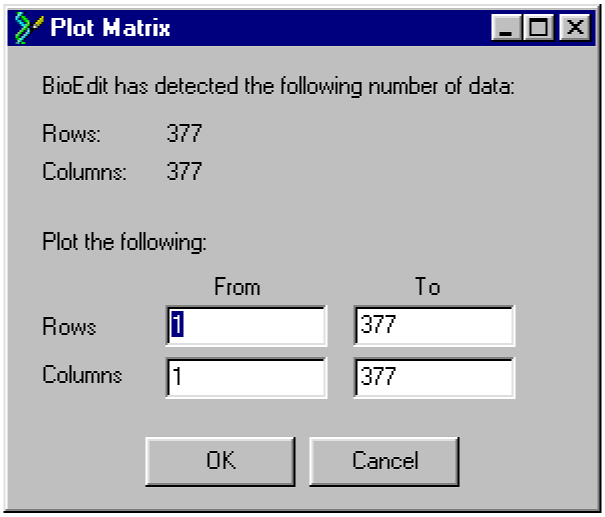
4. Pbest and Nbest options: see using mutual information in BioEdit.

Once the options you would like are chosen, you may either save them, then close the dialog, or simply close the dialog. If you close without saving, the chosen options will remain until the program is closed. If the options are saved, they will become the new defaults.

Using the Matrix Plotter for Mutual Information Data

To plot a 2-D matrix file, choose “matrix plotter” from the “RNA” menu of an open alignment or from the main application window. Once the plotter window is open, choose “plot a matrix” from the “plot” menu.

BioEdit will first look at the file to determine if it’s a file that it can figure out and plot (any fully tab-delimited, symmetric matrix should work). Then, the rows and columns are counted and a dialog is presented which asks what part of the matrix to plot (defaults to all rows and columns). For example, for a matrix representing an M(xy) analysis of 146 bacterial RNase P RNA sequences using *E. coli* as the sequence and numbering masks, the following dialog comes up:



BioEdit has detected the following number of data:

Rows: 377

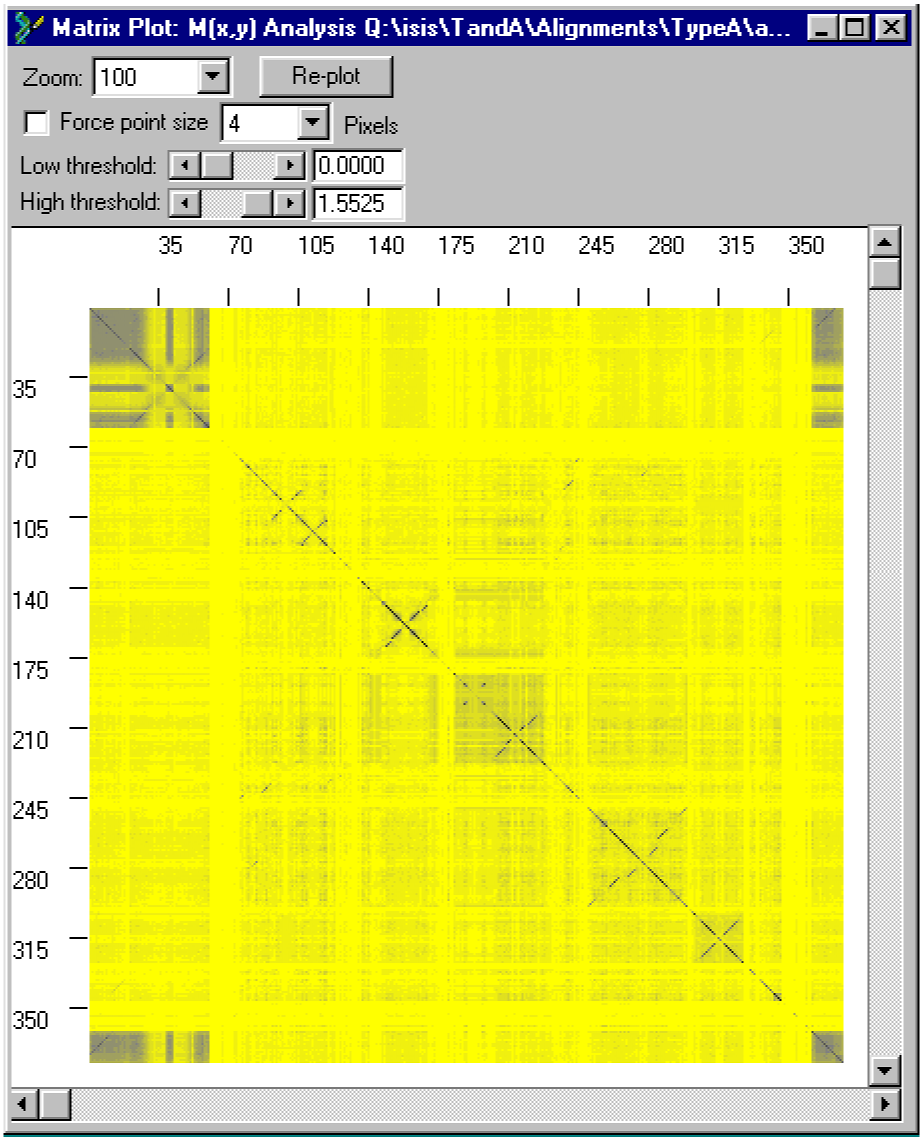
Columns: 377

Plot the following:

	From	To
Rows	1	377
Columns	1	377

Note: the limit for a single graphical plot appears to be less than about 2000 x 2000, so very large matrices must be analyzed in sections.

