**The ACT Manual**

Copyright © 2000-2018 by Genome Research Limited

This document describes ACT - a DNA sequence comparison viewer included as part of release 17 of the Artemis tool set.

ACT 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](http://www.gnu.org/copyleft/gpl.html) 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](#page9) .

**Table of Contents**

1. [Introduction to ACT](#page5)

[What is ACT?](#page5)

[System Requirements](#page5)

[Getting and Installing ACT](#page5)

[Installing Java](#page6)

[Installation Instructions for UNIX and GNU/Linux](#page6) [Installation Instructions for MacOSX](#page6) [Installation Instructions for Windows](#page7)

[Concepts](#page7)

[The "Entry"](#page7) [EMBL/Genbank Features](#page7) [EMBL/Genbank Feature Keys](#page7) [EMBL/Genbank Feature Qualifiers](#page8) ["Tab" Files or "Table" Files](#page8)

[The Active Entries](#page8) [The Default Entry](#page8) [Feature Segments](#page8) [The Selection](#page8) [Feature Colours](#page9)

[Contributions and Suggestions](#page9)

[Distribution Conditions and Acknowledgments](#page9) [Copyright Notice](#page9)

1. [Starting ACT](#page14)

[Running a Comparison](#page14)

[big\_blast.pl](#page14)

[The MSPcrunch Comparison File Format](#page14)

[Running ACT on UNIX and GNU/Linux Systems](#page15)

[UNIX Command Line Arguments for ACT](#page15)

[-options](#page16)

[-Xmsn -Xmxn](#page16)

[-Dblack\_belt\_mode=false](#page16)

[-Doffset=10000](#page16)

[-Duserplot=pathToFile](#page16)

[-Dloguserplot=pathToFile](#page16)

[-Dbam1=pathToFile1 -Dbam2=pathToFile2](#page17) [-Dshow\_snps](#page17)

[-Dshow\_snp\_plot](#page17)

[-Dshow\_cov\_plot](#page17)

[-Dchado="hostname:port/database?username"](#page17)

[-Dread\_only](#page17)

[-Dshow\_forward\_lines=false](#page17)

[-Dshow\_reverse\_lines=false](#page18)

[Running ACT on Macintosh Systems](#page18)

[Running ACT on Windows Systems](#page18)

[The ACT Launch Window](#page18)

[The File Menu](#page18)

[The Options Menu](#page19)

1. [The ACT Main Window](#page21)

[Overview of the Comparison Window](#page21)

[The File Menu](#page22)

[Read An Entry ...](#page22)

[Save Entry](#page22)

[Save All](#page22)

[Write](#page23)

[Read BAM / CRAM / VCF ...](#page24)

[Edit In Artemis ...](#page25)

[Close](#page26)

[The Entries Menu](#page26)

[Set Name Of Entry](#page26)

[Set Default Entry](#page26)

[Remove An Entry](#page26)

[Remove Active Entries](#page26)

[Deactivate All Entries](#page26)

[The Select Menu](#page27)

[Feature Selector ...](#page27)

[All](#page27)

[All Bases](#page28)

[Select All Features in Non-matching Regions](#page28)

[None](#page28)

[By Key](#page28)

[CDS Features](#page28)

[Same Key](#page28)

[Open Reading Frame](#page28)

[Features Overlapping Selection](#page29)

[Features Within Selection](#page29)

[Base Range ...](#page29)

[Feature AA Range ...](#page29)

[Toggle Selection](#page29)

[The View Menu](#page29)

[Selected Features](#page29)

[Selection](#page30)

[Search Results](#page30)

[CDS Genes And Products](#page30)

[Feature Filters](#page30)

[Overview](#page31)

[Forward Strand Overview](#page32)

[Reverse Strand Overview](#page33)

[Feature Bases](#page33)

[Feature Bases As FASTA](#page33)

[Feature Amino Acids](#page33)

[Feature Amino Acids As FASTA](#page33)

[Feature Statistics](#page33)

[Feature Plots](#page33)

[Adjust panel heights ...](#page34)

[The Goto Menu](#page34)

[Navigator ...](#page34)

[Start of Selection](#page35)

[End of Selection](#page35)

[Feature Start](#page35)

[Feature End](#page35)

[Start of Sequence](#page35)

[End of Sequence](#page36)

[Feature Base Position ...](#page36)

[Feature Amino Acid ...](#page36)

[The Edit Menu](#page36)

[Undo](#page36)

[Redo](#page36)

[Selected Features in Editor](#page36)

[Subsequence (and Features)](#page38)

[Find Or Replace Qualifier Text](#page39)

[Qualifier(s) of Selected Feature](#page39)

[Selected Feature(s)](#page40)

[Move Selected Features To](#page41)

[Copy Selected Features To](#page41)

[Trim Selected Features](#page41)

[Extend Selected Features](#page42)

[Fix Stop Codons](#page42)

[Automatically Create Gene Names](#page42)

[Fix Gene Names](#page42)

[Bases](#page43)

[Contig Reordering ...](#page43)

[Header Of Default Entry](#page44)

[The Create Menu](#page44)

[New Feature](#page44)

[Feature From Base Range](#page44)

[Intergenic Features](#page44)

[Features From Non-matching Regions](#page44)

[New Entry](#page45)

[Mark Open Reading Frames ...](#page45)

[Mark Empty ORFs ...](#page45)

[Mark Open Reading Frames In Range ...](#page45)

[Mark From Pattern ...](#page45)

[Mark Ambiguities](#page46)

[The Run Menu](#page46)

[Configuring the Run Menu](#page46)

[The Graph Menu](#page48)

[Hide All Graphs](#page48)

[Add Usage Plots ...](#page48)

[Add User Plot ...](#page48)

[GC Content (%)](#page50)

[GC Content (%) With 2.5 SD Cutoff](#page50)

[AG Content (%)](#page50)

[GC Frame Plot](#page51)

[Correlation Scores](#page51)

[Reverse Correlation Scores](#page51)

[GC Deviation (G-C)/(G+C)](#page51)

[AT Deviation (A-T)/(A+T)](#page51)

[Karlin Signature Difference](#page52)

[Cumulative AT Skew and Cumulative GC Skew](#page52)

[Positional Asymmetry](#page52)

[Informational Entropy](#page52)

[Scaled Chi Square](#page52)

[Mutational Response Index](#page52)

[Effective Codon Number](#page53)

[Intrinsic Codon Deviation Index](#page53)

[The Display Menu](#page53)

[Hide Frame Lines](#page53)

[Show Frame Lines](#page53)

[The DNA Views](#page53)

[Changing the Selection from a View Window](#page54)

[Other Mouse Controlled Functions](#page54)

[The Pop-up Menu](#page54)

[Scrolling The View](#page57)

[Changing The Scale](#page57)

["Direct Editing"](#page57)

[Comparison View](#page57)

[Controls](#page57)

[The Feature List](#page58)

[Changing the Selection from the Feature List](#page58)

[Other Mouse Controlled Functions](#page59)

[The Pop-up Menu](#page59)

[Scrolling The List](#page60)

[Graphs and Plots](#page60)

[Notes on Using The Mouse](#page60)

1. [Secure Shell (SSH) Plugin To ACT](#page61) [Overview of the SSH Plugin](#page61) [Login In](#page61)

[Setting up a SSH tunnel](#page62)

[Using Putty to Set Up A Tunnel](#page65)

[Using the SSH connection to run Fasta and BLAST](#page68) [Configuring Searches to be run via SSH](#page69)

1. [ACT Configuration Options](#page70)

[The Options File](#page70)

[ACT Option Descriptions](#page70)

