The Artemis Manual

Copyright © 1999-2010 by Genome Research Limited

This document describes release 12 of Artemis a DNA sequence viewer and sequence annotation tool.

Artemis is free software; you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation; either version 2 of the License, or (at your option) any later version.

This program is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the GNU General Public License for more details.

You should have received a copy of the GNU General Public License along with this program; if not, write to the Free Software Foundation, Inc., 59 Temple Place, Suite 330, Boston, MA 02111-1307 USA

For the full text of the license see the section called *Copyright Notice* in Chapter 1.

Table of Contents

```
1. Introduction to Artemis
```

What is Artemis?

System Requirements

Getting and Installing Artemis

Installing Java

Installation Instructions for UNIX and GNU/Linux

<u>Installation Instructions for MacOSX</u>

Installation Instructions for Windows

Concepts

The "Entry"

EMBL/Genbank Features

EMBL/Genbank Feature Keys

EMBL/Genbank Feature Qualifiers

"Tab" Files or "Table" Files

The Active Entries

The Default Entry

Feature Segments

The Selection

Feature Colours

Contributions and Suggestions

Distribution Conditions and Acknowledgments

Copyright Notice

2. Starting Artemis

Running Artemis on UNIX and GNU/Linux Systems

UNIX Command Line Arguments for Artemis

<u>-quiet</u>

-options

-Xmsn -Xmxn

Running Artemis on Macintosh Systems

Running Artemis on Windows Systems The Artemis Launch Window The File Menu The Options Menu 3. The Artemis Main Window Overview of the Entry Edit Window A breakdown of the main Artemis edit window The File Menu Show File Manager ... Read An Entry ... Read Entry Into Read BAM ... Save Default Entry Save An Entry Save An Entry As Save All Entries Write Clone This Window Save As Image Files (png/jpeg) **Print Print Preview Preferences** Close The Entries Menu Set Name Of Entry Set Default Entry Remove An Entry Remove Active Entries **Deactivate All Entries** The Select Menu Feature Selector ... All All Bases Select All Features in Non-matching Regions None By Key **CDS** Features Same Key Open Reading Frame Features Overlapping Selection Base Range ... Feature AA Range ... **Toggle Selection** The View Menu **Selected Features** Selection Search Results **CDS Genes And Products** Feature Filters Overview Forward Strand Overview Reverse Strand Overview

Feature Bases

Feature Bases As FASTA Feature Amino Acids Feature Amino Acids As FASTA **Feature Statistics** Feature Plots The Goto Menu Navigator ... Start of Selection End of Selection Feature Start Feature End Start of Sequence End of Sequence Feature Base Position ... Feature Amino Acid ... The Edit Menu Undo Redo Selected Features in Editor Subsequence (and Features) Find Or Replace Qualifier Text Qualifier(s) of Selected Feature Selected Feature(s) Move Selected Features To Copy Selected Features To Trim Selected Features **Extend Selected Features** Fix Stop Codons **Automatically Create Gene Names** Fix Gene Names Bases Contig Reordering ... Header Of Default Entry The Create Menu New Feature Feature From Base Range **Intergenic Features** Features From Non-matching Regions New Entry Mark Open Reading Frames ... Mark Empty ORFs ... Mark Open Reading Frames In Range ... Mark From Pattern ... Mark Ambiguities The Run Menu Configuring the Run Menu The Graph Menu Hide All Graphs Add Usage Plots ... Add User Plot ... GC Content (%) GC Content (%) With 2.5 SD Cutoff

AG Content (%)

```
GC Frame Plot
          Correlation Scores
          Reverse Correlation Scores
          GC Deviation (G-C)/(G+C)
          AT Deviation (A-T)/(A+T)
          Karlin Signature Difference
          Cumulative AT Skew and Cumulative GC Skew
          Positional Asymmetry
          Informational Entropy
          Scaled Chi Square
          Mutational Response Index
          Effective Codon Number
          Intrinsic Codon Deviation Index
     The Display Menu
          Show Entry Buttons
          Show Base View
          Show Feature List
     The Entry Button Line
     The Overview and DNA Views
          Changing the Selection from a View Window
          Other Mouse Controlled Functions
          The Pop-up Menu
          Scrolling The View
          Changing The Scale
          "Direct Editing"
     The Feature List
          Changing the Selection from the Feature List
          Other Mouse Controlled Functions
          The Pop-up Menu
          Scrolling The List
     Graphs and Plots
     Notes on Using The Mouse
4. Secure Shell (SSH) Plugin To Artemis
     Overview of the SSH Plugin
     Login In
     Setting up a SSH tunnel
     Using Putty to Set Up A Tunnel
     Using the SSH connection to run Fasta and BLAST
     Configuring Searches to be run via SSH
5. Artemis Configuration Options
     The Options File
     Artemis Option Descriptions
          font size
          font name
          base plot height
          feature plot height
          draw feature borders
          draw feature arrows
          overview feature labels
          overview one line per entry
          show list
          show base view
          features on frame lines
```

frame line features feature labels one line per entry genetic codes translation table 1 extra keys extra qualifiers common keys hyperlinks undo levels minimum orf size direct edit feature dna programs feature protein programs ncbi dna search ncbi protein search **Setting Colours** Options For Plots and Graphs Options For A Chado Database Options File Format An Example Options File

List of Tables

3-1. IUB Base Codes

Chapter 1. Introduction to Artemis

What is Artemis?

Artemis is a DNA sequence viewer and annotation tool that allows visualisation of sequence features and the results of analyses within the context of the sequence, and its six-frame translation. Artemis is written in Java, reads EMBL or GENBANK format sequences and feature tables, and can work on sequences of any size.

On Unix and GNU/Linux systems, given an EMBL accession number Artemis also can read an entry directly from the <u>EBI</u> using <u>Dbfetch</u>. This feature is still experimental, but copes well with straightforward entries (see <u>the section called *Open from EBI - Dbfetch*</u> ... in Chapter 2).

For more information on Artemis go to the Artemis web pages.

System Requirements

Artemis will run on any machine that has a recent version of Java. This version of Artemis requires Java 1.5 at least. Most of the development has been done using JDK v1.6 and some of the more advanced features

(BamView) now require 1.6. Please note that the version of Java that comes with Netscape and Internet Explorer is not sufficient to run Artemis. See <u>the section called *Getting and Installing Artemis*</u> for details on how to get Java.

Getting and Installing Artemis

The most up to date version of Artemis is always available from the Artemis web pages.

Installing Java

Before installing Artemis you will need to make sure you have Java installed. Please note that the version of Java that comes with Netscape and Internet Explorer is not sufficient to run Artemis.

- Linux, UNIX users should get the current Java version from the <u>Sun</u>.
- Windows users should get latest stable release from <u>Java version</u> from Sun. On some versions of Windows (such as Windows 2000) you will need be have administrator privileges for your machine to successfully install Java.
- Macintosh users with MacOS X should have java installed. You can get the latest version from <u>Apple</u>. Note, however, that Java on the older versions of MacOS X has irritating bugs so we recommend using the latest version of MacOS, which runs Artemis well. Macintosh users with older MacOS versions cannot use this release of Artemis.

Installation Instructions for UNIX and GNU/Linux

- Change directory to the directory you wish to install Artemis in. We will use ~/ in this example and in the next chapter.
- uncompress and untar the artemis compiled.tar.gz file. On UNIX the command is:

 $gzip -d \leq artemis_compiled.tar.gz \mid tar \ xf -$

This will create a directory called ~/artemis which will contain all the files necessity for running Artemis.

For instructions on how to run Artemis on UNIX and GNU/Linux once the archive is unpacked see <u>the section called *Running Artemis on UNIX and GNU/Linux Systems* in Chapter 2.</u>

Installation Instructions for MacOSX

For MacOSX users an archive artemis_act.dmg.gz disk image is provided. This contains both Artemis and ACT applications. This can be uncompressed using gunzip:

```
gunzip artemis_act.dmg.gz
```

Alternatively on OS X an easier solution is provided by StuffIt Expander. Double-click on any file ending in ".gz" and StuffIt Expander will be launched to uncompress that file.



The uncompressed disk image file "artemis_act.dmg" can be mounted by double clicking on it. The mounted image, "artemis_act", can then be opened and the software contents displayed by double clicking on it. Open an read the "Readme.txt" file.

Installation Instructions for Windows

On Windows systems with Java (version 1.5 or higher), installing Artemis is as simple as downloading the the artemis.jar file to an appropriate place (such as the desktop or the Programs folder). When downloading some versions of windows mistake this file for a zip file ensure it does not name this artemis.zip, if it does rename it to artemis.jar (DO NOT UNZIP THIS FILE).

For instructions on how to run Artemis on Windows once it is unpacked see <u>the section called *Running Artemis on Windows Systems* in Chapter 2</u>.

Concepts

Here are some general concepts about Artemis that may make the rest of this manual clearer.

The "Entry"

An "entry" in Artemis-speak is not necessarily a complete EMBL or GENBANK entry. In most places in this manual when we refer to an entry we mean a file that contains just the <u>feature table</u> lines (the FT lines) of an EMBL/GENBANK entry (see <u>the section called "Tab" Files or "Table" Files</u>). After loading a sequence and opening an entry edit window (see <u>the section called *Open* ... in Chapter 2</u>) it is then possible to overlay many feature tables (see <u>the section called *Read An Entry* ... in Chapter 3</u>). Each of these feature table files is called an entry by Artemis and it's features are kept separate from those of other entries.

This meaning of the word "entry" is used by most of the items in the File menu (see the section called *The*

File Menu in Chapter 3) and by the items in the Entry menu (see the section called *The Entries Menu* in Chapter 3).

EMBL/Genbank Features

A "feature" in an EMBL or Genbank file is a region of DNA that has been annotated with a key/type (see the-section called EMBL/Genbank Feature Keys) and zero or more qualifiers (see the-section called EMBL/Genbank Feature Qualifiers). In an EMBL or Genbank formated file the features of a piece of DNA are listed in the feature table section (see the-section called trab" Files or "Table" Files).

EMBL/Genbank Feature Keys

All EMBL and Genbank features have exactly one "key" assigned to them. The key is the type of the feature. Examples include CDS (a CoDing Sequence), intron and misc feature (MISCellaneous feature).

The EBI has a list of all possible feature keys.

EMBL/Genbank Feature Qualifiers

The qualifiers of a feature in an EMBL or Genbank file are the notes and extra information about the feature. For example an exon feature might have a /gene="ratC" qualifier, meaning that the exon feature is part of a gene named ratC.

The EBI has a list of all possible feature qualifiers.

"Tab" Files or "Table" Files

An EMBL or Genbank file that only contains a feature table (just FT lines, no sequence or header lines) is called a "table" file, or sometimes just a "tab" file because the often has a name like "somefile.tab".

The Active Entries

All entries in Artemis are considered to be "active" or "inactive". The overview, DNA view and feature list parts of the main window will only display features from active entries. To find out how to set the active and inactive entries see the section called *The Entries Menu* in Chapter 3.

The Default Entry

Many actions (such as creating features) require an entry to be identified as the source or destination for the action. Some actions, such as "Move Selected Features To ..." in the edit menu, will explicitly ask for an entry, but some assume that the action refers to a "default entry" that was previously set by the user.

The default entry can be set by using the "Set Default Entry ..." menu item in the Entries menu (see <u>the section called Set Default Entry in Chapter 3</u>) or by using the entry buttons (see <u>the section called The Entry Button Line in Chapter 3</u>).

Feature Segments

The term "segment" in the context of a CDS feature means "exon". We use the term "segment", because non-CDS features (such as misc_feature) can have exon-like parts too, but the term "exon" would be inappropriate in that case.

The Selection

In common with applications like word processors and graphics programs, Artemis allows the user to "select" the objects that the program will act on. The objects to act on in Artemis are features, feature segments or bases. If a feature segment is added to the selection, the feature that contains the segment is implicitly added as well. The current selection can be changed with the Select Menu (see the section called *The Select Menu* in Chapter 3) or using the mouse (see the section called *Changing the Selection from a View Window* in Chapter 3 and the section called *Changing the Selection from the Feature List* in Chapter 3).

Feature Colours

Each feature displayed in Artemis can be given a colour. The available colours are set in the options file (see Chapter 5) and are assigned to a feature by adding a /colour qualifier (see the section called Selected Features in Editor in Chapter 3). Currently there are two ways of specifying feature colours. The first way uses a single number. For example red is colour 2, so adding /colour=2 as a feature qualifier will make that feature red. The second way is to specify the red, green and blue components of the colour. Each of the components can take values from 0 to 255, with 255 being the most intense. For example /colour=255 0 is another way to give a feature the colour red. If no /colour qualifier is set for a feature a default colour is used (the default colours are also specified in the options file).

Contributions and Suggestions

We welcome contributions to Artemis, bug reports from users and suggestions for new features. An email

discussion list has been set up for this purpose. To join, send a message to 'majordomo@sanger.ac.uk' with 'subscribe artemis-users' in the body (not the subject). Announcements will also be sent to this list.

Distribution Conditions and Acknowledgments

Artemis may be freely distributed under the terms of the <u>GNU General Public License</u>. See <u>the section called *Copyright Notice*</u> for the full text of the license.

The development of Artemis is funded by the <u>Wellcome Trust's</u> through it's support of the <u>Pathogen Genomics Group</u> at <u>The Sanger Centre</u>.

Copyright Notice

GNU GENERAL PUBLIC LICENSE Version 2, June 1991

Copyright (C) 1989, 1991 Free Software Foundation, Inc. 59 Temple Place, Suite 330, Boston, MA 02111-1307 USA

Everyone is permitted to copy and distribute verbatim copies of this license document, but changing it is not allowed.