A plot of Mxy values for all positions of bacterial RNase P RNA present in *E. coli* is shown below:



Matrix Plot: M(x,y) Analysis Q:\isis\TandA\Alignments\TypeA\a...

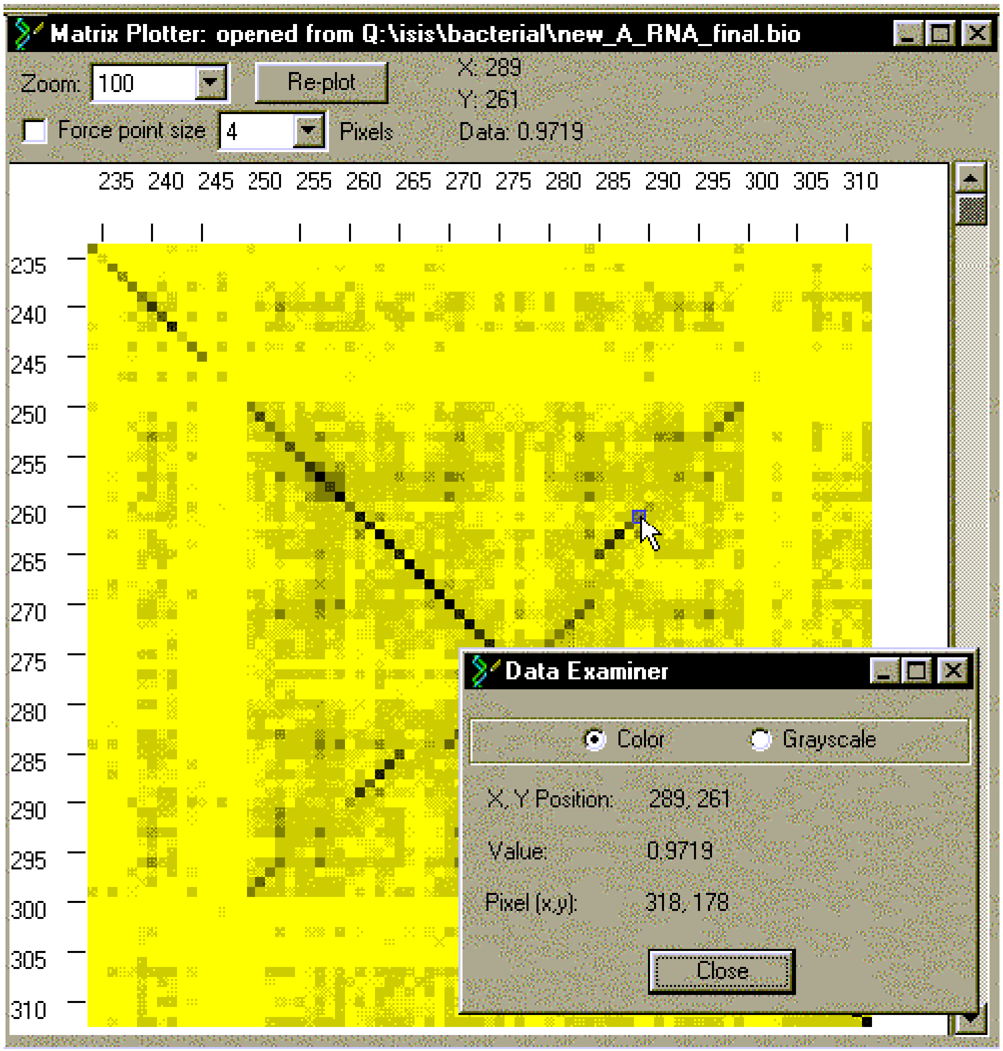
Zoom: 100

☐ Force point size 4 Pixels

Low threshold: 0.0000

High threshold: 1.5525

After the data have been plotted, you may open the data examiner from the “view” menu. To look at the data, simply move the mouse over the image. The x, y coordinates and the data values will show up in the data examiner. You may also click on a data point with the mouse and the data is reported on the top bar of the matrix plotter. Currently, printing is not supported from this window, but may be added later. However, the image may be copied directly to the clipboard and pasted into any application, or it can be saved as a bitmap (*.bmp).