[font\_size](#page70)

[font\_name](#page71)

[base\_plot\_height](#page71)

[feature\_plot\_height](#page71)

[draw\_feature\_borders](#page71)

[show\_forward\_lines](#page71)

[show\_reverse\_lines](#page71)

[draw\_feature\_arrows](#page71)

[overview\_feature\_labels](#page72)

[overview\_one\_line\_per\_entry](#page72)

[show\_list](#page72)

[show\_base\_view](#page72)

[features\_on\_frame\_lines](#page72)

[frame\_line\_features](#page72)

[feature\_labels](#page72)

[one\_line\_per\_entry](#page73)

[genetic\_codes](#page73)

[translation\_table\_1](#page73)

[extra\_keys](#page74)

[extra\_qualifiers](#page74)

[common\_keys](#page74)

[hyperlinks](#page74)

[undo\_levels](#page74)

[minimum\_orf\_size](#page74)

[direct\_edit](#page75)

[feature\_dna\_programs](#page75)

[feature\_protein\_programs](#page75)

[ncbi\_dna\_search](#page75)

[ncbi\_protein\_search](#page75)

[Setting Colours](#page75)

[Options For Plots and Graphs](#page76)

[Options For A Chado Database](#page76)

[Options File Format](#page77)

[An Example Options File](#page77)

**List of Tables**

3-1. [IUB Base Codes](#page45)

**Chapter 1. Introduction to ACT**

**What is ACT?**

ACT is a DNA sequence comparison viewer based on [Artemis](http://sanger-pathogens.github.io/Artemis/Artemis/). The name ACT stands for Artemis Comparison Tool. ACT is written in Java, reads EMBL or GENBANK format sequences and feature tables, and can work on sequences of any size.

ACT understands several different comparison file formats. See [the section called *Running a Comparison*](#page14) [in Chapter 2](#page14) for details. Note that ACT is a comparison viewer only. The comparison information itself must be generated externally. See [the section called *Running a Comparison* in Chapter 2](#page14) for more details.

For other information see the [ACT web pages](http://sanger-pathogens.github.io/Artemis/ACT/) or the [Artemis web pages](http://sanger-pathogens.github.io/Artemis/Artemis/).

**System Requirements**

ACT will run on any machine that has a recent version of Java. This version of ACT requires Java 1.8 at least. Most of the development has been done using JDK v1.8. See [the section called *Getting and Installing*](#page5) [*ACT*](#page5) fordetails on how to get Java.

**Getting and Installing ACT**

ACT is included in the Artemis tool set package. The most up to date version of ACT is always available from the [ACT web pages](http://sanger-pathogens.github.io/Artemis/ACT/)

**Installing Java**

Before installing Artemis/ACT you will need to make sure you have Java installed.

* Linux, UNIX users should get the current Java version from the [Oracle](http://www.java.com/).
* Windows users should get latest stable release from [Java version](http://www.java.com/) from Oracle. On some versions of Windows you will need be have administrator privileges for your machine to successfully install Java.
* For Macintosh users the Java runtime comes packaged with the Artemis software and so does not need to be installed separately.

**Installation Instructions for UNIX and GNU/Linux**

Change directory to the directory you wish to install the Artemis/ACT software 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:

**tar zxf artemis\_compiled.tar.gz**

This will create a directory called ~/artemis which will contain all the files necessity for running Artemis, ACT and associated tools.

For instructions on how to run ACT on UNIX and GNU/Linux once the archive is unpacked see the [section called *Running ACT on UNIX and GNU/Linux Systems* in Chapter 2](#page15) .

**Installation Instructions for MacOSX**

For MacOSX users an archive artemis.dmg.gz disk image is provided. Your Mac may uncompress this automatically on download. If not it can be uncompressed using gunzip:

gunzip artemis.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.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 and read the "Readme.txt" file.

**Installation Instructions for Windows**

On Windows systems with Java (version 1.8 or higher), installing Artemis is as simple as downloading the artemis-windows.zip file to an appropriate place (such as the desktop or the Programs folder). Unzip the file using WinZip or equivalent. The unzipped folder will contain the Artemis tool set executable jar files.

For instructions on how to run ACT on Windows once it is unpacked see [the section called *Running ACT*](#page18) [*on Windows Systems* in Chapter 2](#page18).

**Concepts**

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

**The "Entry"**

An "entry" in ACT-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 [feature table](http://www.ebi.ac.uk/embl/Documentation/FT_definitions/feature_table.html) lines (the FT lines) of an EMBL/GENBANK entry (see [the section called *"Tab" Files or "Table" Files*](#page8) ). After loading a sequence and opening an entry edit window (see [the section called *Open ...* in Chapter 2](#page18) ) it is then possible to overlay many feature tables (see [the section called *Read An Entry ...* in Chapter 3](#page22) ). Each of these feature table files is called an entry by ACT 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](#page22) [*The File Menu* in Chapter 3](#page22))and by the items in the Entry menu (see[the section called *The Entries Menu*](#page26)[in Chapter 3](#page26)).

**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*](#page7) ) and zero or more qualifiers (see [the section called](#page8) [*EMBL/Genbank Feature Qualifiers*](#page8)). In an EMBL or Genbank formatted file the features of a piece ofDNA are listed in the feature table section (see [the section called *"Tab" Files or "Table" Files*](#page8) ).

**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](http://www.ebi.ac.uk/) has a list of [all possible feature keys](http://www.ebi.ac.uk/embl/Documentation/FT_definitions/feature_table.html).

**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](http://www.ebi.ac.uk/) has a list of [all possible feature qualifiers](http://www.ebi.ac.uk/embl/Documentation/FT_definitions/feature_table.html).

**"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 ACT 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](#page26) .

**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 [the](#page26) [section called *Set Default Entry* in Chapter 3](#page26) ).

**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, ACT allows the user to "select" the objects that the program will act on. The objects to act on in ACT 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](#page27) [called *The Select Menu* in Chapter 3](#page27) ) or using the mouse (see [the section called *Changing the Selection*](#page54) [*from a View Window* in Chapter 3](#page54)).

**Feature Colours**

Each feature displayed in ACT can be given a colour. The available colours are set in the options file (see [Chapter 5](#page70)) and are assigned to a feature by adding a /colour qualifier (see [the section called *Selected*](#page36) [*Features in Editor* in Chapter 3](#page36)). Currently there are two ways of specifying feature colours. The first wayuses 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 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 ACT and 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 'artemis - users-join@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**

ACT may be freely distributed under the terms of the [GNU General Public License](http://www.gnu.org/copyleft/gpl.html). See [the section called](#page9) [*Copyright Notice*](#page9) forthe full text of the license.

The development of ACT is funded by the [Wellcome Trust](http://www.wellcome.ac.uk/) through its support of the [Pathogen Informatics Group](https://www.sanger.ac.uk/science/groups/pathogen-informatics) at [The Sanger Institute](http://www.sanger.ac.uk/).

**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

1. 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

1. 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.

1. 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:
   1. You must cause the modified files to carry prominent notices stating that you changed the files and the date of any change.
   2. 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.
   3. 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.

1. 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:
   1. 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,

* 1. 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.

1. 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.
2. 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.
3. 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.
4. 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.

1. 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.
2. 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.