Preamble

The licenses for most software are designed to take away your freedom to share and change it. By contrast, the GNU General Public License is intended to guarantee your freedom to share and change free software—to make sure the software is free for all its users. This General Public License applies to most of the Free Software Foundation's software and to any other program whose authors commit to using it. (Some other Free Software Foundation software is covered by the GNU Library General Public License instead.) You can apply it to your programs, too.

When we speak of free software, we are referring to freedom, not price. Our General Public Licenses are designed to make sure that you have the freedom to distribute copies of free software (and charge for this service if you wish), that you receive source code or can get it if you want it, that you can change the software or use pieces of it in new free programs; and that you know you can do these things.

To protect your rights, we need to make restrictions that forbid anyone to deny you these rights or to ask you to surrender the rights. These restrictions translate to certain responsibilities for you if you distribute copies of the software, or if you modify it.

For example, if you distribute copies of such a program, whether gratis or for a fee, you must give the recipients all the rights that you have. You must make sure that they, too, receive or can get the source code. And you must show them these terms so they know their rights.

We protect your rights with two steps: (1) copyright the software, and

(2) offer you this license which gives you legal permission to copy, distribute and/or modify the software.

Also, for each author's protection and ours, we want to make certain that everyone understands that there is no warranty for this free software. If the software is modified by someone else and passed on, we want its recipients to know that what they have is not the original, so that any problems introduced by others will not reflect on the original authors' reputations.

Finally, any free program is threatened constantly by software patents. We wish to avoid the danger that redistributors of a free program will individually obtain patent licenses, in effect making the program proprietary. To prevent this, we have made it clear that any patent must be licensed for everyone's free use or not licensed at all.

The precise terms and conditions for copying, distribution and modification follow.

GNU GENERAL PUBLIC LICENSE TERMS AND CONDITIONS FOR COPYING, DISTRIBUTION AND MODIFICATION

O. This License applies to any program or other work which contains a notice placed by the copyright holder saying it may be distributed under the terms of this General Public License. The "Program", below, refers to any such program or work, and a "work based on the Program" means either the Program or any derivative work under copyright law: that is to say, a work containing the Program or a portion of it, either verbatim or with modifications and/or translated into another language. (Hereinafter, translation is included without limitation in the term "modification".) Each licensee is addressed as "you".

Activities other than copying, distribution and modification are not covered by this License; they are outside its scope. The act of running the Program is not restricted, and the output from the Program is covered only if its contents constitute a work based on the Program (independent of having been made by running the Program). Whether that is true depends on what the Program does.

1. You may copy and distribute verbatim copies of the Program's source code as you receive it, in any medium, provided that you conspicuously and appropriately publish on each copy an appropriate copyright notice and disclaimer of warranty; keep intact all the notices that refer to this License and to the absence of any warranty; and give any other recipients of the Program a copy of this License along with the Program.

You may charge a fee for the physical act of transferring a copy, and you may at your option offer warranty protection in exchange for a fee.

- 2. You may modify your copy or copies of the Program or any portion of it, thus forming a work based on the Program, and copy and distribute such modifications or work under the terms of Section 1 above, provided that you also meet all of these conditions:
 - a) You must cause the modified files to carry prominent notices stating that you changed the files and the date of any change.
 - b) You must cause any work that you distribute or publish, that in whole or in part contains or is derived from the Program or any part thereof, to be licensed as a whole at no charge to all third parties under the terms of this License.
 - c) If the modified program normally reads commands interactively

when run, you must cause it, when started running for such interactive use in the most ordinary way, to print or display an announcement including an appropriate copyright notice and a notice that there is no warranty (or else, saying that you provide a warranty) and that users may redistribute the program under these conditions, and telling the user how to view a copy of this License. (Exception: if the Program itself is interactive but does not normally print such an announcement, your work based on the Program is not required to print an announcement.)

These requirements apply to the modified work as a whole. If identifiable sections of that work are not derived from the Program, and can be reasonably considered independent and separate works in themselves, then this License, and its terms, do not apply to those sections when you distribute them as separate works. But when you distribute the same sections as part of a whole which is a work based on the Program, the distribution of the whole must be on the terms of this License, whose permissions for other licensees extend to the entire whole, and thus to each and every part regardless of who wrote it.

Thus, it is not the intent of this section to claim rights or contest your rights to work written entirely by you; rather, the intent is to exercise the right to control the distribution of derivative or collective works based on the Program.

In addition, mere aggregation of another work not based on the Program with the Program (or with a work based on the Program) on a volume of a storage or distribution medium does not bring the other work under the scope of this License.

- 3. You may copy and distribute the Program (or a work based on it, under Section 2) in object code or executable form under the terms of Sections 1 and 2 above provided that you also do one of the following:
 - a) Accompany it with the complete corresponding machine-readable source code, which must be distributed under the terms of Sections
 1 and 2 above on a medium customarily used for software interchange; or,
 - b) Accompany it with a written offer, valid for at least three years, to give any third party, for a charge no more than your cost of physically performing source distribution, a complete machine-readable copy of the corresponding source code, to be distributed under the terms of Sections 1 and 2 above on a medium customarily used for software interchange; or,
 - c) Accompany it with the information you received as to the offer to distribute corresponding source code. (This alternative is allowed only for noncommercial distribution and only if you received the program in object code or executable form with such an offer, in accord with Subsection b above.)

The source code for a work means the preferred form of the work for making modifications to it. For an executable work, complete source code means all the source code for all modules it contains, plus any associated interface definition files, plus the scripts used to control compilation and installation of the executable. However, as a special exception, the source code distributed need not include anything that is normally distributed (in either source or binary form) with the major components (compiler, kernel, and so on) of the operating system on which the executable runs, unless that component itself accompanies the executable.

If distribution of executable or object code is made by offering access to copy from a designated place, then offering equivalent

access to copy the source code from the same place counts as distribution of the source code, even though third parties are not compelled to copy the source along with the object code.

- 4. You may not copy, modify, sublicense, or distribute the Program except as expressly provided under this License. Any attempt otherwise to copy, modify, sublicense or distribute the Program is void, and will automatically terminate your rights under this License. However, parties who have received copies, or rights, from you under this License will not have their licenses terminated so long as such parties remain in full compliance.
- 5. You are not required to accept this License, since you have not signed it. However, nothing else grants you permission to modify or distribute the Program or its derivative works. These actions are prohibited by law if you do not accept this License. Therefore, by modifying or distributing the Program (or any work based on the Program), you indicate your acceptance of this License to do so, and all its terms and conditions for copying, distributing or modifying the Program or works based on it.
- 6. Each time you redistribute the Program (or any work based on the Program), the recipient automatically receives a license from the original licensor to copy, distribute or modify the Program subject to these terms and conditions. You may not impose any further restrictions on the recipients' exercise of the rights granted herein. You are not responsible for enforcing compliance by third parties to this License.
- 7. If, as a consequence of a court judgment or allegation of patent infringement or for any other reason (not limited to patent issues), conditions are imposed on you (whether by court order, agreement or otherwise) that contradict the conditions of this License, they do not excuse you from the conditions of this License. If you cannot distribute so as to satisfy simultaneously your obligations under this License and any other pertinent obligations, then as a consequence you may not distribute the Program at all. For example, if a patent license would not permit royalty-free redistribution of the Program by all those who receive copies directly or indirectly through you, then the only way you could satisfy both it and this License would be to refrain entirely from distribution of the Program.

If any portion of this section is held invalid or unenforceable under any particular circumstance, the balance of the section is intended to apply and the section as a whole is intended to apply in other circumstances.

It is not the purpose of this section to induce you to infringe any patents or other property right claims or to contest validity of any such claims; this section has the sole purpose of protecting the integrity of the free software distribution system, which is implemented by public license practices. Many people have made generous contributions to the wide range of software distributed through that system in reliance on consistent application of that system; it is up to the author/donor to decide if he or she is willing to distribute software through any other system and a licensee cannot impose that choice.

This section is intended to make thoroughly clear what is believed to be a consequence of the rest of this License.

8. If the distribution and/or use of the Program is restricted in certain countries either by patents or by copyrighted interfaces, the original copyright holder who places the Program under this License

may add an explicit geographical distribution limitation excluding those countries, so that distribution is permitted only in or among countries not thus excluded. In such case, this License incorporates the limitation as if written in the body of this License.

9. The Free Software Foundation may publish revised and/or new versions of the General Public License from time to time. Such new versions will be similar in spirit to the present version, but may differ in detail to address new problems or concerns.

Each version is given a distinguishing version number. If the Program specifies a version number of this License which applies to it and "any later version", you have the option of following the terms and conditions either of that version or of any later version published by the Free Software Foundation. If the Program does not specify a version number of this License, you may choose any version ever published by the Free Software Foundation.

10. If you wish to incorporate parts of the Program into other free programs whose distribution conditions are different, write to the author to ask for permission. For software which is copyrighted by the Free Software Foundation, write to the Free Software Foundation; we sometimes make exceptions for this. Our decision will be guided by the two goals of preserving the free status of all derivatives of our free software and of promoting the sharing and reuse of software generally.

NO WARRANTY

- 11. BECAUSE THE PROGRAM IS LICENSED FREE OF CHARGE, THERE IS NO WARRANTY FOR THE PROGRAM, TO THE EXTENT PERMITTED BY APPLICABLE LAW. EXCEPT WHEN OTHERWISE STATED IN WRITING THE COPYRIGHT HOLDERS AND/OR OTHER PARTIES PROVIDE THE PROGRAM "AS IS" WITHOUT WARRANTY OF ANY KIND, EITHER EXPRESSED OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. THE ENTIRE RISK AS TO THE QUALITY AND PERFORMANCE OF THE PROGRAM IS WITH YOU. SHOULD THE PROGRAM PROVE DEFECTIVE, YOU ASSUME THE COST OF ALL NECESSARY SERVICING, REPAIR OR CORRECTION.
- 12. IN NO EVENT UNLESS REQUIRED BY APPLICABLE LAW OR AGREED TO IN WRITING WILL ANY COPYRIGHT HOLDER, OR ANY OTHER PARTY WHO MAY MODIFY AND/OR REDISTRIBUTE THE PROGRAM AS PERMITTED ABOVE, BE LIABLE TO YOU FOR DAMAGES, INCLUDING ANY GENERAL, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES ARISING OUT OF THE USE OR INABILITY TO USE THE PROGRAM (INCLUDING BUT NOT LIMITED TO LOSS OF DATA OR DATA BEING RENDERED INACCURATE OR LOSSES SUSTAINED BY YOU OR THIRD PARTIES OR A FAILURE OF THE PROGRAM TO OPERATE WITH ANY OTHER PROGRAMS), EVEN IF SUCH HOLDER OR OTHER PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

Chapter 2. Starting Artemis

Running Artemis on UNIX and GNU/Linux Systems

On Unix and GNU/Linux the easiest way to run the program is to run the script called **art** in the Artemis

installation directory (see the section called *Getting and Installing Artemis* in Chapter 1) like this:

artemis/art

If all goes well you will be presented with a small window with two menus. See <u>the section called *The Artemis Launch Window*</u> to find out what to do next.

Alternatively you start Artemis with the name of a sequence file or embl file eg:

artemis/art artemis/etc/c1215.embl

Or if you have a sequence file and extra feature table files you can read them all with a command like this (the example file c1215.blastn.tab is the result of a BLASTN search against EMBL which has been converted to feature table format):

artemis/art artemis/etc/c1215.embl + artemis/etc/c1215.blastn.tab

Note that any number of feature files can be read by listing them after the plus sign. The "+" must be surrounded by spaces.

See the section called *UNIX Command Line Arguments for Artemis* for a list of the other possible arguments.

Here is an alternative way to run Artemis if the **art** script doesn't work for you:

cd ~/artemis

java -classpath lib/biojava.jar:lib/jemAlign.jar:lib/j2ssh/j2ssh-core.jar:lib/ibatis/ibatis-2.3.4.726.jar:lib/ibatis/log4j-1.2.14.jar:lib/chado-14-interface.jar:lib/postgresql-8.4-701.jdbc3.jar:lib/picard.jar:lib/picard/sam.jar:. -Dartemis.environment=UNIX uk.ac.sanger.artemis.components.ArtemisMain

UNIX Command Line Arguments for Artemis

As well as the listing file names on the command line, the following switches are available to UNIX users:

-quiet

This option tells Artemis to suppress normal informational messages while running.

-options

This option instructs Artemis to read an extra file of options after reading the standard options. (See <u>the section called *The Options File* in Chapter 5</u> for more about the Artemis options file.)

For example -options ./new_options will instruct Artemis to read new_options in the current directory as an options file.

-Xmsn -Xmxn

Use -Xmsn to specify the initial size, in bytes, of the memory allocation pool. This value must be a multiple of 1024 greater than 1MB. Append the letter k or K to indicate kilobytes, or m or M to indicate megabytes.

Use -Xmxn to specify the maximum size, in bytes, of the memory allocation pool. This value must a multiple of 1024 greater than 2MB. Append the letter k or K to indicate kilobytes, or m or M to indicate megabytes.

Running Artemis on Macintosh Systems

On MacOSX machines Artemis can be started by double clicking on the Artemis icon. The icon can be added to the dock by dragging it from a 'Finder' window and dropping it onto the position in the dock you want it. In addition sequence files (with gff, embl, EMBL, genbank, gbk, fasta, seq, art file name extensions) can be dragged and dropped on to the Artemis application image to open them up.

To change the memory allocated to Artemis on MacOSX, set the value in the file Info.plist in the directory Artemis.app/Contents. Towards the bottom of the file there are a couple of lines that look like this:

```
<key>VMOptions</key>
<string>-Xmx800m</string>
```

Changing the value after -Xmx will change the memory used by Artemis.

Running Artemis on Windows Systems

On systems with Java 1.5 (or above) installed Artemis can be started by double clicking on the artemis.jar icon.

The Artemis Launch Window

This is the first window that opens when you start Artemis. The menus at the top of this window allow you to read a new sequence or embl entry.

The File Menu

Open File Manager ...