Matrix Plotter: opened from Q:\isis\bacterial\new_A_RNA_final.bio

Zoom: 100

□ Force point size 4 Pixels

X: 289
Y: 261
Data: 0.9719

Data Examiner

● Color ○ Grayscale

X, Y Position: 289, 261

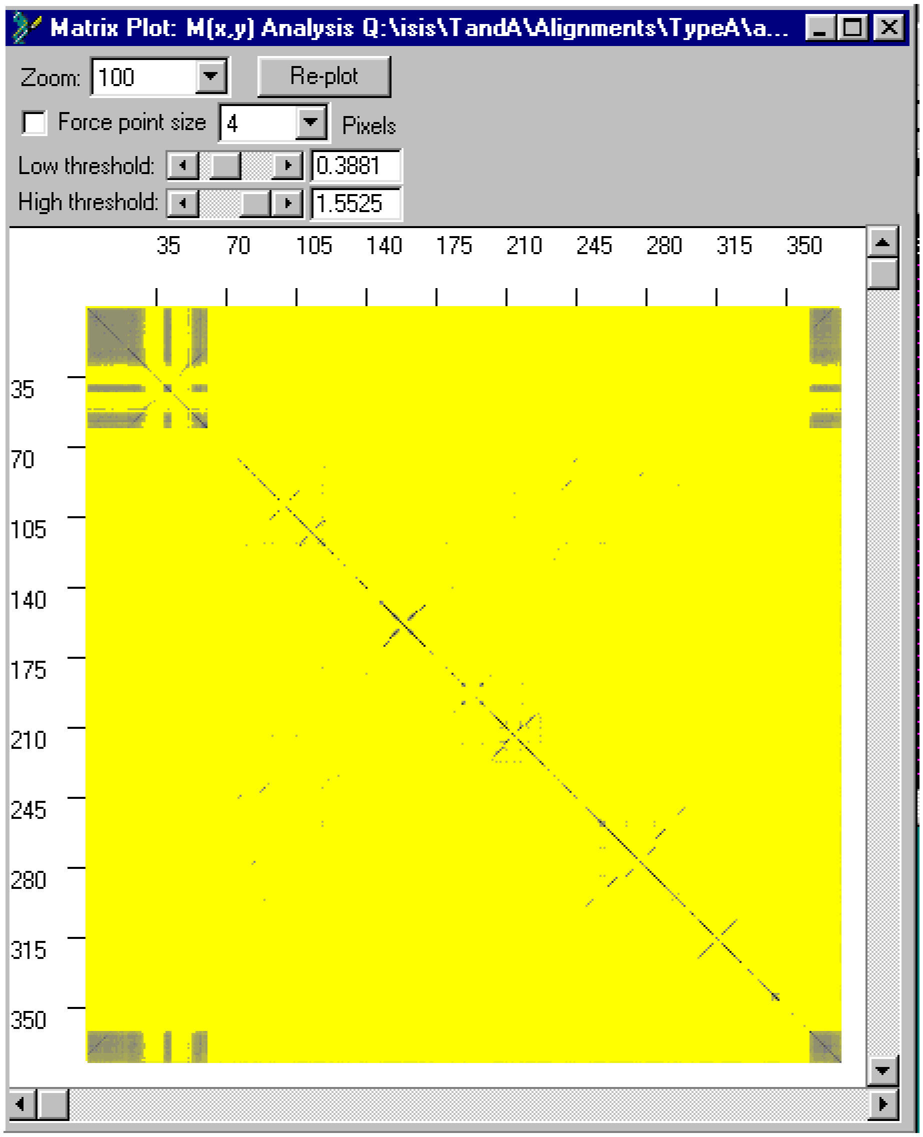
Value: 0.9719

Pixel (x,y): 318, 178

The zoom control may be used to zoom in on the image from 25% to 800%.

The currently selected point may be used as a launch point for a 1-D plot of a rows or columns of matrix data (see 1-D Plots of Matrix Data Rows and Columns)

The threshold controls set the data thresholds for shading in the matrix plotter. This may be useful for bringing out only high scoring data, as in the following example showing the same plot as shown above, with the low threshold set at 0.3881. The high threshold causes anything over the set value to be shaded light blue. This is currently set to max here.



Matrix Plot: M(x,y) Analysis Q:\isis\TandA\Alignments\TypeA\a...