1. 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

1. 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.
2. 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 ACT**

**Running a Comparison**

Before running ACT you will need to obtain a comparison file. ACT supports three different comparison file formats:

* **The output of** [**BLAST**](http://www.ncbi.nlm.nih.gov/BLAST/) **version 2.2.2 or better.** The **blastall** command must be run with the **-m 8** flag which generates one line of information per HSP. If [BLAST+](http://blast.ncbi.nlm.nih.gov/) is used then the legacy\_blast.pl PERL script (bundled along with the BLAST+ applications) can be used to generate the tabular format or run it with the **-outfmt 6** flag. For more details see the [BLAST+ manual](ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/user_manual.pdf).
* **MEGABLAST output.** ACT can also read the output of[MEGABLAST](http://www.ncbi.nlm.nih.gov/blast/megablast.shtml),which is part of the blastdistribution.
* **MSPcrunch output.** [MSPcrunch](http://sonnhammer.sbc.su.se/download/software/MSPcrunch+Blixem/)isprogram for UNIX and GNU/Linux systems which can post-process [BLAST](http://www.ncbi.nlm.nih.gov/BLAST/) version 1 output into an easier to read format. MSPcrunch much be run with the -d flag.

Anthony Underwood and Jonathan Green at the [Health Protection Agency](http://www.hpa.org.uk/) have developed an online tool, [Double ACT](http://www.hpa-bioinfotools.org.uk/pise/double_act.html), to generate ACT comparison files.

Also there is [WebACT](http://www.webact.org/) which was designed and built by James Abbott and David Aanensen at Imperial College, London and is hosted in the laboratory of Professor Brian Spratt at Imperial College London with funding from The Wellcome Trust.

**big\_blast.pl**

At the Sanger Centre we generally use a perl script called [big\_blast.pl](ftp://ftp.sanger.ac.uk/pub/pathogens/software/artemis/extra/big_blast.pl) to run blast and generate a file of results in MSPcrunch format. To run this script you will need to install a copy of the Pathogen Sequencing Unit [internal perl modules](ftp://ftp.sanger.ac.uk/pub/pathogens/software/artemis/extra/zoo_modules.tar.gz) . [big\_blast.pl](ftp://ftp.sanger.ac.uk/pub/pathogens/software/artemis/extra/big_blast.pl) can also be used to process blast results after the fact with a command line like this:

**big\_blast.pl blast\_results.out**

Note that big\_blast.pl has only been tested on UNIX and GNU/Linux machines.

**The MSPcrunch Comparison File Format**

Here is an example input file generated by MSPcrunch -d.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1399 97.00 | 940 2539 | sequence1.dna 1 | | 1596 | AF140550.seq | |
| 1033 93.00 | 9041 10501 sequence1.dna | | | 9420 | 10880 AF140550.seq | |
| 828 95.00 | 6823 7890 | sequence1.dna | 7211 8276 | | | AF140550.seq |
| 773 94.00 | 2837 3841 | sequence1.dna | 2338 3342 | | | AF140550.seq |

The columns have the following meanings (in order): score, percent identity, match start in the query sequence, match end in the query sequence, query sequence name, subject sequence start, subject sequence end, subject sequence name.

The columns are separated by spaces.

**Running ACT on UNIX and GNU/Linux Systems**

On Unix and GNU/Linux the easiest way to run the program is to run the script called **act** in the ACT installation directory (see [the section called *Getting and Installing ACT* in Chapter 1](#page5) ) like this:

**artemis/act**

If all goes well you will be presented with a small window with one menu (the File menu). See [the](#page18) [section called *The ACT Launch Window*](#page18) for a description of this window.

Alternatively you start ACT with the names of two sequence/EMBL/GENBANK files and a file containing the comparison data eg:

**artemis/act sequence1 blast\_output.crunch sequence2**

If you have more than two sequences (there is no upper limit) they should be listed on the command line separated by the corresponding comparison file:

**artemis/act sequence1 seq2\_v\_seq1.crunch sequence2 seq3\_v\_seq2.crunch sequence3**

See [the section called *UNIX Command Line Arguments for ACT*](#page15) for a list of the other possible command line arguments. Also to see a summary of the options type:

**artemis/act -help**

**UNIX Command Line Arguments for ACT**

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

**-options**

This option instructs ACT to read an extra file of options after reading the standard options. (See [the](#page70) [section called *The Options File* in Chapter 5](#page70) for more about the ACT options file.)

For example -options ./new\_options will instruct ACT 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.

**-Dblack\_belt\_mode=false**

If this is set to false then warning messages are kept to a minimum.

**-Doffset=10000**

This sets the base position that ACT opens at, e.g. 10000.

**-Duserplot=pathToFile**

This can be used to open userplots from the command line. The paths are comma separated to open multiple plots (-Duserplot='/pathToFile/userPlot1,/pathToFile/userPlot2').

In ACT numbers are used to associate the file with a particular sequence. For example to add a userplot to the second sequence:

**act -Duserplot2=/pathToFile/userPlot**

**-Dloguserplot=pathToFile**

This is the same as the above userplot option except that the log transform of the data is plotted.

**-Dbam1=pathToFile1 -Dbam2=pathToFile2**

This can be used to open BAM/CRAM files and/or VCF/BCF files (see [the section called *Read BAM / CRAM / VCF ...* in](#page24) [Chapter 3](#page24) for more about the using these file types). This can take a path name to a file or an HTTP address. Numbers are used to associate the file with a particular sequence. For example to add a BAM to the first (top) sequence in ACT:

**act -Dbam1=/pathToFile/file.bam**

**-Dshow\_snps**

Show SNP marks in BAM/CRAM panels.

**-Dshow\_snp\_plot**

Automatically open SNP plots for BAM/CRAM files that are opened.

**-Dshow\_cov\_plot**

Automatically open coverage plots for BAM/CRAM files that are opened.

**-Dchado="hostname:port/database?username"**

This is used to get ACT to look for the database. The address of the database (hostname, port and name) can be conveniently included (e.g. -Dchado="genedb-db.sanger.ac.uk:5432/snapshot?genedb\_ro") and these details are then the default database address in the popup login window.

**-Dread\_only**

For a read only chado connection -Dread\_only is specified on the command line (e.g. art -Dchado="genedb-db.sanger.ac.uk:5432/snapshot?genedb\_ro" -Dread\_only).

**-Dshow\_forward\_lines=false**

Hide/show forward frame lines.

**-Dshow\_reverse\_lines=false**

Hide/show reverse frame lines.

**Running ACT on Macintosh Systems**

On Macintosh machines ACT can be started by double clicking on the ACT 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.

**Running ACT on Windows Systems**

On systems with Java 1.8 (or higher) installed ACT can be started by double clicking on the act.jar icon.

**The ACT Launch Window**

This is the first window that opens when you start ACT.

**The File Menu**

**Open ...**

If you select this menu item a file requester will be displayed which allows you to open the sequence files and the comparison files. If the files you select are read successfully, a new window will open, which shows the sequences and the comparison. See [Chapter 3](#page21) to find out how to use the main window. The comparison file is described in [the section called *The MSPcrunch Comparison File Format*](#page14) .

The "more files ..." button expands the window so that more sequence and comparison files can be read.

**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](#page77) [section called *Options File Format* in Chapter 5](#page77) for more information about options.

**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 [the section called *"Direct Editing"* in Chapter 3](#page57) for more detail about this.

**Genetic Code Tables**

These options make all the [NCBI Genetic Codes](http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=cgencodes) available. The default setting is the Standard Code. This setting effects the display of start codons (see [the section called *Start Codons* in Chapter 3](#page56) ) and the "Suspicious Start Codons ..." feature filter (see [the section called *Suspicious Start Codons ...* in Chapter](#page30) [3](#page30)).

**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 ACT, i.e. art -Dblack\_belt\_mode=yes.

**Highlight Active Entry**

When this option is on and the "One Line Per Entry" is on (see [the section called *One Line Per Entry* in](#page55) [Chapter 3](#page55)) the line that the active entry is on will be highlighted in yellow.