Selecting this shows the files and directories that are in the directory Artemis is launched from. The user home directory and the current working directories are shown and can be navigated.

Open SSH File Manager ...

This opens a window displaying a local file manager at the top and login options to access a remote file system via Secure Shell (SSH). When the login details are typed in and 'Connect' pressed the bottom half of the window will display the other (remote) file system. See <u>Chapter 4</u> to find out how to use this and how to set up the connection.

Open ...

If you select this menu item a file requester will be displayed which allows you to open a flat file containing an entry. If the file you select is successfully read a new window will open, which shows the sequence and features for the entry. See <u>Chapter 3</u> to find out how to use the main window.

Open from EBI - Dbfetch ...

The functions will ask the user for an accession number and then will attempt to read it directly from the <u>EBI</u> using <u>Dbfetch</u>. If all goes well you will be presented with an view/edit window (see <u>Chapter 3</u>).

Quit

This menu item will close all windows and then exit the program

The Options Menu

Re-read Options

Choosing this menu item will discard the current options settings and then re-read the options file. Note that changing the font size in the file and then selecting this menu item will only change the font size for new windows, not existing windows. Currently some options are unaffected by this menu item. See the section

Enable Direct Editing

This menu item will toggle "direct editing" option. It is off be default because it can have surprising results unless the user is expecting it. See <u>the section called "Direct Editing"</u> in Chapter 3 for more detail about this.

Genetic Code Tables

These options make all the <u>NCBI Genetic Codes</u> available. The default setting is the Standard Code. This setting effects the display of start codons (see <u>the section called Start Codons in Chapter 3</u>) and the "Suspicious Start Codons ..." feature filter (see <u>the section called Suspicious Start Codons ... in Chapter 3</u>).

Send Searches Via SSH

This is an advanced option for sites that have set up the ability to submit searches via SSH to another machine.

Black Belt Mode

This is an advanced option that can be used to turn off warning message. This options is displayed if the java property (black belt mode) is specified on opening up Artemis, i.e. art -Dblack belt mode=yes.

Highlight Active Entry

When this option is on and the "One Line Per Entry" is on (see <u>the section called *One Line Per Entry* in Chapter 3)</u> the line that the active entry is on will be highlighted in yellow.

Show Log Window

Show the log of informational messages from Artemis. Currently the log window is only used on UNIX and GNU/Linux systems to show the output of external programs. This menu item is only available when running Artemis on UNIX or GNU/Linux systems. The logging is controlled by log4j.properties file (etc/log4j.properties in the source distribution) sets the level of logging. This can be used to send the logging information to other places such as a file.

Hide Log Window

Hide the log of informational messages. This menu item is only available when running Artemis on UNIX or GNU/Linux systems.

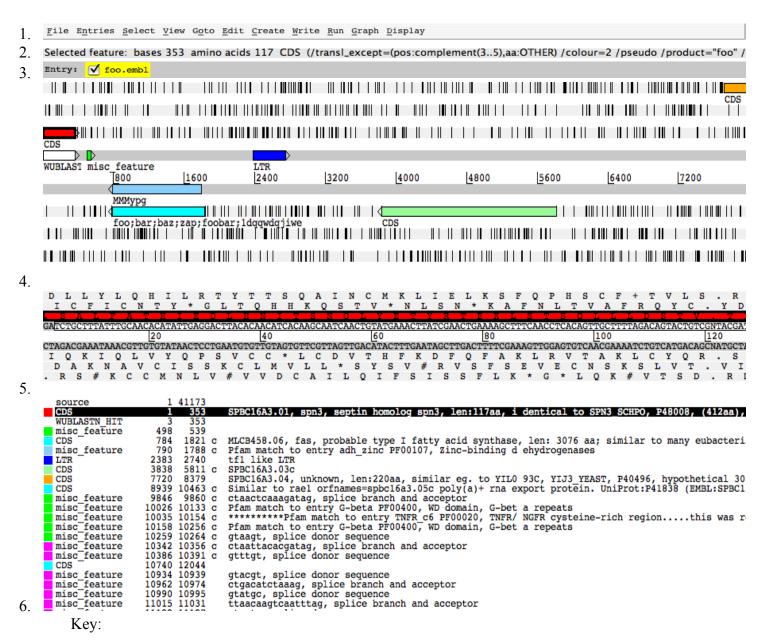
Chapter 3. The Artemis Main Window

Overview of the Entry Edit Window

This window is the main editing and viewing window. See <u>the section called *Open* ... in Chapter 2 or the section called *Open from EBI - Dbfetch* ... in Chapter 2 to find out how to read an entry (and hence open one of these windows).</u>

The following images show a breakdown of the main Artemis edit window. The full screen shot is here.

A breakdown of the main Artemis edit window



- 1. The menus for the main window (described later in this chapter).
- 2. A one line summary of the current selection (see <u>the section called *The Select Menu*</u> and <u>the section</u> called *The Selection* in Chapter 1 for more).
- 3. This line contains one button for each entry that has been loaded. These buttons allow the user to set

the default entry (see <u>the section called *The Default Entry* in Chapter 1)</u> and to set the active entries (see <u>the section called *The Active Entries* in Chapter 1)</u>. For more detail on operating the buttons see <u>the section called *The Entry Button Line*.</u>

- 4. This shows an overview of the sequence and the features from the active entries. (see <u>the section</u> called *The Overview and DNA Views*).
- 5. This is called the "DNA view" to distinguish it from the overview, but in fact it operates in a very similar way. (see the section called *The Overview and DNA Views*).
- 6. A textual summary of the active features. (See the section called *The Feature List*).

The File Menu

Most of the items in this menu are used to read and write entries and parts of entries, the exceptions are Clone and Close.

Show File Manager ...

This will open the file manager, or if it is already open will bring it to the foreground. Entries can be dragged from the file manager into the artemis main window and dropped. When dropped the entry is then read in and displayed.

Read An Entry ...

Read an entry (see <u>the section called *The "Entry"* in Chapter 1</u>), but keep it separate from the others. A new button will be created on the entry button line for the new entry. The new entry will be marked as active (see <u>the section called *The Active Entries* in Chapter 1</u>) and will be the new default entry (see <u>the section called *The Default Entry* in Chapter 1</u>).

This function only reads the feature section of the input file - the sequence (if any) is ignored.

Artemis can read these feature file formats:

- EMBL or GenBank feature tables
- GFF Version 3 files
- The output of **MSPcrunch**. **MSPcrunch** must be run with the **-x** or **-d** flags.
- The output of <u>blastall version 2.2.2</u> or better. **blastall** must be run with the **-m 8** flag which generates one line of information per HSP. Note that currently Artemis displays each Blast HSP is a separate feature rather than displaying each BLAST hit as a feature.

Read Entry Into

Read the features from an entry (see <u>the section called *The "Entry"* in Chapter 1</u>) chosen by the user and then insert them into the entry selected by the user.

Read BAM ...

Read a BAM file that has been sorted and indexed using <u>samtools</u>. This provides an integrated <u>BamView</u> panel displaying sequence alignment mappings to a reference sequence. This require Artemis to be run with Java 1.6 as it makes use of <u>picard</u> to read from the BAM file.

Save Default Entry

Save the default entry to the file it came from, unless the entry has been given a new name, in which case the entry is saved to a file with that name. If the entry has no name, Artemis will prompt the user for a new name. [shortcut key: S]

Save An Entry

This item will do the same as "Save Default Entry" for the chosen entry.

Save An Entry As

This sub-menu contains the less frequently used save functions.

New File

Ask for the name of file to save the given entry to. The name of entry (as displayed in the entry button line) will change to the new name.

EMBL Format

This does the same as "Save An Entry As -> New File ...", but will write the features and sequence of the entry in EMBL format. Note that currently the header of a GENBANK entry can't be converted to the equivalent EMBL header (it will be discarded instead).

GENBANK Format

This does the same as "Save An Entry As -> New File ...", but will write the features and sequence of the entry in GENBANK format. Note that currently the header of a EMBL entry can't be converted to the equivalent GENBANK header (it will be discarded instead).

Sequin Table Format

This saves a file in Sequin table format which is used by Sequin.

GFF Format

Writes the features in GFF format and sequence of the entry in FASTA format to a file selected by the user. Note that if you use this function on an EMBL or GENBANK entry the header will discarded.

EMBL Submission Format

This does the same as "Save An Entry As -> EMBL Format ...", but will write an entry/tab file that contains only valid EMBL qualifiers (see the section called extra_qualifiers in Chapter 5) and valid EMBL keys (see the section called extra_keys in Chapter 5). It will also check that the start and stop codons of each CDS are sensible, that no two features have the same key and location and that all required EMBL qualifiers are present.

Save All Entries

This acts like "Save Default Entry", but save all the entries.

Write

Amino Acids Of Selected Features

Prompt for a file name and then write the translation of the bases of the selected features to that file. The file is written in FASTA format.

PIR Database Of Selected Features

Prompt for a file name and then write the translation of the bases of the selected features to that file. The file is written in PIR format (similar to FASTA, but with a * as the last line of each record).

Bases Of Selection

Prompt for a file name and then write the bases of the selection to that file in the selected format. If the selection consists of features (rather than a base range) then the bases of each feature will be written to the file as a separate record. If the selection is a range of bases, then those bases will be written.

Upstream Bases Of Selection

Prompt for a number and a file name, then write that many bases upstream of each selected feature to the file in the selected format. For example if the selected feature has a location of "100..200", then asking for 50 upstream will write the bases in the range 50 to 99. Writing upstream bases of a feature on the complementary strand will work in the expected way.

Downstream Bases Of Selection

Prompt for a number and a file name, then write that many bases downstream of each selected feature to the file in the selected format.

All Bases

Prompt for a file name, then write the complete sequence to that file in the selected format.

Codon Usage of Selected Features

Prompt for a file name, then write a codon usage table for the selected features. The file in written in the same format as the data at <u>Kazusa codon usage database site</u>. In the output file each codon is followed by it's occurrence count (per thousand) and it's percentage occurrence. (See <u>the section called *Add Usage Plots*</u> ... to find out how to plot a usage graph).

Clone This Window

Make a new main window with the same contents as the current window. All changes in the old window will be reflected in the new window, and vice versa. The exception to this rule is the selection (see <u>the section called *The Selection* in Chapter 1</u>), which is not shared between the old and new window.

Save As Image Files (png/jpeg)

Print out the contents of the current window. All or some of the window panels can be select for printing to an image file.

Print

This option can be used to print the contents of the current window to a file as PostScript or to a printer.

Print Preview

This opens the print image in a preview window. This shows what the image will look like when printed to a file.

Preferences

This enables the user to define their own shortcut preferences.

Close

Close this window.

The Entries Menu

The items in this menu are used to change which entry is the default entry and which entries are active (see the section called *The "Entry"* in Chapter 1). At the bottom of the menu there is a toggle button for each entry which controls whether the entry is active or not. These toggle buttons work in a similar way the the buttons on the entry button line (see the section called *The Entry Button Line*).

Here is a description of the other menu items:

Set Name Of Entry

Set the name of an entry chosen from a sub-menu. The name of the entry is used as the name of the file when the entry is saved.

Set Default Entry

Set the default entry by choosing one of the entries from the sub-menu. (See <u>the section called *The Default Entry* in Chapter 1).</u>

Remove An Entry

Remove an entry from Artemis by choosing one of the entries from the sub-menu. The original file that this entry came from (if any) will not be removed.

Remove Active Entries

Remove the entries that are currently active. (See the section called *The Active Entries* in Chapter 1).

Deactivate All Entries

Choosing this menu item will deactivate all entries. (See the section called *The Active Entries* in Chapter 1.)

The Select Menu

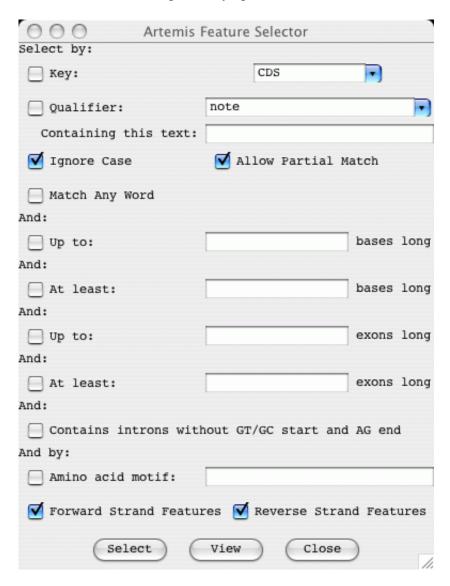
The items in this menu are used to modify the current selection (see <u>the section called *The Selection* in Chapter 1</u>). Artemis A short summary of the current selection is shown at the top of the main window (see <u>2</u> for details)..

Feature Selector ...

Open a new Feature Selector window. This window allows the user to choose which features to select or view based on feature keys (see <u>the section called *EMBL/Genbank Feature Keys* in Chapter 1)</u>, qualifier values (see the section called *EMBL/Genbank Feature Qualifiers* in Chapter 1) and amino acid motifs.

The Select button will set the selection to the contain those features that match the given key, qualifier and amino acid motif combination.

The View button will create a new feature list (see <u>the section called *The Feature List*</u>) containing only those features that match the given key, qualifier and amino acid motif combination.



All

Reset the selection so that nothing is selected then select all the features in the active entries. [shortcut key: A]

All Bases

Reset the selection so that nothing is selected then select all the bases in the sequence.

Select All Features in Non-matching Regions

Select all features that have no corresponding match in ACT. This is used to higlight regions that are different between sets of sequence. It will only take into account matches that have not been filtered out using the score, identity or length cut-off.

None

Clear the selection so that nothing is selected. [shortcut key: N]

By Key

Ask the user for a feature key, reset the selection so that nothing is selected, then select all the features with the key given by the user.

CDS Features

Reset the selection so that nothing is selected, then select all the CDS features that do not have a /pseudo qualifier.