Zoom: 100

□ Force point size 4 Pixels

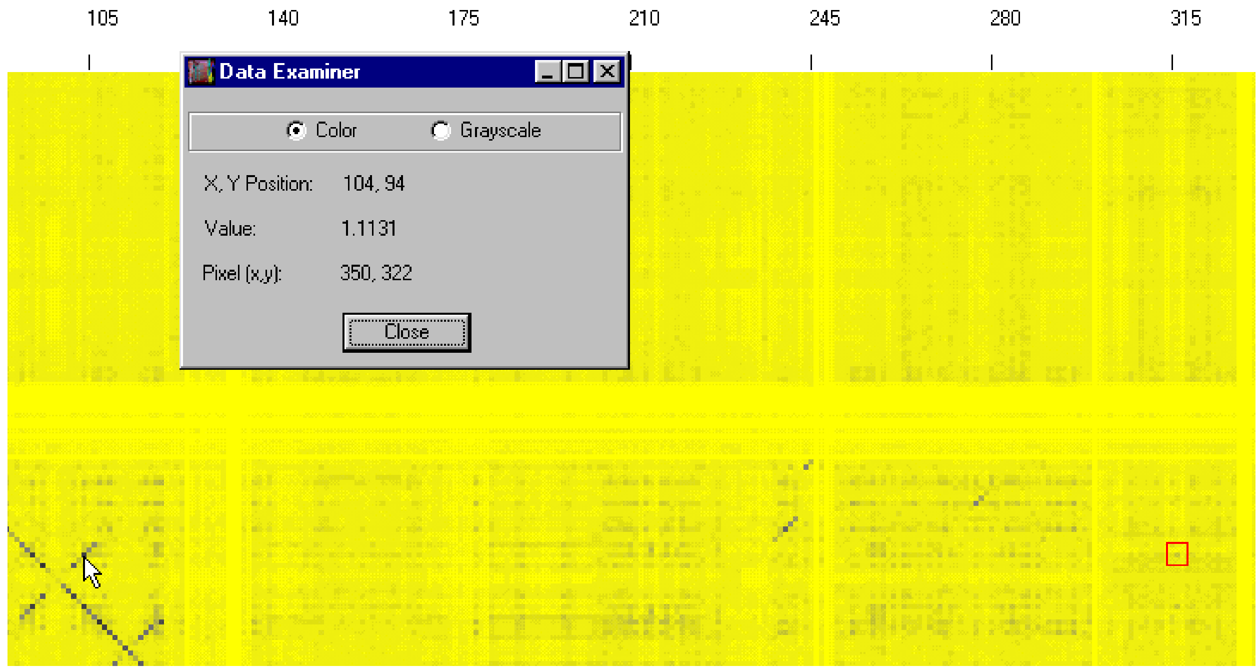
Low threshold: 0.3881

High threshold: 1.5525

1-D Plots of Matrix Data Rows and Columns

When looking at data from a 2-D matrix, such as that generated from a mutual information analysis, it can be tedious or even overwhelming to look at tables of numbers to pick out high scores. To help view this type of data, the matrix plotter was created. The matrix plotter, however, plots data as a darkness intensity on a scale of 1 byte (0 to 255). Subtle differences between different data points are sometimes difficult to see, such as the mutual information often seen between the two residues of a base-pair and a third residue of a nucleotide triplet. For this reason, BioEdit offers the option to plot rows or columns of a matrix along a 1-dimensional line area graph. Consider the following example:

A matrix plot of mutual information data from an alignment of bacterial RNaseP RNAs is shown below (*E. coli* serving as the mask) In this plot, it would be extremely difficult to pick out a particular base triplet, namely a triplet between base pair 94-104 and nucleotide 316, although the base pair 94-104 is clearly visible. The figure below is a partial view of a full Mxy table from a bacterial RNase P RNA alignment, plotted with a forced data point size of 3x3 pixels. Position 94-104 is shown at the mouse arrow. Position 94-316 is in the center of the small red box toward the right side.