**Show Log Window**

Show the log of informational messages from ACT. 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 ACT on UNIX or GNU/Linux systems. The logging is controlled by [log4j](http://logging.apache.org/log4j/1.2/index.html). The 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**

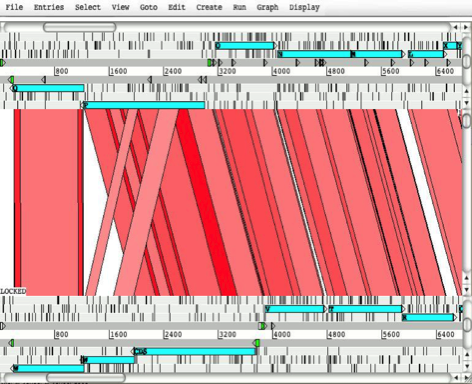
Hide the log of informational messages. This menu item is only available when running ACT on UNIX or GNU/Linux systems.

**Chapter 3. The ACT Main Window**

This chapter describes the main viewing window of ACT. First is an overview of the window and a description of the menus. The menus are also accessible from the pop-up menu in the view windows (see [the section called *Other Mouse Controlled Functions*](#page54) and [the section called *The Pop-up Menu*](#page54) for more).

**Overview of the Comparison Window**

The following images show a breakdown of the main ACT window taken from a screen shot of a two sequence comparison.



Key:

1. The menus for the main window (described later in this chapter). Each menu contains a sub-menu to control each of the sequences in the comparison. The Display menu (see [the section called *The*](#page53) [*Display Menu*](#page53))is the exception since it controls the overall display. Note that all the menus apartfrom the File menu and the Display menus can also be accessed via a pop-up menu on the third mouse button. See [the section called *Other Mouse Controlled Functions*](#page54) and [the section called](#page54) [*The Pop-up Menu*](#page54) formore.
2. This a view of the subject sequence of the comparison. See [the section called The DNA Views](#page53) for more information on how to operate this part of the window.
3. This part of the window shows the comparison between the two sequences. See [the section called](#page57) [Comparison View](#page57) for more information.
4. The query sequence of the comparison. See [the section called *The DNA Views*](#page53) for more.

**The File Menu**

**Read An Entry ...**

Read an entry for this sequence, but keep it separate from the others. See [the section called *The "Entry"* in](#page7) [Chapter 1](#page7) to see what we mean when we say "entry". The new entry will be marked as active (see [the](#page8) [section called *The Active Entries* in Chapter 1](#page8) ) and will be the new default entry (see [the section called](#page8) [*The Default Entry* in Chapter 1](#page8)).

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

ACT can read these feature files formats:

* [EMBL or GenBank feature tables](http://www.ebi.ac.uk/embl/Documentation/FT_definitions/feature_table.html)
* [GFF files](http://www.sequenceontology.org/resources/gff3.html)
* The output of [**MSPcrunch**](http://www.cgr.ki.se/cgr/groups/sonnhammer/MSPcrunch.html). **MSPcrunch** must be run with the **-x** or **-d** flags.
* The output of [blastall](http://www.ncbi.nlm.nih.gov/BLAST/) version 2.2.2 or better. **blastall** must be run with the **-m 8** flag which generates one line of information per HSP. Note that currently ACT displays each Blast HSP as a separate feature rather than displaying each BLAST hit as a feature.

**Save Entry**

Save the 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, ACT will prompt the user for a new name.

**Save All**

Save all the entries that have been loaded on top of this sequence.

**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 [Kazusa codon usage database site](http://www.kazusa.or.jp/codon/). In the output file each codon is followed by it's occurrence count (per thousand) and it's percentage occurrence. (See [the section called *Add Usage*](#page48) [*Plots ...*](#page48) tofind out how to plot a usage graph).

**Read BAM / CRAM / VCF ...**

ACT can read in and visualise BAM, CRAM, VCF and BCF files. These files need to be indexed as described below. Some examples can be found [here](https://www.sanger.ac.uk/science/projects/artemis-visualising-analysing-and-browsing-next-generation-sequence-data).

[BAM](http://samtools.github.io/hts-specs/SAMv1.pdf) files need to be sorted and indexed using [SAMtools](http://www.htslib.org/). The index file should be in the same directory as the BAM file. This provides an integrated [BamView](http://sanger-pathogens.github.io/Artemis/BamView/) panel in ACT, displaying sequence alignment mappings to a reference sequence. Multiple BAM files can be loaded in from here either by selecting each file individually or by selecting a file of path names to the BAM files. The BAM files can be read from a local file system or remotely from an HTTP server.

BamView will look to match the length of the sequence in ACT with the reference sequence lengths in the BAM file header. It will display a warning when it opens if it finds a matching reference sequence (from these lengths) and changes to displaying the reads for that. The reference sequence for the mapped reads can be changed manually in the drop down list in the toolbar at the top of the BamView.

In the case when the reference sequences are concatenated together into one (e.g. in a multiple FASTA sequence) and the sequence length matches the sum of sequence lengths given in the header of the BAM, ACT will try to match the names (e.g. locus \_tag or label) of the features (e.g. contig or chromosome) against the reference sequence names in the BAM. It will then adjust the read positions accordingly using the start position of the feature.

When open the BamView can be configured via the popup menu which is activated by clicking on the BamView panel. The 'View' menu allows the reads to be displayed in a number of views: stack, strand-stack, paired-stack, inferred size and coverage.

In ACT the BamView display can be used to calculate the number of reads mapped to the regions covered by selected features. In addition the reads per kilobase per million mapped reads (RPKM) values for selected features can be calculated on the fly. Note this calculation can take a while to complete.

[CRAM](https://github.com/samtools/hts-specs/blob/master/CRAMv3.pdf) files can be loaded in a similar fashion to BAMs. They can be created, sorted and indexed using [SAMtools](http://www.htslib.org/).

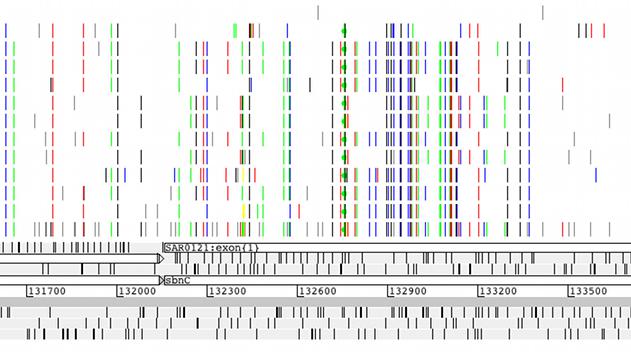
Variant Call Format ([VCF](http://1000genomes.org/wiki/doku.php?id=1000_genomes:analysis:vcf4.0)) files can also be read. The VCF files need to be compressed and indexed using bgzip and tabix respectively (see the [tabix manual](http://samtools.sourceforge.net/tabix.shtml) and [download page](http://sourceforge.net/projects/samtools/files/) ). The compressed file gets read in (e.g. file.vcf.gz) and below are the commands for generating this from a VCF file:

bgzip file.vcf

tabix -p vcf file.vcf.gz

Alternatively a Binary VCF ([BCF](http://samtools.sourceforge.net/mpileup.shtml)) can be indexed with BCFtools and read into Artemis or ACT.

As with reading in multiple BAM/CRAM files, it is possible to read a number of (compressed and indexed) VCF files by listing their full paths in a single file. They then get displayed in separate rows in the VCF panel.