Same Key

Select all the features that have the same key as any of the currently selected features.

Open Reading Frame

Extend the current selection of bases to cover complete open reading frames. Selecting a single base or codon and then choosing this menu item has a similar effect to double clicking the middle button on a base or residue (see <u>the section called *Changing the Selection from a View Window*</u> for details).

Features Overlapping Selection

Select those (and only those) features that overlap the currently selected range of bases or any of the currently selected features. The current selection will be discarded.

Base Range ...

Ask the user for a range of bases, then select those bases. The range should look something like this: 100–200, complement (100..200), 100.200 or 100..200. If the first number is larger than the second the bases will be selected on the forward strand, otherwise they will be selected on the reverse strand (unless there is a complement around the range, in which case the sense is reversed).

Feature AA Range ...

Ask the user for a range of amino acids in the selected feature and select those bases. The range should look something like this: 100-200, or 100..200.

Toggle Selection

Invert the selection - after choosing this menu item the selection will contain only those features that were not in the selection beforehand.

The View Menu

Selected Features

Open a view window for each selected feature showing it's feature table entry. [shortcut key: V]

Selection

Open a view window that will show the current selection. The window is updated as the selection changes, so it can be left open.

When one feature is selected the window will show the text (EMBL, GenBank or GFF format) of the feature, the base composition, GC percentage, correlation score (see <u>the section called *Correlation Scores*</u>), and the bases and translation of the sequence of the feature.

When two or more features are selected the window will show the text (EMBL, GenBank or GFF format) of the features, the base composition, average GC percentage, average correlation score, minimum/maximum GC content and minimum/maximum correlation score of the feature sequence.

When a range of bases is selected the window will show the base composition, GC content percentage and the bases and translation of the sequence of the feature.

Search Results

On this sub-menu allows the user to view the results of feature searches that are launched from the run menu in Artemis (see the section called *The Run Menu*).

CDS Genes And Products

Pop up a feature list (see <u>the section called *The Feature List*</u>) of the CDS showing the gene names (from the /gene qualifier) and the product (from the /product qualifier). This list includes pseudo genes.

Feature Filters

Each of the items in this sub-menu each allow the user to view a subset of the active features. An example of a subset is all those features with misc_feature as a key. The features are displayed in a new window that contains a menu bar with possible actions to apply to the subset, and feature list (see the section called *The Feature List*). Most of the possible actions will apply only to the features in the list. For example "Show Overview" in the View menu (see the section called *Overview*) will include statistics only on the features in the list.

Suspicious Start Codons ...

Show those CDS features that have a suspicious start codon. ie. the first codon of the feature isn't ATG (in eukaroytic mode) or ATG, GTG and TTG (in prokaryotic mode). This function is effected by the setting of the "Eukaroytic Mode" option in the main options menu (see the section called *Genetic Code Tables* in Chapter 2 for more).

Suspicious Stop Codons ...

Show those CDS features that have a suspicious stop codon. ie. the last codon of the feature isn't one of TAA, TAG or TGA.

Non EMBL Keys ...

Show those features that have a key that isn't valid for EMBL/GenBank entries.

Duplicated Features ...

Show those features that are duplicated (ie. features that have the same key and location as another feature). These sort of duplicates aren't allowed by the EMBL database.

Overlapping CDS Features ...

Show those CDS features that overlap another CDS feature (on either strand).

Features Missing Required Qualifiers ...

Show those features that are missing a qualifier that is required by the EMBL database.

Filter By Key ...

Show those features that have a key chosen by the user.

Selected Features ...

Show the currently selected features in a new feature list. The contents of the list will remain the same even if selection subsequently changes. This is useful for bookmarking a collection of features for later use.

Overview

Open a new window the will show a summary of the active entries and some statistics about the sequence (such as the GC content). [shortcut key: O]

Sequence Statistics

The overview window show the following statistics about the sequence:

- Number of bases.
- GC percentage.
- The number of each nucleotide in the sequence.
- GC percentage of non-ambiguous bases ie. the GC content percentage ignoring bases other than A,T,C and G. This should be the same as the "GC percentage" above.

Feature Statistics

The overview window also shows the following statistics about the features in the active entries (if there are any features). Note that the "genes" are the non-pseudo CDS features.

- Number of features in the active entries (see the section called *The Active Entries* in Chapter 1).
- Gene density the average number of non-pseudo CDS features per 1000 bases.
- Average gene length the average length of non-pseudo CDS features (not including introns).
- Number of non-spliced genes.
- Number of spliced genes.
- Number of pseudo genes (ie. CDS features with a /pseudo qualifier).
- Protein coding (CDS) features.
- Protein coding (CDS) bases.

- Protein coding percentage ie. the number of coding bases excluding introns.
- Coding percentage (including introns).
- A summary of the number of features of each key (type) and their colours.

Forward Strand Overview

Open a new window the will show a summary of the features and bases of the forward strand.

Reverse Strand Overview

Open a new window the will show a summary of the features and bases of the reverse strand.

Feature Bases

Create a view window for each selected feature, which shows bases of the feature.

Feature Bases As FASTA

Create a view window for each selected feature, which shows bases of the feature in FASTA format.

Feature Amino Acids

Create a view window for each selected feature, which shows amino acids of the feature.

Feature Amino Acids As FASTA

Create a view window for each selected feature, which shows amino acids of the feature in FASTA format.

Feature Statistics

Show some statistics about each selected feature. On the left on the feature information window is the amino acid composition of the feature. On the right is the codon composition of the feature.

Feature Plots

Open a window for each selected feature that shows a plot of the Kyte-Doolittle Hydrophobicity [short name: hydrophobicity], the Hopp-Woods Hydrophilicity [short name: hydrophilicity], and an approximation of the GCG Coiled Coils algorithm [short name: coiled_coil]. (For more detail about the coiled coils algorithm see "Predicting Coiled Coils from Protein Sequences", Science Vol. 252 page 1162.) [shortcut key: W]

Some general information about graphs and plots in Artemis can be found in the section called *Graphs and Plots*. Configuration options for graphs are described in the section called *Options For Plots and Graphs* in Chapter 5.

The Goto Menu

The items in this menu allow the user to navigate around the sequence and features.

Navigator ...

Open a new navigation window. [shortcut key: G]

This window allows the user to perform five different tasks:

- 1. **Scroll all the views so that a particular base is in the centre of the display**. To use this function, type a base position into the box to the right of the "Goto Base:" label then press the goto button at the bottom of the window. The requested base will be selected and then the overview display and the DNA display will scroll so that the base is as near as possible to the middle of the main window.
- 2. **Find the next feature that has the given gene name**. To use this function, type a gene name into the box to the right of the "Goto Feature With This Gene Name:" label and then press the goto button. Artemis will select the first feature with the given text in any of it's qualifiers and will then scroll the display so that feature is in view.
- 3. **Find the next feature that has a qualifier containing a particular string.** To use this function, type a string into the box to the right of the "Goto Feature With This Qualifier Value:" label and then press the goto button. Artemis will select the first feature with the given string in any of it's qualifier values (see the section called *EMBL/Genbank Feature Qualifiers* in Chapter 1) and will then scroll the display so that feature is in view.

- 4. **Find the next feature that has a particular key**. To use this function, type a key into the box to the right of the "Goto Feature With This Key:" label and then press the goto button. Artemis will select the first feature with the given key and will then scroll the display so that feature is in view.
- 5. **Find the next occurrence of a particular base pattern in the sequence**. To use this function, type a base pattern into the box to the right of the "Find Base Pattern:" label and then press the goto button. Artemis will select the first contiguous group of bases on either strand that match the given base pattern and will then scroll the display so that those bases are in view. Any IUB base code can be used in the pattern, so for example searching for aanntt will match any six bases that start with "aa" and ends with "tt". See Table 3-1 for a list of the available base codes.
- 6. **Find the next occurrence of a particular residue pattern in the sequence**. To use this function, type a amino acid pattern into the box to the right of the "Goto Amino Acid String:" label and then press the goto button. Artemis will select the first contiguous group of bases on either strand that translate to the given amino acids and will then scroll the display so that those bases are in view. The letter 'X' can be used as an ambiguity code, hence 'AAXXXDD' will match 'AALRTDD' or 'AATTTDD' etc.

Note that for all the functions above except the first ("Goto Base"), if the "Start search at beginning" option is set or if there is nothing selected the search will start at the beginning of the sequence. Otherwise the search will start at the selected base or feature. This means that the user can step through the matching bases or features by pressing the goto button repeatedly.

If the "Ignore Case" toggle is on (which is the default) Artemis will ignore the difference between upper and lower case letters when searching for a gene name, a qualifier value or a feature key.

The "Allow Substring Matches" toggle affects 2 and 3. If on Artemis will search for qualifier values that contain the given characters. For example searching for the genename CDC will find CDC1, CDC2, ABCDC etc. If the toggle is off Artemis will only find exact matches, so searching for the gene CDC will only find features that have /gene="CDC" not /gene="CDC11".

Start of Selection

Scroll all the views so that the first base of the selection is as close to the centre as possible. If the a range of bases is selected the views will move to the first base of the range. If one or more features are selected, then the first base of the first selected feature will be centred. Otherwise, if one or more segments (see the selected feature will be centred. [shortcut key: control-left]

End of Selection

This does the same as "Goto Start of Selection", but uses the last base of the selected range or the last base of the last selected feature or segment. [shortcut key: control-right]

Feature Start

Scroll the views to the start of the first selected feature.

Feature End

Scroll the views to the end of the first selected feature.

Start of Sequence

Scroll the views so that the start of the sequence is visible. [shortcut key: control-up]

End of Sequence

Scroll the views so that the end of the sequence is visible. [shortcut key: control-down]

Feature Base Position ...

Ask the user for a base position within the first selected feature, then scroll the views so that that base position is centred.

Feature Amino Acid ...

Ask the user for an amino acid position within the first selected feature, then scroll the views so that that position is centred.

The Edit Menu

This menu contains most of the functions that change the entries. Note that the changes will not be saved back to the original files until one of the save functions in the File menu is used (see <u>the section called Save An Entry</u>).

Undo

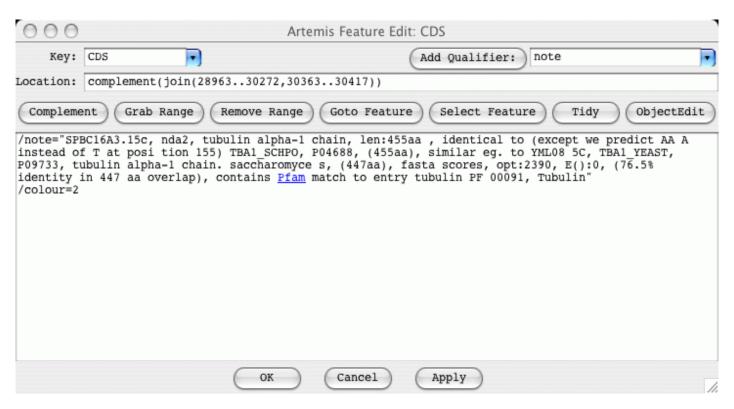
This function will undo the last change that was made using the Edit or Create menus. Up to 20 changes can be undone. This menu item is only enabled when there is something to undo. This limit can be changed in the options file (see the section called <u>undo levels</u> in Chapter 5). [shortcut key: U]

Redo

This function will redo the last undo operation.

Selected Features in Editor

Open an edit window for each selected feature. [shortcut key: E]



From the top down the edit window has these parts:

- 1. At the top left is a selector for choosing the key of the feature. This only contains a subset of the legal keys. The subset can be changed by changing the common_keys option in the options file (see the section called common_keys in Chapter 5).
 - At the top right of the edit window is a selector for adding a qualifier. For example choosing note from the menu will insert /note="" into the qualifier edit area.
- 2. Just below the key and qualifier selector is the location entry field. Artemis understands most of the EMBL location syntax, including joins, complements, ranges with non-exact ends (eg.

```
(100.200)..>350) and references to other entries (eg. join (100..200, SPB23A1:100..200)).
```

- 3. Below the location is a row of buttons:
 - The Complement button will complement the contents of the location field.
 - The Grab Range button will grab the currently selected range into the location field.
 - The Remove Range button will remove the selected bases from the location string. This is normally used to create an intron in a feature.
 - Pressing the Goto Feature button has the same effect as the "Start of Selection" item in the "Goto" menu. (See the section called *Start of Selection* for more).
 - The Select Feature button selects this feature (in the same way as clicking on the feature in one of the views).
- 4. The centre of the edit window contains the qualifier entry section. The qualifiers should be entered the in same way the appear in the feature table part of an EMBL entry, but without the leading FT and spaces.
- 5. The bottom of the window contains three buttons. The OK button will update the feature with the changes that have been made by the user and will then close the edit window. The Cancel button will discard the changes and then close the window. The Apply will make the changes, but will not close the window. Before any changes are made the location and the qualifiers are checked for formatting errors. Any errors will brought to the attention of the user through the use of annoying pop-up boxes. No changes will be performed until there all errors have been fixed.

Subsequence (and Features)

Make a copy (in a new edit window) of the selected bases and the features in that range. Any features that overlap the end of the range will be truncated.

Find Or Replace Qualifier Text

This opens a search window with options to find or replace qualifier text. The search can be restricted to features with a given key and/or it can be restricted to a given qualifier name.

Boolean operators (and/or) can be used in the search. Clicking on the Show Boolean Search Options displays 4 options.

- 1. The Use boolean operators (and, or, & |) means that it uses any of these operators that are in the Find text field.
- 2. The Match any string (i.e. x OR y) means that the words in the Find text field will be

separated by an OR condition. So that it finds those features with qulaifiers that contain any of the words.

- 3. The Match all string (i.e. \times AND y) means that the words in the Find text field will be separated by an AND condition. So that it finds those features with qulaifiers that contain all of the words.
- 4. The No boolean search option is the default. This means it searches for those features with qualifiers that contain the complete text from the Find text field.