● Color ○ Grayscale

X, Y Position: 104, 94

Value: 1.1131

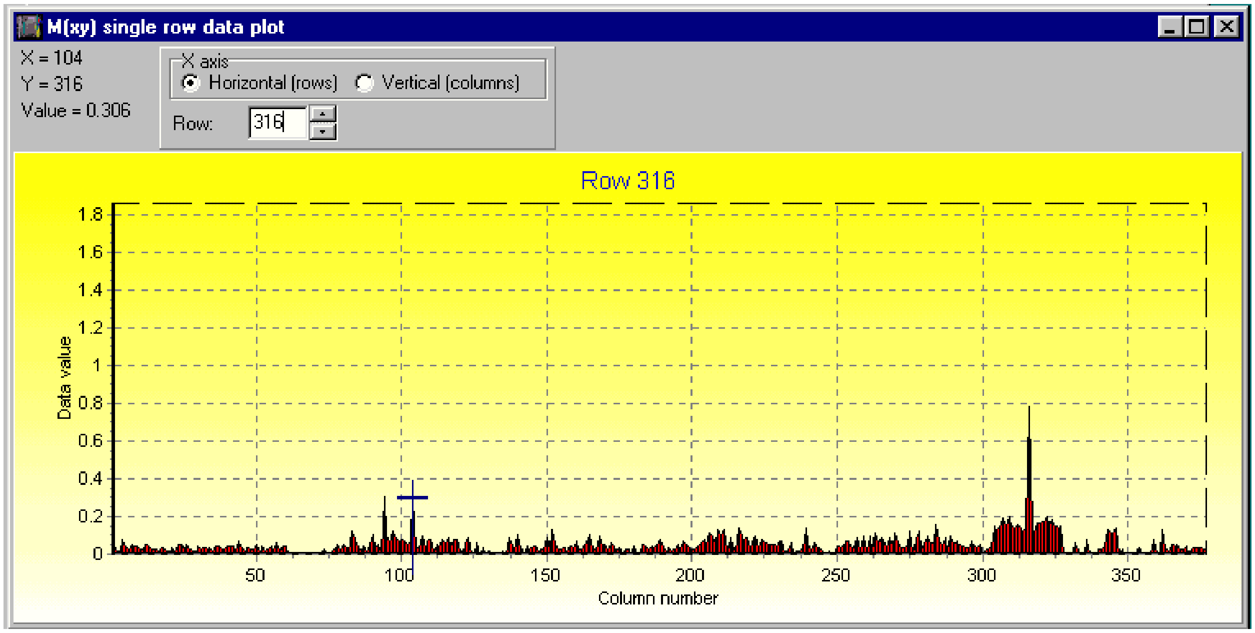
Pixel (x,y): 350, 322

That nucleotide 316 might interact with positions 94 and 104 is not obvious on this plot. A 1-D area graph of rows of this matrix may be produced by choosing “Line Graph of Rows” from the “Plot” menu of an open plot.

Note: This option is only available from within an open matrix plot.

When the graph comes up, it will show a contour plot of Row 1. To examine the data in another row, the up and down arrow keys may be used to scroll through the rows, or the row to examine may be entered directly into the “Row” window at the top of the graph form.

A plot of row 316 is shown below. Note the position of the blue cross. The data at any position may be viewed by clicking the mouse on the graph for that value. The lines of the blue cross will intersect at the peak of the data point, and x, y, and the data value will be shown in the upper left corner of the form.



X = 104
Y = 316
Value = 0.306

X axis: ● Horizontal (rows) ○ Vertical (columns)

Row: 316

This plot shows that there is a relatively high level of information between 316 and 104, and between 316 and 94. In the above example, position 104 has been selected, and the upper left shows that the Mxy value for 104-316 is 0.306. The data may also be vied by column by selecting "columns" for the X-axis (above).

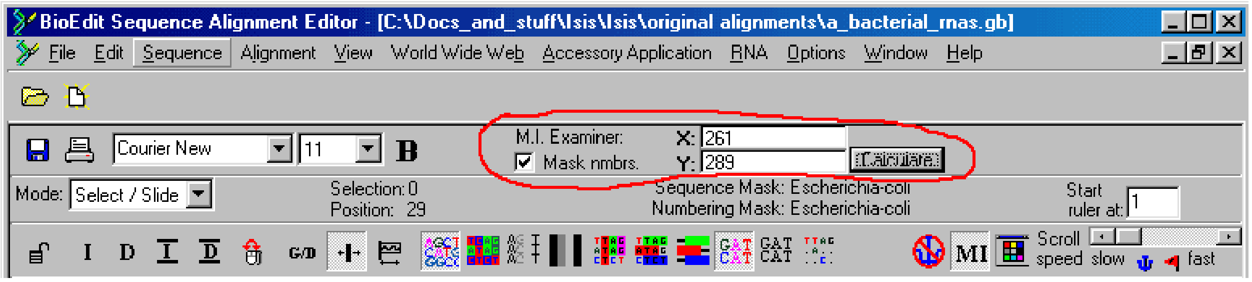
The current version of BioEdit allows the use of a numbering mask which differs from the sequence mask when running comparative analyses. This allows for easier navigation of data when, for example, only a part of a molecule is analyzed, and it is convenient to refer to the numbering scheme of a reference sequence or structure that represents the whole molecule. Also, the numbering in the actual alignment may be used even when a sequence mask is used, to allow for reference to the alignment when analyzing positional data. Because of this, the latest release (v5.0.0) has updated the line plot to report the row or column number which actually appears in the data file, rather than the absolute row or column (for example, the first row in the matrix might be 234, rather than 1, and the second may be 300, rather than 2).

For information on this type of analysis, see mutual information and The basis of phylogenetic comparative analysis.

The mutual information examiner

If you would like to view the mutual information between any two positions from within the alignment window, make the "mutual information examiner" visible by choosing this option under the "View" menu. This is an idea stolen directly from Dr. J. Brown's Macintosh hypercard program "Covariation".

To use the mutual information examiner, first make the control visible by choosing View->Mutual Information Examiner. The control comes up at the top of the form:



Courier New 11

M.I. Examiner: X: 261

☑ Mask nmbrs. Y: 289

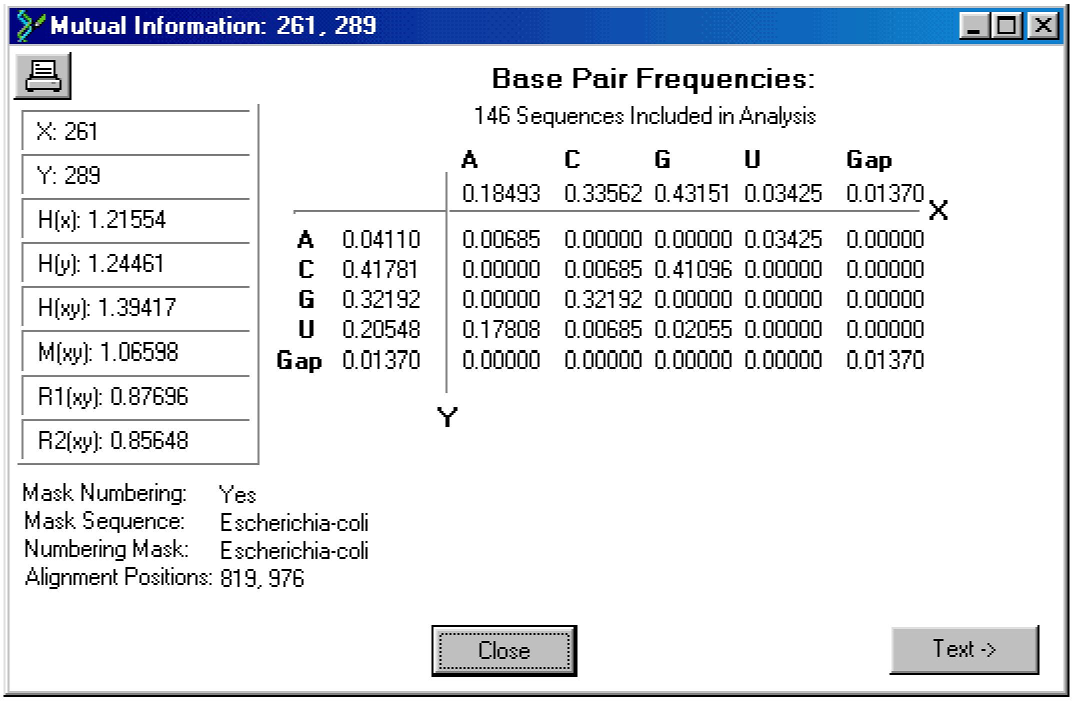
Mode: Select / Slide

Selection: 0
Position: 29

Sequence Mask: Escherichia-coli
Numbering Mask: Escherichia-coli

Start ruler at: 1

Enter the numbers of the positions (x and y) you would like to analyze. If you want these to reflect the positions of a particular sequence (not gapped), set this sequence as the *numbering* mask and enter the positions accordingly. The above positions correspond to the position selected on the matrix plot example. ***Make sure to select all the sequences you would like to include in the analysis***. If for some reason you would like to exclude sequences from the analysis, either do not select them or deselect them by Ctrl-mouse-clicking them. After the positions are entered, press calculate. A window with the following information will appear:



X: 261
Y: 289
H(x): 1.21554
H(y): 1.24461
H(xy): 1.39417
M(xy): 1.06598
R1(xy): 0.87696
R2(xy): 0.85648

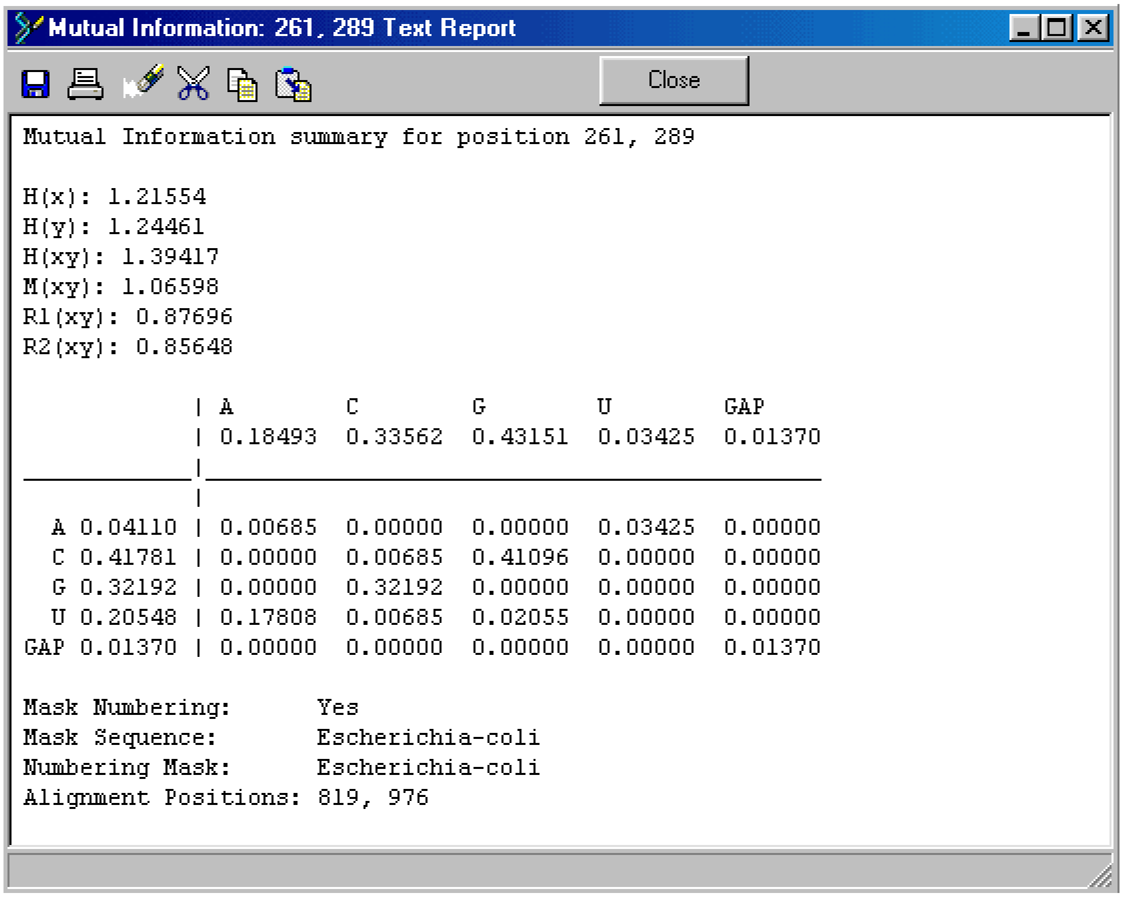
Base Pair Frequencies:

146 Sequences Included in Analysis

		A	C	G	U	Gap
		0.18493	0.33562	0.43151	0.03425	0.01370
A	0.04110	0.00685	0.00000	0.00000	0.03425	0.00000
C	0.41781	0.00000	0.00685	0.41096	0.00000	0.00000
G	0.32192	0.00000	0.32192	0.00000	0.00000	0.00000
U	0.20548	0.17808	0.00685	0.02055	0.00000	0.00000
Gap	0.01370	0.00000	0.00000	0.00000	0.00000	0.01370

Mask Numbering: Yes
Mask Sequence: Escherichia-coli
Numbering Mask: Escherichia-coli
Alignment Positions: 819, 976

To get a text summary which can be copied and pasted easily, press the Text-> button. The following will appear in a text editor window:



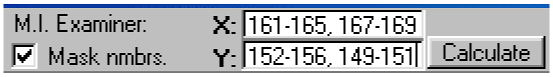
```
Mutual Information summary for position 261, 289

H(x): 1.21554
H(y): 1.24461
H(xy): 1.39417
M(xy): 1.06598
R1(xy): 0.87696
R2(xy): 0.85648

            | A        C        G        U        GAP
            | 0.18493  0.33562  0.43151  0.03425  0.01370
____________|_____________________________________________
            |
  A 0.04110 | 0.00685  0.00000  0.00000  0.03425  0.00000
  C 0.41781 | 0.00000  0.00685  0.41096  0.00000  0.00000
  G 0.32192 | 0.00000  0.32192  0.00000  0.00000  0.00000
  U 0.20548 | 0.17808  0.00685  0.02055  0.00000  0.00000
GAP 0.01370 | 0.00000  0.00000  0.00000  0.00000  0.01370

Mask Numbering:      Yes
Mask Sequence:       Escherichia-coli
Numbering Mask:      Escherichia-coli
Alignment Positions: 819, 976
```

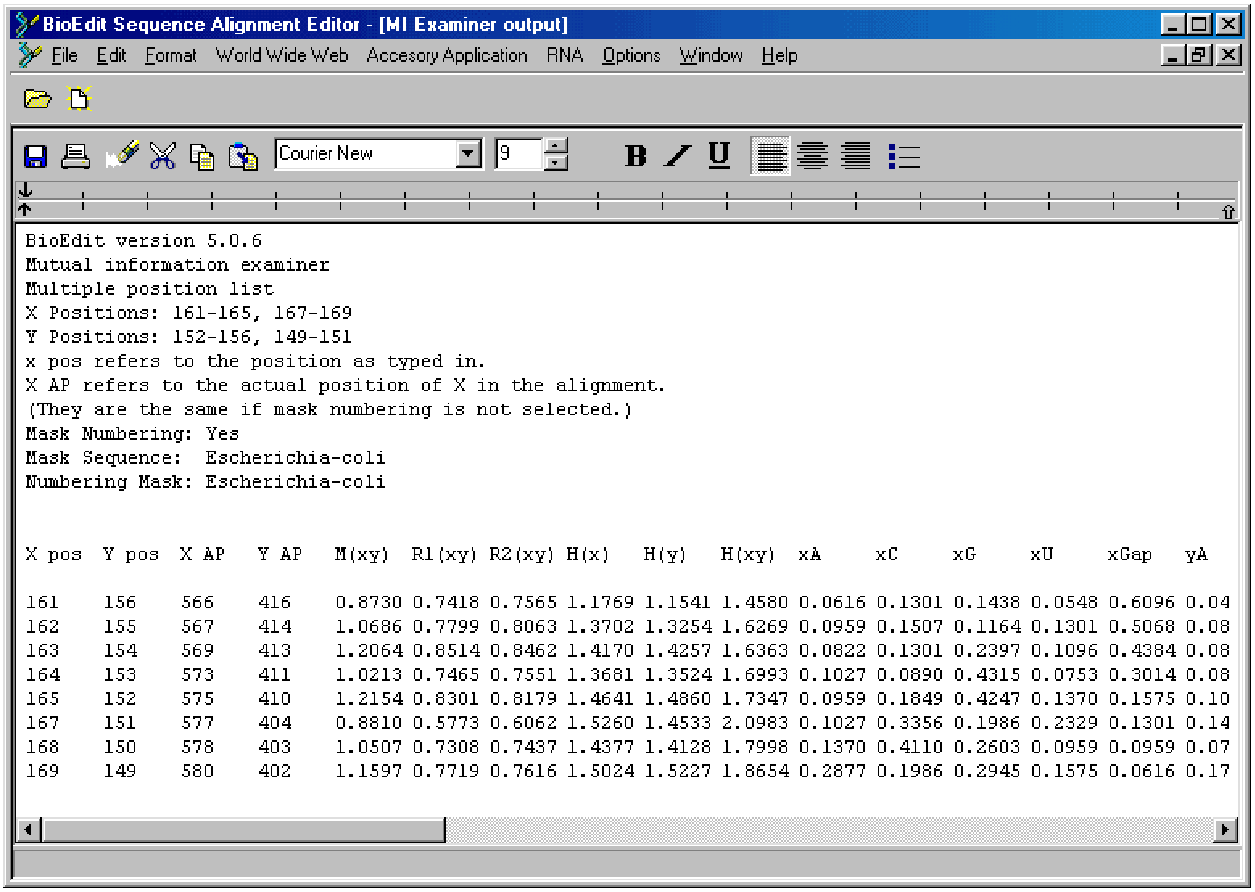
If you would like to analyze several positions at once, you may specify the positions with commas and dashes. For example, you could enter the following:



M.I. Examiner: X: 161-165, 167-169

☑ Mask nmbrs. Y: 152-156, 149-151

You will get a list such as on the following page:


BioEdit Sequence Alignment Editor - [MI Examiner output]
File Edit Format World Wide Web Accesory Application RNA Options Window Help
Courier New
9
B
U

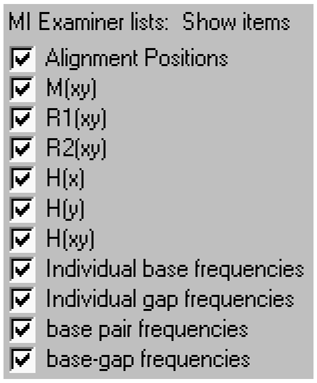
```
BioEdit version 5.0.6
Mutual information examiner
Multiple position list
X Positions: 161-165, 167-169
Y Positions: 152-156, 149-151
x pos refers to the position as typed in.
X AP refers to the actual position of X in the alignment.
(They are the same if mask numbering is not selected.)
Mask Numbering: Yes
Mask Sequence:  Escherichia-coli
Numbering Mask: Escherichia-coli


X pos  Y pos  X AP   Y AP   M(xy)  R1(xy) R2(xy) H(x)   H(y)   H(xy)  xA     xC     xG     xU     xGap   yA

161    156    566    416    0.8730 0.7418 0.7565 1.1769 1.1541 1.4580 0.0616 0.1301 0.1438 0.0548 0.6096 0.04
162    155    567    414    1.0686 0.7799 0.8063 1.3702 1.3254 1.6269 0.0959 0.1507 0.1164 0.1301 0.5068 0.08
163    154    569    413    1.2064 0.8514 0.8462 1.4170 1.4257 1.6363 0.0822 0.1301 0.2397 0.1096 0.4384 0.08
164    153    573    411    1.0213 0.7465 0.7551 1.3681 1.3524 1.6993 0.1027 0.0890 0.4315 0.0753 0.3014 0.08
165    152    575    410    1.2154 0.8301 0.8179 1.4641 1.4860 1.7347 0.0959 0.1849 0.4247 0.1370 0.1575 0.10
167    151    577    404    0.8810 0.5773 0.6062 1.5260 1.4533 2.0983 0.1027 0.3356 0.1986 0.2329 0.1301 0.14
168    150    578    403    1.0507 0.7308 0.7437 1.4377 1.4128 1.7998 0.1370 0.4110 0.2603 0.0959 0.0959 0.07
169    149    580    402    1.1597 0.7719 0.7616 1.5024 1.5227 1.8654 0.2877 0.1986 0.2945 0.1575 0.0616 0.17
```

When regions are specified as X = *a*-*b* and Y = *c*-*d*, it is assumed that what you are interested in is helices, and the positions are analyzed in antiparallel order. regardless of whether they are written as *c*-*d* or *d*-*c*. To force the comparison of positions in a particular order, specify them such as X = *a*, *b*, *c*, *d*   Y = *e*, *f*, *g*, *h*.

For the list output, you may not want to weed through all of the reporting options. Therefore, under Options->Preferences->Mutual Information, you have the following list formatting options:



MI Examiner lists: Show items

- ☑ Alignment Positions
- ☑ M(xy)
- ☑ R1(xy)
- ☑ R2(xy)
- ☑ H(x)
- ☑ H(y)
- ☑ H(xy)
- ☑ Individual base frequencies
- ☑ Individual gap frequencies
- ☑ base pair frequencies
- ☑ base-gap frequencies