For single base changes the colour represents the base it is being changed to, i.e. T black, G blue, A green, C red. There are options available to filter the display by the different types of variants. Right clicking on the VCF panel will display a pop-up menu in which there is a 'Filter...' menu. This opens a window with check boxes for a number of variant types and properties that can be used to filter on. This can be used to show and hide synonymous, non-synonymous, deletion (grey), insertion (magenta), and multiple allele (orange line with a circle at the top) variants. In this window there is a check box to hide the variants that do not overlap CDS features. There is an option to mark variants that introduce stop codons (into the CDS features) with a circle in the middle of the line that represents the variant. There are also options to filter the variants by various properties such as their quality score (QUAL) or their depth across the samples (DP).

Placing the mouse over a vertical line shows an overview of the variation as a tooltip. Also right clicking over a line then gives an extra option in the pop-up menu to show the details for that variation in a separate window. There are also alternative colouring schemes. It is possible to colour the variants by whether they are synonymous or non-synonymous or by their quality score (the lower the quality the more faded the variant appears).

There is an option to provide an overview of the variant types (e.g. synonymous, non-synonymous, insertion, deletion) for selected features. Also, filtered data can be exported in VCF format, or the reconstructed DNA sequences of variants can be exported in FASTA format for selected features or regions for further analyses. These sequences can be used as input for multiple sequence alignment tools.

**Edit In Artemis ...**

Open an Artemis edit window for this sequence and features. All changes made in the Artemis window will be immediately visible in the ACT window and vice versa.

See [the Artemis web pages](http://sanger-pathogens.github.io/Artemis/Artemis/) for more about Artemis.

**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](#page7) ). At the bottom of the menu there is a toggle button for each entry which controls whether the entry is active or not.

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 [the section called *The*](#page8) [*Default Entry* in Chapter 1](#page8)).

**Remove An Entry**

Remove an entry from ACT 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](#page8) ).

**Deactivate All Entries**

Choosing this menu item will deactivate all entries. (See [the section called *The Active Entries* in Chapter](#page8) [1](#page8).)

**The Select Menu**

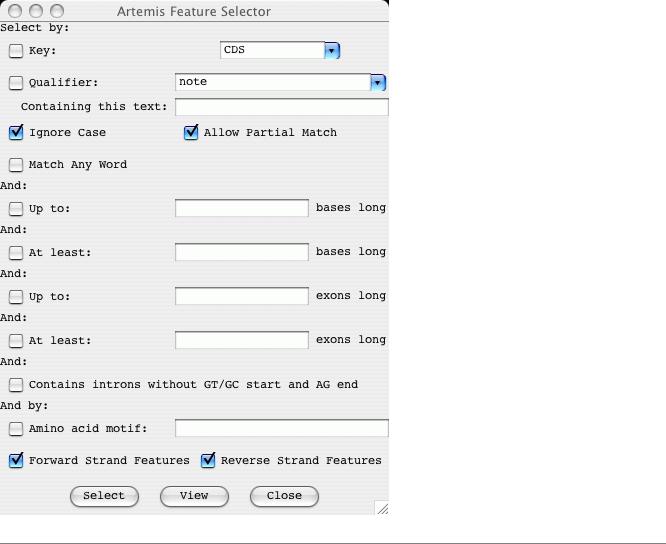
The items in this menu are used to modify the current selection (see [the section called *The Selection* in](#page8) [Chapter 1](#page8)). Artemis.

**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 [the section called *EMBL/Genbank Feature Keys* in Chapter 1](#page7) ), qualifier values (see [the section called *EMBL/Genbank Feature Qualifiers* in Chapter 1](#page8) ) 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 [the section called *The Feature List*](#page58) ) 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 highlight 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 [the section called *Changing the Selection from a View Window*](#page54) 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.

**Features Within Selection**

Select those (and only those) features that are fully contained by 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 [the section called *Correlation*](#page51) [*Scores*](#page51)),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.

**CDS Genes And Products**

Pop up a feature list (see [the section called *The Feature List*](#page58) ) 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](#page58) [section called *The Feature List*](#page58) ). 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*](#page31) ) 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](#page19) [Chapter 2](#page19) for more).

**Suspicious Stop Codons ...**

Show those CDS features that have a suspicious stop codon. i.e. 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 – i.e. 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](#page8) ).
* 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 ACT can be found in [the section called *Graphs and*](#page60) [*Plots*](#page60).Configuration options for graphs are described in[the section called *Options For Plots and Graphs*](#page76)[in Chapter 5](#page76).

**Adjust panel heights ...**

Option in ACT for adjusting the panel heights (BAM, CRAM, VCF, plots, comparisons) by giving them different weights in order to distribute the space between each component.

**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 thisfunction, 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 intothe box to the right of the "Goto Feature With This Gene Name:" label and then press the goto button. ACT will select the first feature with the given text in any of its 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. ACT will select the first feature with the given string in any of its qualifier values (see [the section called *EMBL/Genbank Feature Qualifiers* in Chapter 1](#page8) ) 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 tothe right of the "Goto Feature With This Key:" label and then press the goto button. ACT 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. ACT 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](#page45) 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 an amino acid pattern into the box to the right of the "Goto Amino Acid String:" label and then press the goto button. ACT 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](#page34) and [3](#page34). If on ACT 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 ACT 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](#page8) [section called *Feature Segments* in Chapter 1](#page8) ) is selected then the first base of the first selected segment 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.

**Undo**

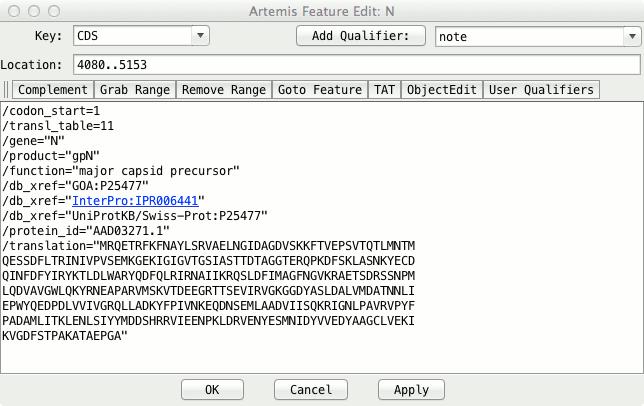
This function will undo the last change that was made using the Edit or Create menus. Up to 20 changes can be undone. This menu item is only enabled when there is something to undo. This limit can be changed in the options file (see [the section called *undo\_levels* in Chapter 5](#page74) ). [shortcut 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](#page74) ).

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.

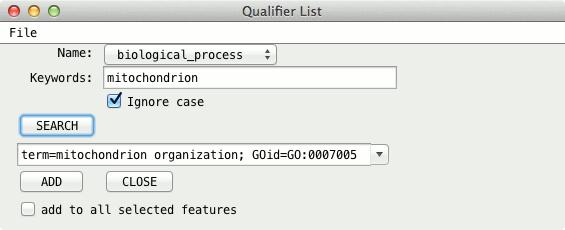
1. Just below the key and qualifier selector is the location entry field. ACT understands most of the EMBL location syntax, including joins, complements, ranges with non-exact ends (eg.

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

1. 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*](#page35) for more).
   * The User Qualifiers button opens a tool for maintaining user defined lists of qualifiers (i.e. qualifiers in the form tag = value pairs on separate lines) and the option to read qualifiers from [OBO](http://www.geneontology.org/GO.format.shtml) formatted files or URLs. In the intial screen (see below) you are invited to import your qualifier list from the import options in the "File" menu. These lists can be optionally saved between sessions in the file '.artemis.qualifiers' in the home directory.