In addition selecting the Duplicate Qualifiers tab provides options to search for or delete duplicate qualifiers.

Qualifier(s) of Selected Feature

Change ...

This function allows the user to add or change qualifiers on all the selected features in one operation. The main part of the window acts like the qualifier editing field of the feature edit window (see <u>the section called Selected Features in Editor</u>).

Remove ...

This function allows the user to remove all qualifiers with a particular name from all the selected features.

Convert ...

This function allows the user to convert all qualifiers of a particular type to another qualifier for all the selected features.

Selected Feature(s)

Duplicate

Make a copy of each selected feature. Each new feature will be added just after the original in the same entry as the original. [shortcut key: D]

Merge

Create a new feature that con	ntains all the exons	and qualifiers	of the selected	features.	The selected	features
must all have the same key.	[shortcut key: M]					

Unmerge

If the selection contains exactly two exons and those exons are adjacent in the same feature, split the feature into two pieces between the exons. The original feature is truncated and a new feature is created. The qualifiers of the old feature are copied to new feature.

Unmerge All Segments

All exons in a feature are unmerged.

Delete

Remove each selected feature from it's entry. [shortcut key: control-delete]

Delete Exons

Delete the selected exons. The last exon of a feature can't be deleted (delete the whole feature instead).

Remove Introns

Delete the selected introns.

Move Selected Features To

Move the selected features to another entry. Choose the destination entry from the sub-menu.

Copy Selected Features To

Copy the selected features to another entry. Choose the destination entry from the sub-menu.

Trim Selected Features

To Met

For each of the selected features this function will attempt to move the start position to the first ATG in the feature if the feature does not already start on a ATG codon. If there is no ATG in the first thirty percent of the bases of the feature the start position will be unchanged. The search will stop at the end of the first segment of a multi-segment feature.

To Any

This works in the same way as "Trim Selected Features To Met", but will attempt to move the start position of the feature to the first TTG, ATG or GTG in the feature if it does not already start on one of those codons. As above it will only search the first thirty percent of the feature bases and will only search the first segment of a multi-segment feature.

To Next Met

For each of the selected features this function will attempt to move the start position to the next ATG in the feature (the first codon is skipped). If there is no ATG in the first thirty percent of the bases of the feature the start position will be unchanged. The search will stop at the end of the first segment of a multi-segment feature. [shortcut key: T]

To Next Any

This works in the same way as "Trim Selected Features To Next Met", but will attempt to move the start position of the feature to the next TTG, ATG or GTG in the feature (the first codon is skipped). As above it will only search the first thirty percent of the feature bases and will only search the first segment of a multisegment feature. [shortcut key: Y]

Extend Selected Features

To Previous Stop Codon

Extend each of the selected features which do not start on a stop codon so that each feature starts just after the previous stop codon in this reading frame. [shortcut key: Q]

To Next Stop Codon

Extend each of the selected features which do not end on a stop codon so that each feature ends just before the next stop codon in this reading frame.

To Next Stop Codon and Fix

Same as above but in addition this fixes the stop codons.

Fix Stop Codons

Check and fix the stop codons to all the selected features. For each feature if the last codon is a stop codon, then all is well, nothing further is done to the feature. If the last codon is not a stop codon, but the very next codon is a stop codon, then the end of the feature is moved forward by three bases. If both the last codon and the very next codon after the feature are not stop codons, the feature is selected, an error message is displayed and processing stops immediately.

Automatically Create Gene Names

Ask for a gene name prefix (using a text requester), and then give a unique gene name to each CDS feature in the active entries using that prefix. For example if there are four CDS features with locations: "1..500", "complement (100..600)", "200..700" and "complement (300..800)", entering SPBC16A3 will give the four features these names: SPBC16A3.01, SPBC16A3.02c, SPBC16A3.03 and SPBC16A3.04c.

Fix Gene Names

For each selected CDS, add the gene name from the CDS to neighbouring/overlapping mRNA, intron, exon, gene, 5'UTR and 3'UTR features. Warn about inconsistencies such as overlapping CDSs.

Bases

Reverse And Complement

Reverse and complement the sequence and all the features in all the entries (active and inactive).

Reverse And Complement Selected Contig

Reverse and complement the sequence and all the features in a selected contig feature. If this option is used in ACT then all the matches within the contig are also reversed. Any matches extending past the boundary of the contig are deleted. The changes to the comparison file can be saved by right clicking in the comparison window and selecting "Save Comparison File...". However, ideally the comparison between the two sequences should be recalculated.

Delete Selected Bases

Deletes the selected range of bases (if any) from both strands. The deletion will not proceed if the selected range overlaps any features.

Add Bases At Selection

Prompt the user for some bases to insert just before the selected bases. The operation will not proceed if there is no selected range, but bases can be inserted anywhere in the sequence, including inside a feature. The same bases, reversed and complemented, will be inserted at the corresponding place on the opposite strand.

Add Bases From File ...

Prompt the user for the name of a file containing the bases to insert just before the selected bases.

Replace Bases At Selection

Prompt the user for some bases to replace the selected bases.

Contig Reordering ...

Opens a 'Contig Tool' displaying contigs, i.e. with feature keys 'fasta_record' or 'contig'. The former being created automatically for each sequence when a mutiple fasta sequence file is read in. The contigs in this tool can then individually be selected and dragged and dropped to another location. In this way the order of contigs and features within a contig can be changed.

If this is used in ACT then the matches are also reordered with respect to the change in the sequence. If a match spans the boundary of a contig that is being moved then if possible it is split. In some situations where there is a match with 'indels' then this is not possible and the match is deleted. The changes to the comparison file can be saved by right clicking in the comparison window and selecting "Save Comparison File...". However, ideally the comparison between the two sequences should be recalculated.

Header Of Default Entry

Open a edit window containing the header of the default entry. Changes made in the edit window will be applied immediately to the entry provided there are no errors in the formatting of the header.

The Create Menu

This menu contains functions for creating new features (see <u>the section called *EMBL/Genbank Features* in Chapter 1)</u> or entries (see <u>the section called *The "Entry"* in Chapter 1)</u>.

New Feature

Create a new feature in the default entry with a key of "misc_feature" (see <u>the section called EMBL/Genbank</u> <u>Feature Keys in Chapter 1</u>), a location of that spans the whole sequence and which has no qualifiers (see <u>the section called EMBL/Genbank Feature Qualifiers in Chapter 1</u>).

Feature From Base Range

Create a new feature in the default entry with a key of "misc_feature", no qualifiers and a location that exactly matches the selected range of bases. If no bases are selected an error will be reported. [shortcut key: C]

Intergenic Features

Create new features between CDS features in the default entry all with the "misc_feature" key.

Features From Non-matching Regions

Create features in ACT spanning all the regions where a match is not to be found.

New Entry

Create a new entry with no name and no features. The new entry will become the default entry (see <u>the section called *The Default Entry* in Chapter 1)</u>.

Mark Open Reading Frames ...

Create a feature for each "large" open reading frame in the sequence. The default minimum size of a "large" open reading frame can be changed by changing the minimum_orf_size option (see the section called minimum_orf_size in Chapter 5). If a codon usage file (see the section called Add Usage Plots ...) has been read each new ORF will have a codon usage score added as a /score qualifier. The new features can then be filtered from the display (see "Set Score Cutoffs ..." in the section called The Pop-up Menu).

Mark Empty ORFs ...

Create a feature for each open reading frame that doesn't already contain a feature.

Mark Open Reading Frames In Range ...

Create a feature for each "large" open reading frame in a range of bases. A range must be selected before using this command.

Mark From Pattern ...

Open a text requester to ask the user for a base pattern, then create a feature for each group of bases that matches that pattern. A new entry will be created to hold the features with the name "matches: <pattern>",

where <pattern> is the pattern that was entered be the user. Any IUB base code can be used in the pattern, so for example, annut will match any six bases that start with "aa" and ends with "tt".

Table 3-1. IUB Base Codes

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

Mark Ambiguities

Create a new feature for each block of ambiguous bases. The new features will have a key of misc feature and will created in a new entry called "ambiguous bases".

The Run Menu

This menu is primarily used for running external programs on UNIX. Additionally there are menu options to send sequences from selected features to the <u>NCBI web BLAST</u> and <u>Pfam</u>.

Once configured correctly, running an external program should be as simple as selecting some features of interest, then choosing one of the items from the run menu. When the external programs finishes the results can viewed using the "Search Results" item in the View menu (see the section called *Search Results*).

Configuring the Run Menu

To use this feature the run_blastp, run_fasta etc. scripts that are supplied with Artemis will need to be changed to reflect the paths and databases that are configured at each site. Note that the run scripts are stored in the etc/ directory.

Each external program that is listed in the options file (see the section called <u>feature_dna_programs</u> in <u>Chapter 5</u> and the section called <u>feature_protein_programs</u> in <u>Chapter 5</u>) gets a "run" menu item and a "set options" menu item. For each external program (such as blastp) there must be a shell script available that sets any necessary environment variables and then launches the search/analysis program (for blastp the script is called run_blastp).

Taking blastp as an example, this is the sequence of events that occurs when the user selects the "Run blastp on selected features" menu item:

- 1. Artemis creates a new directory in the current directory called blastp.
- 2. A protein FASTA sequence file is written in the new directory for each selected feature. (For a DNA search program such as blastn the file will be a DNA FASTA file). The sequence file name will be something like: blastp/features.tab.seq.00001.
- 3. The name of the expected output file is stored in the feature in a qualifier called <code>/blastp_file</code>. If the entry is called <code>features.tab</code> then the qualifier will be set to something like: <code>/blastp_file="blastp/features.tab.seq.00001.out"</code>. Note that because the file name is stored in the entry you will need to save the entry to keep the association between the features and the output files.
- 4. A file is then written (called something like blastp/file_of_filenames.1) that contains the names of all the newly created sequence files in the blastp directory.
- 5. Artemis then tries to read the run_blastp script from the Artemis installation directory. The script is executed like this:

```
run blastp blastp/file of filenames.1 [options]
```

where <code>[options]</code> currently must be a single word (normally a database to search). In the case of blastp/blastn/fasta etc. the second argument of the script is passed directly to the blast/fasta as the database name. For testing purposes it is possible to run <code>run_blastp</code> on the command line with the same arguments as above.

```
run_blastp will run blastp on each of the sequence files listed in file of filenames.blastp and save the output in the corresponding .out file.
```

6. If the program is successfully started, control will immediately return to the user. When run_blastp finishes a message will be displayed to alert the user.

If necessary, it is possible to exit once Artemis indicates that the external program has been started and the entry has been saved. run blastp will keep running in the background.

The Graph Menu

Some general information about the graphs can be found in <u>the section called *Graphs and Plots*</u>. Configuration options for graphs are described in <u>the section called *Options For Plots and Graphs* in Chapter 5.</u>

Hide All Graphs

This item will turn off all the visible graphs.

Add Usage Plots ...

This menu item prompts the user for the name of a file which should contain codon usage data in the same format as the data at this web site. If Artemis successfully loads the codon usage file two new plots will be added to the display menu and will be immediately visible. One plot shows the codon scores (in a sliding window) for each of the forward reading frames and the other shows the same thing for the reverse reading frames. [short name: codon usage]

The graph is calculated using the codon preference statistic from <u>Gribskov et al. (Nucl. Acids Res. 12; 539-549 (1984))</u>.

Here is an example usage file:

```
UUU 32.2( 48423) UCU 30.5( 45913) UAU 21.8( 32829) UGU 8.9( 13371)
UUC 13.0( 19519) UCC 12.1( 18149) UAC 11.8( 17721) UGC 5.6( 8372)
UUA 26.0(39138) UCA 17.9(26850) UAA 1.3(1944) UGA 0.5(733)
UUG 24.0(36134) UCG 8.0(12055) UAG 0.5(705) UGG 10.9(16364)
CUU 25.3( 38015) CCU 21.9( 32964) CAU 16.3( 24577) CGU 16.3( 24495)
CUC 7.3 (10922) CCC 8.4 (12619) CAC 6.4 (9653) CGC 6.2 (9316)
CUA 8.6( 12957) CCA 12.7( 19075) CAA 27.3( 41066) CGA 7.9( 11896)
CUG 6.3( 9503) CCG 4.6( 6910) CAG 10.9( 16457) CGG 3.0( 4487)
AUU 35.0(52636) ACU 22.9(34419) AAU 33.9(51009) AGU 14.7(22108)
AUC 12.6( 19000) ACC 10.9( 16378) AAC 17.9( 26895) AGC 9.2( 13905)
AUA 13.1( 19726) ACA 13.9( 20898) AAA 39.3( 59079) AGA 11.1( 16742)
AUG 20.9 ( 31376) ACG 6.5 ( 9744) AAG 25.2 ( 37825) AGG 5.1 (
GUU 29.3 (44015) GCU 30.2 (45397) GAU 38.1 (57240) GGU 22.0 (33101)
GUC 11.0 (16497) GCC 11.6 (17518) GAC 15.8 (23749) GGC 8.5 (12717)
GUA 12.3 (18451) GCA 15.7 (23649) GAA 44.3 (66550) GGA 15.7 (23623)
GUG 8.3(12422) GCG 5.3(8011) GAG 21.3(31979) GGG 4.3(6497)
```

Add User Plot ...

Artemis is able to display some types of user data in a graph that looks like the GC content graph (see <u>the section called GC Content (%)</u>). This menu item will prompt the user for the name of a data file which should contain one of the following possible graph file formats:

1. one line per base and one or more columns of integer or floating point values per line. The number of lines should match the number of bases in the sequence. Artemis will plot each data point for the corresponding base. Each column represents a data set for a line. Example extract:

```
21.4 1910.7
44 1126.1
1911.7 0
0 0
0 1782.0
1937.5 65.4
```

2. the first column corresponds to the base position and is an integer. Note to distinguish this format from the previous format the first line of the file must start with a '#'. Line colours can be specified in

the header using the keyword colour followed by space separated R:G:B values for each line. The next rows(s) are the data values. Example extract:

```
# BASE VAL1 VAL2 VAL3 VAL4 VAL5 VAL6
# colour 5:150:55 255:0:0 0:255:0 0:0:255 100:100:100 50:150:50
176 2204.8 848.23 0
                   0
                            0
                                    536.04
278 804.99 0
                   837.2
                          0
                                681.63 0
452 0 699.98 0
                                251.18
                   0
                          0
                          0
553 0
      0
             0
                   0
                                52.4
654 0 0
             0
                   0
                          334.2 0
686 0 0
             652.78 0
                          0
                                0
                                67.97
831 0 0
           0 0
                          0
. . .
```

3. the next two formats are types of <u>Wiggle formats</u>. The first is variableStep. Note that Artemis only supports the colour element in the track line.

```
track type=wiggle_0 color=255,200,0
variableStep chrom=chr19 span=10
310 1
320 12
330 18
340 6
350 5
430 3
440 1
```

Right clicking on the graph and selecting the 'Configure...' option will display the 'Plot style' option for wiggle plots. The plots can be displayed as histograms or as a heat map.

4. the next format supported by Artemis is fixedStep and is again a Wiggle format.

```
track type=wiggle_0 name="fixedStep" description="fixedStep format"
visibility=full autoScale=off viewLimits=0:1000 color=0,200,100
maxHeightPixels=100:50:20 graphType=points priority=20
fixedStep chrom=chr19 start=7401 step=300 span=200
1000
900
800
700
600
500
400
300
200
100
```

5. Blast tabular format. The blastall command must be run with the -m 8 flag which generates one line of information per HSP. Alternatively the MSPcrunch file format can be read in as a graph file format. Artemis will prompt the user to determine whether it uses the query or subject coordinates to plot the graph.

When a file is prompted for there is an option which if selected will mean the log transform is plotted.

GC Content (%)

Controls whether the GC content plot is visible. This is a graph of the average GC content of a moving window (default size 120 base), across the bases visible in the overview window. [Default: off] [short name: gc_content]

GC Content (%) With 2.5 SD Cutoff

Controls whether the cutoff GC content plot is visible. This is similar to the GC content graph, but the plot is clipped so that the GC content of each algorithm window is shown only when it is more than 2.5 times the standard deviation of the GC content in all the windows. [Default: off] [short name: sd gc content]

AG Content (%)

Controls whether the AG content plot is visible. This is a graph of the average AG content of a moving window (default size 120 base), across the bases visible in the overview window. [Default: off] [short name: ag content]

GC Frame Plot

Controls whether the GC frame plot is visible. This graph is similar to the GC content graph but shows the GC content of the first, second and third position independently. For more information on the algorithm and on how to interpret the result see <u>this web page</u>.

See <u>Ishikawa</u>, <u>J. and Hotta</u>, <u>K. FEMS Microbiol</u>. <u>Lett. 174:251-253 (1999)</u> and <u>GC frame plot</u> for more information on the algorithm.

[Default: off] [short name: gc frame]

Correlation Scores

Controls whether the (forward) correlation scores plot is visible. The graph shows the correlation between the amino acid composition of the globular proteins in TREMBL and the composition of the base translation in each of the three reading frames. The green line represents forward frame 1, blue represents frame 2 and red represents frame 3. [Default: off] [short name: correlation_score]

Reverse Correlation Scores

This does the same as "Correlation Scores", but does the calculation on the reverse strand. The green line represents reverse frame 1 (the bottom frame line), blue represents frame 2 and red represents frame 3. [Default: off] [short name: correlation_score]

GC Deviation (G-C)/(G+C)

Controls whether the GC deviation plot is visible. This graph shows the difference between the "G" content of the forward strand and the reverse strand.

See "Asymmetric substitution patterns in the two DNA strands of bacteria" Lobry JR. - Mol Biol Evol 1996 May;13(5):660-5.

[Default: off] [short name: gc deviation]

AT Deviation (A-T)/(A+T)

Controls whether the AT deviation plot is visible. This graph shows the difference between the "A" content of the forward strand and the reverse strand. [Default: off] [short name: at_deviation]

Karlin Signature Difference

This menu item toggles the display of the graph of the dinucleotide absolute relative abundance difference between the whole sequence and a sliding window.

For details of the algorithm see "Global dinucleotide signatures and analysis of genomic heterogeneity" Samuel Karlin - <u>Current Opinion in Microbiology 1998, 1:598-610</u>.

[Default: off] [short name: karlin_sig]

Cumulative AT Skew and Cumulative GC Skew

AT skew is calculated as ([A]-[T])/([A]+[T]), where [A] and [T] are the counts of these bases in the window. Grigoriev A (1999) <u>Strand-specific compositional asymmetries in double-stranded DNA viruses. Virus Research 60, 1-19</u>.

Positional Asymmetry

Shulman MJ, Steinberg CM, Westmoreland N (1981) <u>The coding function of nucleotide sequences can be discerned by statistical analysis</u>. J Theor Biol 88:409-20.

Informational Entropy

Konopka Andrzej (1984) <u>Is the information content of DNA evolutionarily significant? J Theor Biol</u> <u>107:697-704</u>. Informational entropy is calculated from a table of overlapping DNA triplet frequencies, using equation 1 in the above reference. The use of overlapping triplets smooths the frame effect.

Scaled Chi Square

Shields DC, Sharp PM (1987) Synonymous codon usage in Bacillus subtilis reflects both translational selection and mutational biases. Nucleic Acids Res 15:8023-40.

Mutational Response Index

Gatherer D, McEwan NR (1997) <u>Small regions of preferential codon usage and their effect on overall codon bias--the case of the plp gene. Biochem Mol Biol Int 43:107-14</u>.

Effective Codon Number

Wright F (1990) The 'effective number of codons' used in a gene. Gene 87:23-9, and Fuglsang A (2004) The 'effective number of codons' revisited. Biochem Biophys Res Commun. May 7;317(3):957-64.

Intrinsic Codon Deviation Index

Freire-Picos MA, Gonzalez-Siso MI, Rodriguez-Belmonte E, Rodriguez-Torres AM, Ramil E, Cerdan ME (1994) Codon usage in Kluyveromyces lactis and in yeast cytochrome c-encoding genes. Gene 139:43-9.

The Display Menu

The buttons in this menu are toggles controlling which parts of the Artemis display are visible. All the

Show Entry Buttons

This toggle controls whether the entry button line is visible. (see <u>the section called *The Entry Button Line*</u>). [Default: on]

Show Base View

This toggle controls whether the base view is visible. (see <u>the section called *The Overview and DNA Views*</u>). [Default: on]

Show Feature List

This toggle controls whether the feature view is visible. (see <u>the section called *The Feature List*</u>). [Default: on]

The Entry Button Line

There is one button on this line for each currently loaded entry. The buttons indicate which entries are active and allow the user to toggle each entry between the active and inactive states. The default entry can be set by clicking the right mouse button (see <u>the section called *Notes on Using The Mouse*</u>) on an entry.

See also 3, the section called *The Default Entry* in Chapter 1 and the section called *The Active Entries* in Chapter 1).

The Overview and DNA Views

The overview and DNA view take up most of the main window (see the section called *A breakdown of the main Artemis edit window*). Both views act in a very similar way, so they are described in the same section. In particular they both show the forward and reverse strands of the sequence and a representation of the three translation frames in each direction. The forward sequence is read from the EMBL entry, the reverse sequence is derived from the forward one by complementing it. In the overview shows only the stop codons of each translation frame, but in the DNA view the shows the complete six frame translation.

The horizontal scrollbar controls which part of the sequence is currently visible. The scroll bar at the left controls the zoom level.

Changing the Selection from a View Window

To select a feature just click on it with the first mouse button. This will unselect anything that is currently selected. To add a feature to the selection rather than replacing the current selection, hold the shift key while clicking. A single feature can be removed from the selection in the same way, because shift-clicking acts as a toggle. The situation is made slightly more complicated by the existence of spliced genes. When a feature segment (exon) is added to the selection the feature that contains the segment is implicitly added as well. When the last segment of a feature is removed from the selection, the feature is implicitly removed.

A single base or amino acid can be selected simply by clicking on it. A range of bases can be added by clicking on the base at one end of the range then shift-click on the base at the other end of the range. Alternatively you can drag out a range of bases: click on a base then hold the mouse button down and move to the other end. To select a complete open reading frame (ORF), double click the middle mouse button (see the section called *Notes on Using The Mouse*) anywhere inside the ORF (see the section called *Open Reading Frame* for another way to select an ORF).

See the section called *The Selection* in Chapter 1 for more about the selection.

Other Mouse Controlled Functions

Double clicking on a feature with the first mouse button causes both views and the feature list to centre themselves on that feature. Similarly, double clicking the first mouse button on a base or amino acid will centre both views on that base/amino acid.

A double click of the middle mouse button on a feature will open an edit window for that feature. This is the same as clicking once and then choosing the Edit Selected Features menu item (see <u>the section called</u> <u>Selected Features in Editor</u>).

The Pop-up Menu

The pop-up menu is activated by pressing the third mouse button (see <u>the section called Notes on Using The Mouse</u>) on a feature view. The menu is split into three sections. The top contains functions that will act on the current view. The middle contains shortcuts to some of the main window menus. The bottom contains four toggle buttons which influence the appearance of the view.

Pop-up Menu Functions

Note that not all of these functions are available all the time (the first two are only shown when there are some selected features).

• Raise Selected Features. Raise the selected features so that they appear in front of all other features.

- Lower Selected Features. Make the selected features go behind all the other features.
- Smallest Features In Front. Sort the visible features so that the smallest features appear in front of the larger ones. This is only necessary when the user has manually rearranged the features using "Raise Selected Features" or "Lower Selected Features".
- **Zoom to Selection.** Scroll and scale the display so that the current selection is centred and full width.
- Select Visible Range. Select the currently visible bases on the forward strand.
- **Select Visible Features.** Select those (and only those) features that currently visible in this view. Any features that are off screen or have been filtered out with the "Set Score Cutoffs ..." control.
- Set Score Cutoffs ... The score cutoffs panel allows the user to filter the features of the active entries so that features with low or high scores are not shown. The "score" of a feature is the value of the /score qualifier and should be a number from 0 to 100. The cutoffs window has two sliders. Any feature that has a score less than the value of the top controller or more than the value of the bottom controller will not be shown. Features with no /score qualifier will always be shown.

Toggle Buttons

Feature Labels

This toggle button controls whether the feature labels are displayed on their own line (when the toggle is on) or on the top of the features (when the toggle is off). The default setting for this toggle can be set in the options file (see the section called feature labels in Chapter 5).

One Line Per Entry

This toggle controls whether the entries are shown in the context of the three frame translation or one entry per line on screen. In the first case the entries will be overlaid, in second case they will be shown in parallel. The default setting for this toggle can be set in the options file (see the section called one_line_per_entry in Chapter 5).

Forward Frame Lines

This toggle button controls whether or not to show the 3 frame translation of the forward sequence.

Reverse Frame Lines

This toggle button controls whether or not to show the 3 frame translation of the reverse sequence.

Start Codons

Toggle the display of start codons in the view. See <u>the section called *Genetic Code Tables* in Chapter 2</u> to find out how to change which start codons to use.

Stop Codons

Toggle the display of stop codons in the view.

Feature Arrows

Toggle the display of directional arrows on each feature. The default setting for this toggle can be set in the options file (see the section called <u>draw feature arrows</u> in Chapter 5).

Feature Borders

Toggle the display of black borders around each feature. The default setting for this toggle can be set in the options file (see the section called <u>draw feature borders</u> in Chapter 5).

All Features On Frame Lines

Normally non-protein features are drawn on the DNA lines. This toggle allows the user to force all features to be drawn on the frame lines, which can sometimes improve readability. The default setting for this toggle can be set in the options file (see the section called features on frame lines in Chapter 5).

Flip Display

If selected the sequence and features will be drawn on screen as if they are reverse complemented with the first base to the right of the screen.

Colourise Bases

This toggle turn base colouring on or off. (Note that this feature is completely unless, it exists for amusement only).

Scrolling The View

Moving the horizontal scrollbar will change the part of the sequence that is visible. The position of the view can also be changed by using the Goto menu (see the section called *The Goto Menu*), by double clicking the first mouse button on a feature in one of the sequence views or in the feature list or by double clicking on a base or amino acid.

Changing The Scale

The vertical scrollbar at the right edge of the view controls the scale - moving the scrollbar up will zoom in and moving the scrollbar down will zoom out. When the scrollbar is at the top (at it's most "zoomed-in" position), the complete six frame translation is shown. Otherwise only the stop codons are shown. The main difference between the overview and the DNA view is that the DNA view initially shows the full translation, whereas the overview shows only the stop codons. The other difference is that the labels are on in the overview by default (see the section called *The Pop-up Menu*).

"Direct Editing"

The direct editing option (See the section called *Enable Direct Editing* in Chapter 2) enables the user to change the start or end position of a segment by dragging it around with the mouse. This works best in the DNA view window. As an example, to move the start position, click the mouse button on the first base of the feature or exon, hold the button down, move the mouse pointer to the desired position, then release the button.

The Feature List

The feature list is bit at the bottom of the main window of Artemis (see the section called *Overview of the Entry Edit Window*). The feature list can be toggled on and off from the display menu (see the section called *Show Feature List*). The same component is used by the two items in the view menus that show a sub-set of components (see the section called *CDS Genes And Products* and the section called *Feature Filters*). The list contains information about each feature in each active entry (see the section called *The Active Entries* in Chapter 1), one per line.