When a qualifier list or OBO file has been added then it is possible to search for keywords within a list. The qualifier selected in the drop down list (under the SEARCH button) can then be added to the current feature annotation or added to selected features in ACT. If the keywords text field is left blank then all qualifiers are available from the drop down list of qualifier values.



1. The centre of the edit window contains the qualifier entry section. The qualifiers should be entered in the same way they appear in the feature table part of an EMBL entry, but without the leading FT and spaces.
2. 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. x 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 [the section](#page36) [called *Selected Features in Editor*](#page36) ).

**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 contains 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 its 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 multi-segment 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 multiple 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 [the section called *EMBL/Genbank Features*](#page7) [in Chapter 1](#page7)) or entries (see [the section called *The "Entry"* in Chapter 1](#page7) ).

**New Feature**

Create a new feature in the default entry with a key of "misc\_feature" (see [the section called](#page7) [*EMBL/Genbank Feature Keys* in Chapter 1](#page7)), a location of that spans the whole sequence and which hasno qualifiers (see [the section called *EMBL/Genbank Feature Qualifiers* in Chapter 1](#page8) ).

**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 [the](#page8) [section called *The Default Entry* in Chapter 1](#page8) ).

**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](#page74) [section called *minimum\_orf\_size* in Chapter 5](#page74) ). If a codon usage file (see [the section called *Add*](#page48) [*Usage Plots ...*](#page48))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](#page54) [*The Pop-up Menu*](#page54)).

**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, aanntt 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 [NCBI web BLAST](http://blast.ncbi.nlm.nih.gov/Blast.cgi), [Pfam](http://pfam.sanger.ac.uk/) and [Rfam](http://rfam.sanger.ac.uk/).

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*](#page30) [*Results*](#page30)).

**Configuring the Run Menu**

To use this feature the run\_blastp, run\_fasta etc. scripts that are supplied with ACT 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](#page75)

[*feature\_dna\_programs* in Chapter 5](#page75)and[the section called *feature\_protein\_programs* in](#page75)[Chapter 5](#page75)) 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. ACT 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 /blastp\_file. If the entry is called features.tab then the qualifier will be set to something like: /blastp\_file="blastp/features.tab.seq.00001.out". 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. ACT then tries to read the run\_blastp script from the ACT installation directory. The script is executed like this:

run\_blastp blastp/file\_of\_filenames.1 [options]

where [options] 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 run\_blastp 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 ACT 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 [the section called *Graphs and Plots*](#page60) . Configuration options for graphs are described in [the section called *Options For Plots and Graphs* in](#page76) [Chapter 5](#page76).

**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](http://www.kazusa.or.jp/codon/). If ACT 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 [Gribskov et al. (Nucl. Acids Res. 12;](http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=6694906&dopt=Abstract) [539-549 (1984))](http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=6694906&dopt=Abstract).

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( 7615)

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 ...**

ACT is able to display some types of user data in a graph that looks like the GC content graph (see [the](#page50) [section called *GC Content (%)*](#page50) ). 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. ACT will plot each data point for the corresponding base. Each column represents a data set for a line. Example extract:

21.4 1910.7

1. 1126.1

1911.7 0

0 0

0 1782.0

1937.5 65.4

...

1. 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 |  | 0 |  | 0 | 251.18 |  |
| 553 | 0 | 0 |  | 0 |  | 0 |  | 0 | 52.4 |  |
| 654 | 0 | 0 |  | 0 |  | 0 |  | 334.2 | 0 |  |
| 686 | 0 | 0 |  | 652.78 | | 0 |  | 0 | 0 |  |
| 831 | 0 | 0 |  | 0 |  | 0 |  | 0 | 67.97 |  |
| ... |  |  |  |  |  |  |  |  |  |  |

1. indexed tab delimited file. For this tabix is used (see the [tabix manual](http://samtools.sourceforge.net/tabix.shtml)) to create an index. This is especially useful for large data sets as it is memory efficient and only reads the data corresponding to the visible region in ACT. The first columns contain the sequence name and base position and this is then followed by the values to be plotted.

For example 'file.plot' is a tab delimited file with column 1 containing the sequence name and column 2 the positions, this is sorted and then indexed with tabix:

(grep ^"#" file.plot; grep -v ^"#" file.plot | sort -k1,1 -k2,2n) | bgzip > sorted.plot.gz ;

tabix -s 1 -b 2 -e 2 sorted.plot.gz

Example extract:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| foo | 1 | 5 | 5 | 129 | 5 | 5 | 239 |
| foo | 2 | 1 | 10 | 124 | 12 | 10 | 234 |
| foo | 3 | 5 | 16 | 129 | 12 | 15 | 229 |
| foo | 4 | 0 | 23 | 124 | 20 | 20 | 414 |
| foo | 5 | 5 | 22 | 121 | 28 | 25 | 419 |
| foo | 6 | 30 | 36 | 124 | 32 | 30 | 412 |
| ... |  |  |  |  |  |  |  |

1. the next two formats are types of [Wiggle formats](https://cgwb.nci.nih.gov/goldenPath/help/wiggle.html). The first is variableStep. Note that ACT only supports the colour element in the track line.

track type=wiggle\_0 color=255,200,0

variableStep chrom=chr19 span=10

1. 1
2. 12
3. 18
4. 6
5. 5
6. 3
7. 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.

5. the next format supported by ACT 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

6. 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. ACT 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 [this web page](http://www.nih.go.jp/~jun/cgi-bin/frameplot.pl).

See [Ishikawa, J. and Hotta, K. FEMS Microbiol. Lett. 174:251-253 (1999)](http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?uid=10339816&form=6&db=m&Dopt=b) and [GC frame plot](http://www.sanger.ac.uk/Software/Artemis/gc_plot/GC_frame.html) 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](http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8676740&dopt=Abstract) [1996 May;13(5):660-5](http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8676740&dopt=Abstract).

[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 - [Current Opinion in Microbiology 1998, 1:598-610](http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10066522&dopt=Abstract).

[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) [Strand-specific compositional asymmetries in double-stranded DNA viruses.](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10225270) [Virus Research 60, 1-19](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10225270).

**Positional Asymmetry**

Shulman MJ, Steinberg CM, Westmoreland N (1981) [The coding function of nucleotide sequences can be](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6456380) [discerned by statistical analysis. J Theor Biol 88:409-20](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6456380).

**Informational Entropy**

Konopka Andrzej (1984) [Is the information content of DNA evolutionarily significant? J Theor Biol](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6738090) [107:697-704](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6738090). 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](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=3118331) [selection and mutational biases. Nucleic Acids Res 15:8023-40](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=3118331).

**Mutational Response Index**

Gatherer D, McEwan NR (1997) [Small regions of preferential codon usage and their effect on overall](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=9315288) [codon bias--the case of the plp gene. Biochem Mol Biol Int 43:107-14](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=9315288).

**Effective Codon Number**

Wright F (1990) [The 'effective number of codons' used in a gene. Gene 87:23-9](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=2110097), and Fuglsang A (2004) [The 'effective number of codons' revisited. Biochem Biophys Res Commun. May 7;317(3):957-64](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15081433).

**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](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8112587) [139:43-9](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8112587).

**The Display Menu**

The items in this menu control the global appearance of the display.

**Hide Frame Lines**

Selecting this menu item will turn off the frame lines of the query and subject views. The frame lines can be turned on selectively using the pop up menu in the view (see [the section called *The Pop-up Menu*](#page54) ).

**Show Frame Lines**

Selecting this menu item will turn on the frame lines of the query and subject views.