The lines show this information about the feature: the colour (see the section called *Feature Colours* in Chapter 1), the key, start base, end base, the note field (if any) and then the remaining qualifiers in EMBL/GENBANK format. If the correlation scores option is on then the scores will be shown just before the note in each CDS line.

Changing the Selection from the Feature List

To select a feature from the list just click on it with the first mouse button. This will unselect anything that is currently selected. To add a feature to the selection rather than replacing the current selection, hold the shift key while clicking. A single feature can be removed from the selection in the same way, because shift-clicking acts as a toggle.

See the section called *The Selection* in Chapter 1 for more about the selection.

Other Mouse Controlled Functions

Double clicking on a feature with the first mouse button causes both views and the feature list to centre themselves on that feature.

A double click of the middle mouse button on a feature will open an edit window for that feature. This is the same as clicking once and then choosing the Edit Selected Features menu item (see <u>the section called</u> <u>Selected Features in Editor</u>).

The Pop-up Menu

The pop-up menu is activated by pressing the third mouse button (see <u>the section called *Notes on Using The Mouse*</u>) anywhere on the feature list. The menu contains shortcuts to some of the main window menus and three toggle buttons:

Save List to File

Save the list of features to a file.

Show Selected Qualifier

A qualifier can be selected to be displayed in the feature list.

Show Correlation Scores

If this toggle is on the correlation scores for each feature will be show between the end base and the note.

Show Gene Names

This button controls whether the key or gene name is shown for each feature. The "gene name" is actually the /label qualifier of the feature. If there is no /label then the /gene qualifier is used. If neither of the qualifiers exists then the key is used.

Show Qualifiers

If this option is on the feature qualifiers will be displayed after the note.

Show Product

This button controls whether the /note or /product is shown for each feature. [default: /note]

Scrolling The List

The scrollbar on the right of the list controls which part of the list of active features is visible. The list will also move if the user double clicks on a feature in overview or DNA view areas, in which case the list will scroll to that feature

Graphs and Plots

The DNA plots are accessed from the Graph menu (see the section called *The Graph Menu*). When a graph is turned on it is locked to the overview window, so it will follow the overview window when the overview is scrolled. It will also automatically change the viewing scale to match the scale of the overview window. The vertical scrollbar to the right of the graph controls the window size of algorithm.

The feature plots window (see the section called *Feature Plots*) has a horizontal scroll bar that sets the position of the plot in the feature. As with the DNA plots, the vertical scrollbar to the right of the graph controls the window size of algorithm.

Note that clicking the left mouse button on the plot will show the base or residue at that position. Clicking the right mouse button will pop-up a menu. The menu contains a toggle which controls whether the graph is scaled and some menu items which allow the maximum window size to be set.

Notes on Using The Mouse

Artemis makes use of all three buttons of the mouse under X, but other windowing systems often have less than three buttons. To make life easier when running Artemis on those systems, there are some alternative key bindings.

On Windows pressing the alt or meta key and the first mouse button will give the same effect as pressing mouse button three (usually a pop-up menu). Pressing the control key and the first mouse button will do the same thing as mouse button two.

On Macintosh systems pressing the control key and the mouse button will give the same effect as pressing mouse button three (usually a pop-up menu). Pressing the alt key and the mouse button will do the same thing as mouse button two.

Chapter 4. Secure Shell (SSH) Plugin To Artemis

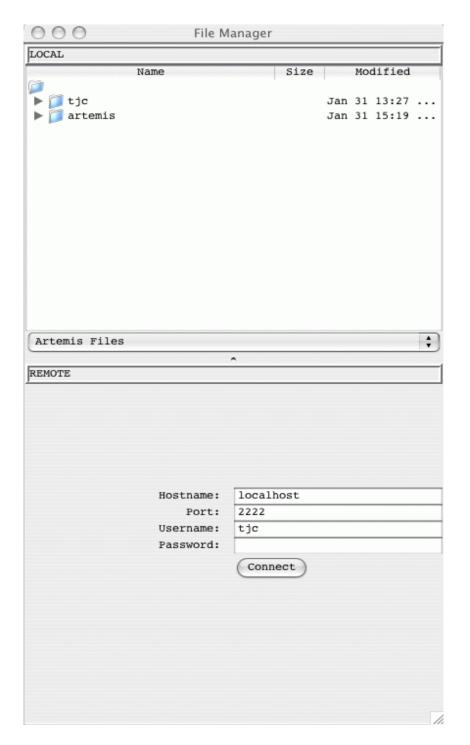
Overview of the SSH Plugin

This is a plug-in to Artemis that enables you to view and transfer files from a local machine (e.g. PC/MacOSX/UNIX) to another remote file system (i.e. any other file system that you have ssh access to). The main areas where this is useful are:

- file management for both local and remote files. Files can be transferred by dragging and droping between file systems. Multiple files can be selected and transferred in this way.
- launching Artemis and ACT with sequences and tab files from files on the remote machines.
- running BLAST and Fasta searches can be sent from the local machine that is running Artemis and run on the remote machine. To do this the software (BLAST and Fasta) need to be set up on the remote side (see the section called Configuring Searches to be run via SSH).

Login In

From the Artemis launch window go to the 'File' menu and select 'Open SSH File Manager'. A file manager window will then open with a local file manager at the top. Underneath this is a remote file manager with a section to enter login details.



If you can connect directly to the remote machine give the hostname, username and password. Alternatively if you are using a SSH tunnel (see <u>the section called *Setting up a SSH tunnel*)</u> then use localhost and the appropriate port number.

Setting up a SSH tunnel

The tunneling capability of SSH Secure Shell allows people to access their email, intraweb pages and files securely even when working away from the office. This can be used with Artemis and this section provides some examples of setting up tunnelling. It may be necessary to consult your friendly system administrator about the details on how best to do this.

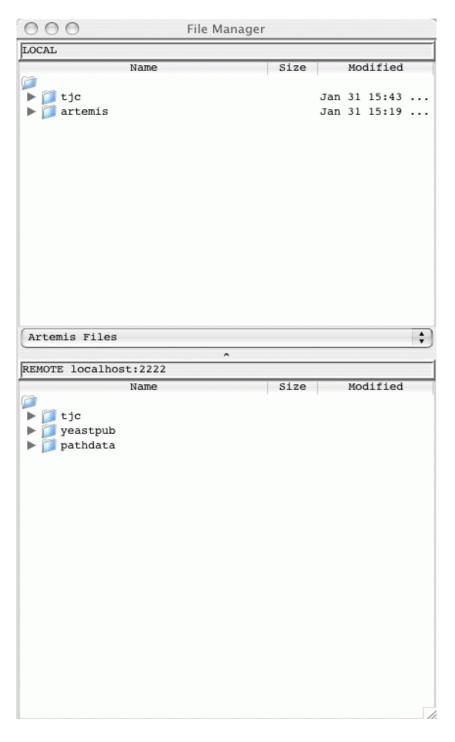
On UNIX the ssh configuration can be set in the file $\sim/.ssh/config$. This contains the information to set up a tunnel that looks like this:

```
Host *.sanger.ac.uk
  LocalForward 2222 machine.sanger.ac.uk:22
  User tjc
```

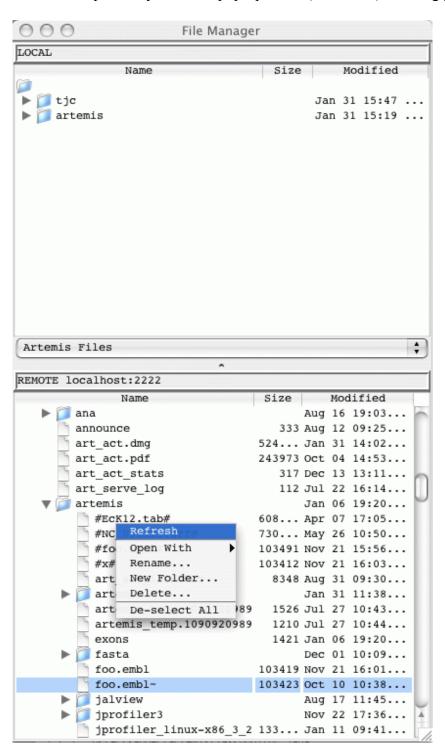
Once this configuration file is in place you can login from a UNIX window to set up a tunnel.

On Windows you can use Putty, see the section called Using Putty to Set Up A Tunnel.

When the tunnel has been established you can start artemis and enter 'localhost' into the Hostname field in the login window and the port number (e.g. 2222 in the above example).



The file manager can be used to drag and drop files between each file system. If you right click on a selected file this will provide you with a pop-up menu (see below) enabling you to delete, rename and open files.



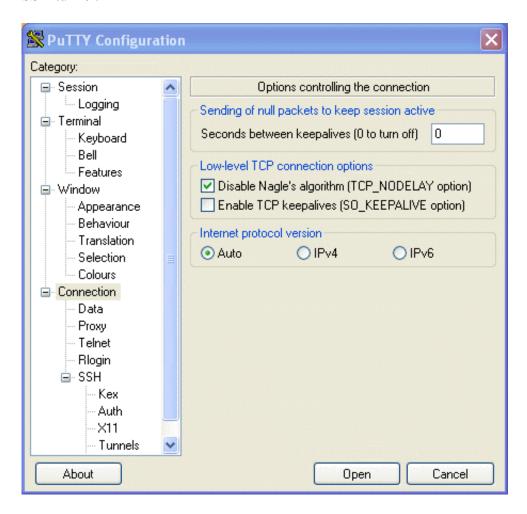
Files on the remote file system can be double clicked on and opened in Artemis (in the same way they can be opened from the local file manager). Tab files can be read in by dragging them from the remote file manager and dropping them into the Artemis window.

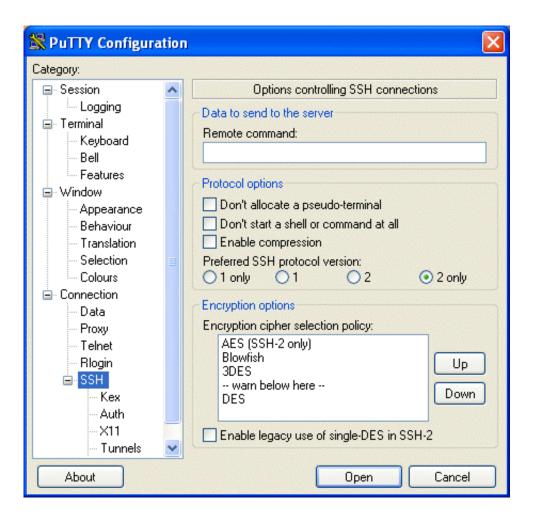
Note for ACT the sequence filename can be dragged from the file manager into the file selection box (if the files are not found locally the are then located on the server). The tab files in ACT can be dragged from the file manager and dropped on the relevant sequence display.

When files are saved, if the entry orginated from the remote file system then this is saved locally in the

Using Putty to Set Up A Tunnel

On *Windows* you can use <u>Putty</u> to set up a ssh tunnel. Below are settings that can be used in Putty to set up a SSH tunnel.

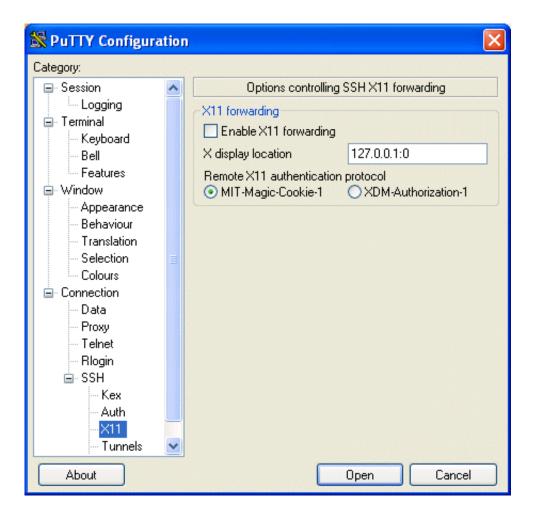




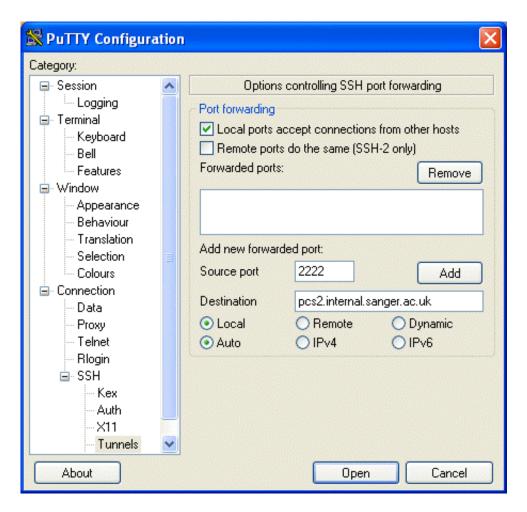
SSH 2 only is probably recommended here.



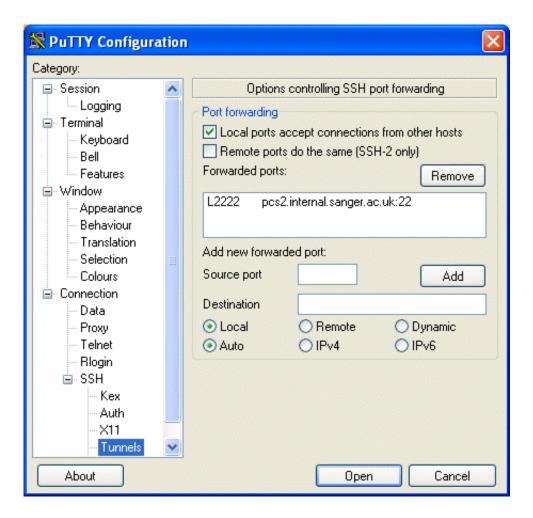
X11 forwarding is not necessary.



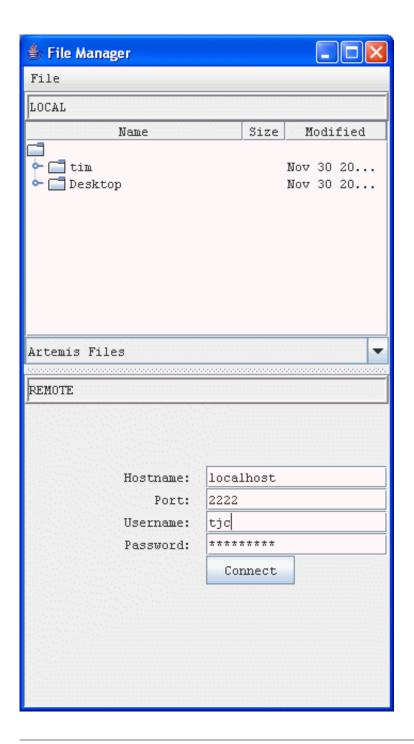
Set the source port to 2222 and set the destination to hostname:port (e.g. pcs2.internal.sanger.ac.uk:22).



Click 'Add' to add this to the list of forwarded ports.



Run Artemis and select the 'SSH File Manager' from the 'File' menu. At the bottom fill in login details and click on the 'Connect' button. Loging in may take a minute (depending on the connection).



Using the SSH connection to run Fasta and BLAST

Using the SSH connection you can send Fasta and BLAST searches to be run on the remote machine. If you have not already be prompted to login (i.e. when opening up the SSH file manager), you will then be asked for your login details. The sequence files that are to be searched against the database are then sent to the remote machine. Once the search has finished the results are transferred back afterwards. If Artemis detects that you have opened up an entry from the remote file system the analysis will be run in the directory you open the entry from. The results in this case are stored on both the local and remote machines.

To keep Fasta and BLAST searches on your local machine and the remote file system *in-sync*:

• copy any previously run fasta and blast directories from the remote machine to your local file system. This can be done by making a tar ball of the directory: e.g. creating a tar file of a 'fasta' directory

```
tar cvf tar ball name.tar fasta
```

• Then transfer this file to the local project directory and extract with:

```
tar xvf tar ball name.tar
```

- when launching Artemis, make sure you first change directory to the directory above the 'fasta' directory that has been unpacked.
- open the files from the remote file system in the file manager. It then knows where to save it back to.

Configuring Searches to be run via SSH

This would most likely be done by a systems administrator. The databases and fasta and BLAST software need to be set up on the remote machine. Once this is done the commands that are sent from the Artemis/ACT client to the remote machine are controlled by a 'j2ssh.properties' file, see below for example of this file. This needs to be put in the CLASSPATH for Artemis/ACT or the one in the distribution (lib/j2ssh/j2ssh.properties) modified.

The j2ssh.properties file also hold default information. For example default parameters can be set for the login prompt, the working directory (for searches when sequences are opened on the local side) and any extra directories (other than the users home directory) to display in the file manager.

```
#
# Configuration file for using SSH file management and
# database searches.
# default login options
port=2222
host=localhost
# Generic default options
blastp=blastall -p blastp
blastx=blastall -p blastx
tblastx=blastall -p tblastx
blastn=blastall -p blastn
fasta=fasta33 t -B -S -q -b 100 -H -l /data/fasta/pubseqqbs
fastx=fastx33 t -B -S -q -b 40 -H -l /data/fasta/pubseqqbs
# default working directory
wdir=/scratch
# aliases used for fasta
%uniprot=%U
%uniprot archaea=%A
```

```
%uniprot_bacteria=%B
%uniprot_eukaryota=%E
%uniprot_viruses=%V
%uniprot_rest=%R
%malaria=%M
%kineto_aa=%K
default_db=%uniprot_bacteria
#
#
#
# remote directories to use as roots in the file manager
remotedir.PD=/nfs/pathdata
remotedir.YP=/nfs/disk222/yeastpub
# local directories to use as roots in the file manager
localdir.PD=/nfs/pathdata
```

Chapter 5. Artemis Configuration Options

The Options File

When started on Artemis will potentially look in 5 different places for files to examine for configuration information. Artemis reads from the locations in order, so the user can override the default options. Note that on Macintosh and Windows systems the "current directory" mentioned below is the directory where the Artemis application has been installed.

This is the search order:

- The standard Artemis options are read first. On UNIX this file is in the file etc/options in the Artemis distribution directory. Changing that file will change the options settings for all users. On Macintosh and Windows system the standard options are stored in the Artemis application itself and can't be changed (easily).
- If the user has specified an options file on the command line with the -options argument, it will be read next. (UNIX only see the section called *UNIX Command Line Arguments for Artemis* in Chapter 2 for more).
- If there is a file called diani.ini in the current directory it will be read. This is for backwards compatibility.
- If there is a file called options, options.txt or options.text in the current directory it will be read.
- If there is a file in the user's home directory called .artemis_options it will be read. (UNIX only).

Artemis Option Descriptions

This section describes the possible options for Artemis.

font_size

This option will set the font size for all the Artemis windows. [default: 14]

font name

This option is used to choose the font for all the Artemis windows. The font must be fixed-width. [default: "Monospaced"]

base_plot_height

The height (in pixels) of each of the base plots. [default: 150]

feature plot height

The height (in pixels) of each of the feature plots. [default: 160]

draw_feature_borders

If set to yes, borders will be drawn around each feature and each exon. if set to no, borders will only be drawn around the selected features. This can also be set in the views popup menu (see <u>the section called Feature Borders in Chapter 3</u>). [default: "yes"]

draw_feature_arrows

If set to yes, a direction arrow will be drawn around at the end of each feature. if set to no, no arrows will be drawn. This can also be set in the views popup menu (see the section called *Feature Arrows* in Chapter 3). [default: "yes"]

overview feature labels

If this option is no then the feature labels in the overview (see <u>the section called *Overview of the Entry Edit Window*</u> in Chapter 3) will be off at startup. [default: "yes"]

overview_one_line_per_entry

If this option is set yes then the overview (see <u>the section called *Overview of the Entry Edit Window* in <u>Chapter 3</u>) will start in one line per entry mode. [default: "no"]</u>

show list

If set to yes then the feature list (see <u>the section called *The Feature List* in Chapter 3</u>) will be shown on startup. [default: "yes"]

show_base_view

If set to yes then the DNA base view (see the section called *The Overview and DNA Views* in Chapter 3) will be shown on startup. [default: "yes"]

features on frame lines

If set to yes then the "All Features On Frame Lines" option will be set to yes on startup. (See <u>the section called *All Features On Frame Lines* in Chapter 3) [default: "no"]</u>

frame_line_features

This is a list of the feature keys that should be shown by default on the frame lines. e.g.

```
frame_line_features = \
  CDS \
  polypeptide
```

feature labels

If set to yes then the feature labels will be shown on startup. (See <u>the section called *All Features On Frame Lines* in Chapter 3) [default: "yes"]</u>

one line per entry

If set to yes then the "One Line Per Entry" option will be set to yes on startup. (See <u>the section called *All Features On Frame Lines* in Chapter 3) [default: "no"]</u>

genetic codes

This option gives the list of names of the available genetic code tables. For each name in the list there is a translation_table_NUMBER entry (see below) where NUMBER is its location in the genetic_codes list. Similarly the start codons are defined as start_codons_NUMBER for each code.

translation_table_1

The translation_table option is used to lookup codon translations. The table must have exactly 64 entries, and there is one entry for each codon.

The entries should appear in this order:

```
TTT TTC TTA TTG
```

This is the default setting for this option is the Standard Code:

```
translation table 1 = \setminus
    f f l l \
    ssss\
    уу * * \
    C C * W \
    1 1 1 1 \
    pppp\
    hhqq\
    rrrr\
    i i i m \
    tttt\
    nnkk\
    ssrr\
    v v v v \
    a a a a \setminus
    ddee \
    gggg
```

The other translation table entries are defined by giving the differences to the Standard Code. These are given as the bases that make up the codon immediately followed by the translation (e.g. atam, so that 'ata' codes for 'm').

extra keys

This contains a list of keys (separated by spaces) that are allowed in addition to those specified by EMBL. The official EMBL keys are listed in the feature keys file in the Artemis code directory.

extra qualifiers

This contains a list of qualifiers (and their associated type) that are allowed in addition to those specified by EMBL. The official EMBL qualifiers and qualifier types are listed and described in the qualifier_types file in the Artemis code directory.

common keys

This is a list of the keys that should be shown by default in the feature edit window. (see <u>the section called</u> *Selected Features in Editor* in Chapter 3).

The default setting for this option is:

```
common_keys = \
   allele attenuator CDS conflict exon intron LTR misc_feature misc_RNA mRNA \
   mutation polyA_signal polyA_site promoter protein_bind RBS repeat_region \
   repeat_unit rRNA scRNA snRNA source stem_loop STS TATA_signal terminator \
   tRNA unsure variation -10_signal -35_signal CDS_motif gene \
   BLASTN_HIT CDS_BEFORE CDS_AFTER BLASTCDS
```

hyperlinks

This is a list of databases and their URL's for Artemis to provide hyperlinks in the Feature Editor (see <u>the</u> section called *Selected Features in Editor* in Chapter 3).

undo_levels

The number of levels of undo to save or 0 to disable undo. More undo levels will require more memory. [default: 20]

minimum orf size

This option is used to set the minimum size (in amino acid residues) of a "large" open reading frame, which controls which ORFS are marked by the "Mark Open Reading Frames" menu item (see <u>the section called Mark Open Reading Frames</u> ... in Chapter 3).

direct edit

Set the default value for the direct edit option. A value of "yes" will turn direct edit on by default. See <u>the section called *Enable Direct Editing* in Chapter 2</u>

feature_dna_programs

This is a list of the possible external programs that can be run on the bases of a feature. Each pair in the list is a program name and a default database to use for that program. For each program name there must be a corresponding shell script called "run_something". eg. run_blastn. See the Run_Menu in Chapter 3 for more information.

feature_protein_programs

This is a list of the possible external programs that can be run on the translation of a feature. Each pair in the list is a program name and a default database to use for that program. For each program name there must be a corresponding shell script called "run_something". eg. run_blastp for blastp or run_fasta for fasta. See the_Run Menu in Chapter 3 for more information.

ncbi_dna_search

This lists the NCBI web-BLAST applications that the bases of a selected feature can be sent to. See <u>the section called *The Run Menu* in Chapter 3.</u>

ncbi protein search

This lists the NCBI web-BLAST applications that the translation of a selected feature can be sent to. See <u>the</u> section called *The Run Menu* in Chapter 3.

Setting Colours

The feature colours (see the section called *Feature Colours* in Chapter 1) used by Artemis can be changed using the options file. By default there are 18 possible colours (numbered 0 to 17), but any number can be used. The option names for the colours are colour_0, colour_1, etc. The value of each of these qualifiers should be the three numbers separated by spaces. The numbers correspond to red, green and blue respectively and each number is an intensity from 0 to 255. As an example, to change colour 2 to white put this line in the options file:

```
colour 2 = 255 255 255
```

Here is a list of the default colour numbers:

```
      0
      white
      (RGB values: 255 255 255)

      1
      dark grey
      (RGB values: 100 100 100)

      2
      red
      (RGB values: 255 0 0)

      3
      green
      (RGB values: 0 255 0)

      4
      blue
      (RGB values: 0 255 255)

      5
      cyan
      (RGB values: 255 0 255)

      6
      magenta
      (RGB values: 255 255 0)

      7
      yellow
      (RGB values: 255 255 0)

      8
      pale green
      (RGB values: 152 251 152)

      9
      light sky blue
      (RGB values: 135 206 250)

      10
      orange
      (RGB values: 255 165 0)

      11
      brown
      (RGB values: 255 200 200)

      12
      pale pink
      (RGB values: 255 200 200)

      13
      light grey
      (RGB values: 170 170 170)

      14
      black
      (RGB values: 255 63 63)

      16
      light red
      (RGB values: 255 127 127)

      17
      pink
      (RGB values: 255 191 191)
```

Options For Plots and Graphs

Each graph type has three option settings associated with it: the default minimum window size, default maximum window size and the default window size. The option names have the following form: [short_name]_default_min_window, [short_name]_default_max_window and [short_name]_default_window (respectively). [short_name] should be replaced with the short name of the graph. (The available short names are documented in the section called *The Graph Menu* in Chapter 3 and the section called *Feature Plots* in Chapter 3).

As an example the short name of the GC content graph is gc_content so to set the initial minimum window size to 100, the maximum to 1000 and the initial window size to 150 use these setting:

```
gc_content_default_min_window = 100
gc_content_default_max_window = 1000
gc content default window size = 150
```

See also the section called *Graphs and Plots* in Chapter 3.

Options For A Chado Database

```
chado_exon_model
```

This is the feature key used when chado exons are joined and displayed in Artemis. For example:

```
chado_exon_model=CDS
```

[default: "exon-model"]

chado transcript

This is the feature key used when chado transcripts are created in a gene model. [default: "mRNA"]

chado_servers

This can be used to provide a list of available databases. For example:

```
chado_servers = \
          test localhost:5432/test?userName \
          genedb_ro db.genedb.org:5432/snapshot?genedb_ro
```

Options File Format

The option files it should contain settings that look like this:

```
option_name = option_value
```

If the value of an options is too long to fit on one line it can be split over several lines by ending each line with a backslash like this:

```
option_name = option_value another_option_value \
    a third option value a forth option value
```

An Example Options File

Here is an example options file:

```
# this line is a comment

font_size = 14

minimum_orf_size = 100

common_keys = \
    allele attenuator CDS conflict exon intron LTR misc_feature misc_RNA mRNA \
    mutation polyA signal polyA site promoter protein bind RBS repeat region \
```

repeat_unit rRNA scRNA snRNA source stem_loop STS TATA_signal terminator $\$ tRNA unsure variation -10_signal -35_signal CDS_motif gene