**The DNA Views**

The two sequence views in ACT show the forward and reverse strands of the sequence and a representation of the three translation frames in each direction. The top view shows the subject sequence of the comparison (see [2](#page21)), the bottom view shows the query sequence (see [4](#page21)). The horizontal scrollbar controls which part of the sequence is currently visible. The scroll bar at the left controls the zoom level. By default the view shows only the stop codons in each translation frame, but if the view is zoomed in it will show the complete six frame translation.

See also [the section called *Overview of the Comparison Window*](#page21) .

**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*](#page60) ) anywhere inside the ORF (see [the section](#page28) [called *Open Reading Frame*](#page28) for another way to select an ORF).

See [the section called *The Selection* in Chapter 1](#page8) for more about the selection.

**Other Mouse Controlled Functions**

Double clicking on a feature with the first mouse button causes the view to centre itself on that feature. Similarly, double clicking the first mouse button on a base or amino acid will centre the view 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 [the section called](#page36) [*Selected Features in Editor*](#page36)).

The third button activates a pop-up menu with functions specific to this view. See [the section called *The*](#page54) [*Pop-up Menu*](#page54) formore.

**The Pop-up Menu**

The pop-up menu is activated by pressing the third mouse button (see [the section called *Notes on Using*](#page60) [*The Mouse*](#page60))on a feature view. The menu is split into three sections. The top contains functions that willact 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 otherfeatures.
* **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 frontof 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 fullwidth.
* **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 activeentries 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](#page72) ).

**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](#page73) [*one\_line\_per\_entry* in Chapter 5](#page73)).

**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 [the section called *Genetic Code Tables* in Chapter 2](#page19) 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 *draw\_feature\_arrows* in Chapter 5](#page71) ).

**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 *draw\_feature\_borders* in Chapter 5](#page71) ).

**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](#page72) [Chapter 5](#page72)).

**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*](#page34) ), by double clicking the first mouse button on a feature in one of the sequence views 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.

**"Direct Editing"**

The direct editing option (See [the section called *Enable Direct Editing* in Chapter 2](#page19) ) 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.

**Comparison View**

This part of the window shows the comparison between two sequences. Each red/pink box corresponds to single match, with red representing a good match and white/pale pink a lower scoring match. This view cannot be scrolled directly, instead it tracks the movement of the subject and query sequence views (see [the section called *The DNA Views*](#page53) ).

See [3](#page21) to see where this view fits in the main window.

**Controls**

The vertical scrollbar at the right of the view sets the minimum size (in bases on the subject sequence) of the matches that are displayed. The default is 1, so when started ACT will show all matches.

Clicking the left button on a match will highlight the match. Double clicking the mouse on one of the matches will cause the sequence views to align around that match.

Clicking the middle mouse button toggles the locking of the two sequences that are being compared by this comparison view. When locked the sequences will scroll in parallel. When unlocked the sequences can be scrolled independently. (See [the section called *Notes on Using The Mouse*](#page60) if you do not have a middle mouse button)

**The Comparison View Pop-up Menu**

The pop-up menu is activated by pressing the third mouse button within the comparison view. The menu items are as follows:

* **View Selected Matches.** Creates a new window which contains a list of currently selected (ie.yellow) matches. Clicking on a match in the list will centre the comparison view on that match.
* **Flip Query Sequence.** Flips the query sequence display so that it appears to be reversecomplemented. (See [the section called *Flip Display*](#page56) .)
* **Flip Subject Sequence.** Flips the subject sequence display so that it appears to be reversecomplemented. (See [the section called *Flip Display*](#page56) .)
* **Lock Sequences.** Lock the two sequences so that they scroll together.
* **Unlock Sequences.** Allow the two sequences to scroll independently.
* **Set Score Cutoffs ...** Pop-up a gadget that allows the matches to be filtered on "score", generallythe BLAST score .
* **Set Percent ID Cutoffs ...** Pop-up a gadget that allows the matches to be filtered on thepercentage identity.
* **Offer To RevComp.** When this option is "on", double clicking on a reverse complemented matchwill cause ACT to ask the user whether to flip the query sequence before centering on the match. If "off" the match will just be centred.
* **Ignore Self Matches.** When this option is "on", matches that have the same start and end positionson both the subject and query sequences will not be shown.

**The Feature List**

The feature list is bit at the bottom of the main window of Artemis (see [the section called *Overview of the*](#page21) [*Comparison Window*](#page21)). 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*](#page30) and [the section called *Feature*](#page30) [*Filters*](#page30) ). The list contains information about each feature in each active entry (see[the section called *The*](#page8)[*Active Entries* in Chapter 1](#page8)), one per line.

The lines show this information about the feature: the colour (see [the section called *Feature Colours* in](#page9) [Chapter 1](#page9)), 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](#page8) 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 [the section called](#page36) [*Selected Features in Editor*](#page36)).

**The Pop-up Menu**

The pop-up menu is activated by pressing the third mouse button (see [the section called *Notes on Using*](#page60) [*The Mouse*](#page60) )anywhere on the feature list. The menu contains shortcuts to some of the main windowmenus 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 Graphs(top) and Graphs(bottom) menus (see [the section called *The*](#page53) [*Display Menu*](#page53)). When a graph is turned on it is locked to the overview window, so it will follow theoverview 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*](#page33) ) 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**

ACT 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 ACT 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 ACT Overview of the SSH Plugin**

This is a plug-in to ACT that enables you to view and transfer files from a local machine (e.g.

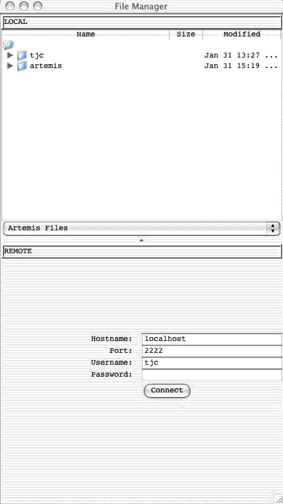
PC/MacOSX/UNIX) to another remote file system (i.e. any other file system that you have ssh access to).

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 ACT 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*](#page69) ).

**Login In**

From the ACT 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 [the section called *Setting up a SSH tunnel*](#page62) ) 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 ACT 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 ~/.ssh/config. This contains the information to set up a tunnel that looks like this:

Host \*.sanger.ac.uk

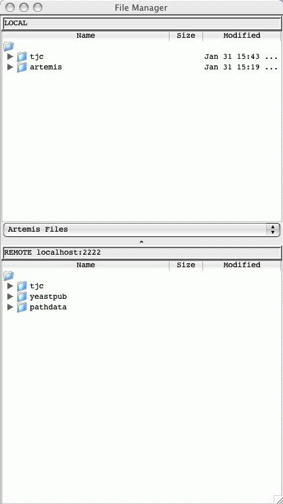
LocalForward 2222 machine.sanger.ac.uk:22

User tjc

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

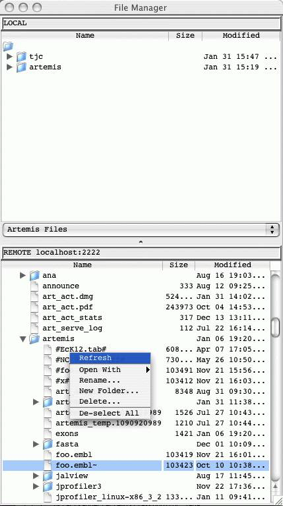
On *Windows* you can use Putty, see [the section called *Using Putty to Set Up A Tunnel*](#page65) .

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.



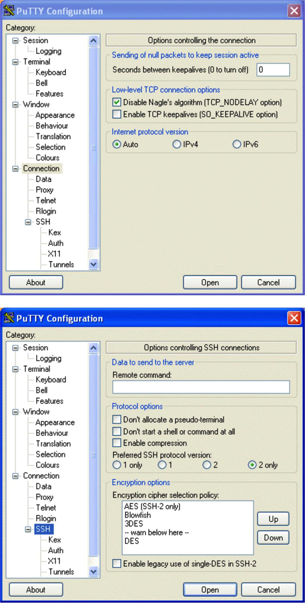
Files on the remote file system can be double clicked on and opened in Artemis (in the same way they can be opened from the local file manager). Tab files can be read in by dragging them from the remote file manager and dropping them into the Artemis window.

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.

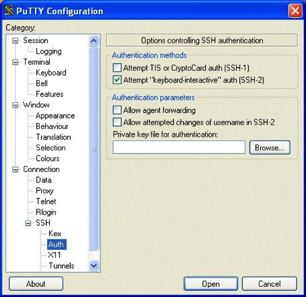
When files are saved, if the entry orginated from the remote file system then this is saved locally in the current working directory and to the remote file as well.

**Using Putty to Set Up A Tunnel**

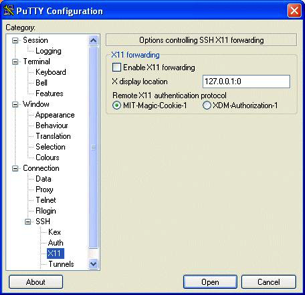
On *Windows* you can use [Putty](http://www.chiark.greenend.org.uk/~sgtatham/putty/) to set up a ssh tunnel. Below are settings that can be used in Putty to set up a SSH tunnel.



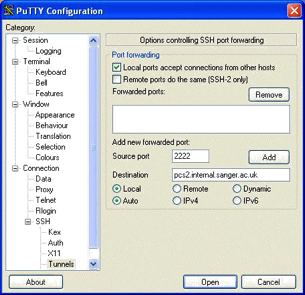
SSH 2 only is probably recommended here.



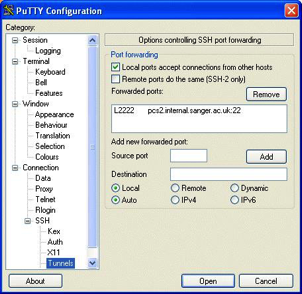
X11 forwarding is not necessary



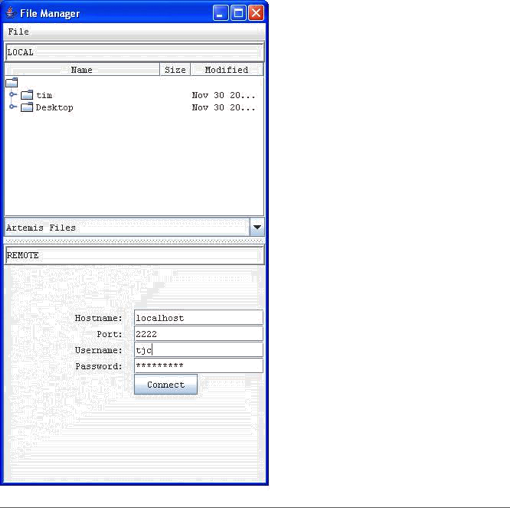
Set the source port to 2222 and set the destination to hostname:port (e.g. pcs2.internal.sanger.ac.uk:22).



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 ACT 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/pubseqgbs fastx=fastx33\_t -B -S -q -b 40 -H -l /data/fasta/pubseqgbs

* 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 localdir.YP=/nfs/disk222/yeastpub

**Chapter 5. ACT Configuration Options The Options File**

When started on ACT will potentially look in 5 different places for files to examine for configuration information. ACT 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 ACT application has been installed.

This is the search order:

* The standard ACT options are read first. On UNIX this file is in the file etc/options in the ACT 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 ACT 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 ACT* in](#page15) [Chapter 2](#page15) 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.

**ACT Option Descriptions**

This section describes the possible options for ACT.

**font\_size**

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

**font\_name**

This option is used to choose the font for all the ACT 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 [the section called](#page56) [*Feature Borders* in Chapter 3](#page56)). [default: "yes"]

**show\_forward\_lines**

If set to yes, the forward frame lines are shown by default. This can also be set in the views popup menu. [default: "yes"]

**show\_reverse\_lines**

If set to yes, the reverse frame lines are shown by default. This can also be set in the views popup menu. [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](#page56) [3](#page56)). [default: "yes"]

**overview\_feature\_labels**

If this option is no then the feature labels in the overview (see [the section called *Overview of the*](#page21) [*Comparison Window* in Chapter 3](#page21))will be off at startup. [default: "yes"]

**overview\_one\_line\_per\_entry**

If this option is set yes then the overview (see [the section called *Overview of the Comparison Window* in](#page21) [Chapter 3](#page21)) will start in one line per entry mode. [default: "no"]

**show\_list**

If set to yes then the feature list (see [the section called *The Feature List* in Chapter 3](#page58) ) will be shown on startup. [default: "yes"]

**show\_base\_view**

If set to yes then the DNA base view (see [the section called *The DNA Views* in Chapter 3](#page53) ) 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 [the section](#page56) [called *All Features On Frame Lines* in Chapter 3](#page56) ) [default: "no"]

**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 [the section called *All Features On*](#page56) [*Frame Lines* in Chapter 3](#page56))[default: "yes"]

**one\_line\_per\_entry**

If set to yes then the "One Line Per Entry" option will be set to yes on startup. (See [the section called *All*](#page56) [*Features On Frame Lines* in Chapter 3](#page56))[default: "no"]

**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:

1. TTC TTA TTG TCT TCC ...

...

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

translation\_table\_1 = \

f f l l \

s s s s \

y y \* \* \

c c \* w \

\

l l l l \

p p p p \

h h q q \

r r r r \

\

i i i m \

t t t t \

n n k k \

s s r r \

\

v v v v \

a a a a \

d d e e \

g g g g

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 ACT 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 ACT code directory.

**common\_keys**

This is a list of the keys that should be shown by default in the feature edit window. (see [the section called](#page36) [*Selected Features in Editor* in Chapter 3](#page36)).

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 [the](#page36) [section called *Selected Features in Editor* in Chapter 3](#page36) ).

**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 [the section](#page45) [called *Mark Open Reading Frames ...* in Chapter 3](#page45) ).

**direct\_edit**

Set the default value for the direct edit option. A value of "yes" will turn direct edit on by default. See [the](#page19) [section called *Enable Direct Editing* in Chapter 2](#page19)

**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 section called *Configuring*](#page46) [*the Run Menu* in Chapter 3](#page46)formore 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 section called *Configuring the Run Menu* in Chapter 3](#page46) 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 [the](#page46) [section called *The Run Menu* in Chapter 3](#page46) .

**ncbi\_protein\_search**

This lists the NCBI web-BLAST applications that the translation of a selected feature can be sent to. See [the section called *The Run Menu* in Chapter 3](#page46) .

**Setting Colours**

The feature colours (see [the section called *Feature Colours* in Chapter 1](#page9) ) used by ACT 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 0 255)

5 cyan (RGB values: 0 255 255)

6 magenta (RGB values: 255 0 255)

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: 200 150 100)

12 pale pink (RGB values: 255 200 200)

13 light grey (RGB values: 170 170 170)

14 black (RGB values: 0 0 0)

15 mid red: (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*](#page48) [*Menu* in Chapter 3](#page48)and[the section called *Feature Plots* in Chapter 3](#page33)).

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](#page60) .

**Options For A Chado Database**

**chado\_exon\_model**

This is the feature key used when chado exons are joined and displayed in ACT. 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