Artemis

Introduction

Artemis is a free DNA viewer and annotation tool written by Kim Rutherford (Rutherford *et al*., 2000). It is routinely used by the Parasite Genomics Group at the Wellcome Sanger Institute for annotation and analysis of both prokaryotic and eukaryotic genomes. The program allows the user to view simple sequence files, EMBL/Genbank entries and the results of sequence analyses in a highly interactive and intuitive graphical format. Artemis is designed to present multiple sets/types of information within a single context. This manifests itself as the ability to zoom in to inspect DNA sequence motifs and zoom out to view local gene architecture, several kilobases of a genome or even an entire genome in one screen. It is also possible to perform some analyses within Artemis with the output stored for later access.

Aims

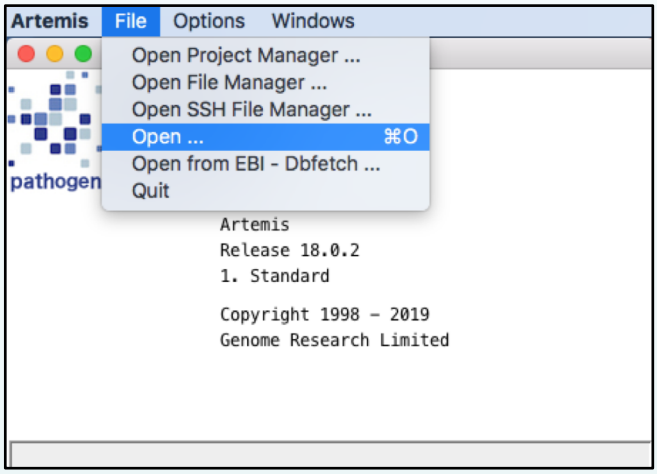
The aim of this Module is for you to become familiar with the basic functions of Artemis using a series of worked examples. These examples are designed to take you through the most immediately useful functions. However, there will be time, and encouragement, for you to explore other menus; nooks and crannies of Artemis that are not featured in the exercises in this manual. Like all the Modules in this workshop, the key is ‘if you don’t understand please ask’.

Artemis Exercise 1 Part I

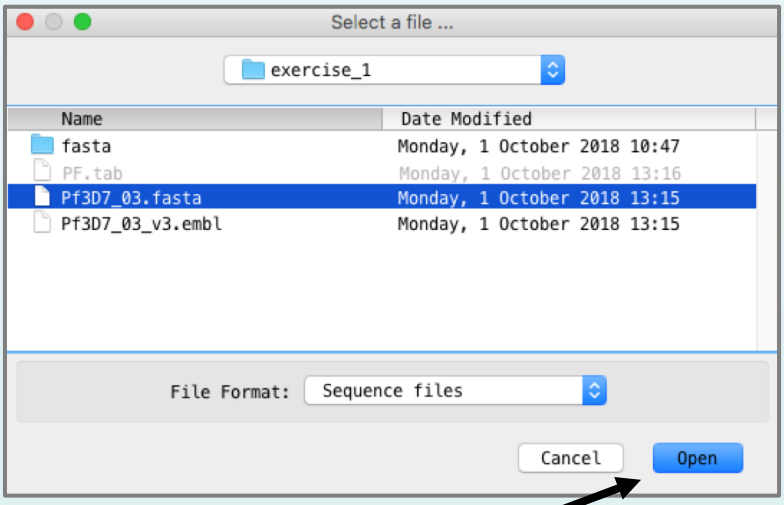
1. Starting up the Artemis software

Double click the ARTEMIS Icon on your Desktop
A small start-up window will appear (see below).
Navigate to the directory Module_1_Artemis, exercise_1 containing the file Pf3D7_03.fasta using the file manager.

1 2 Click 'File' then 'Open'



For simplicity it is a good idea to open a new start up window for each Artemis session and close down any sessions once you have finished an exercise.



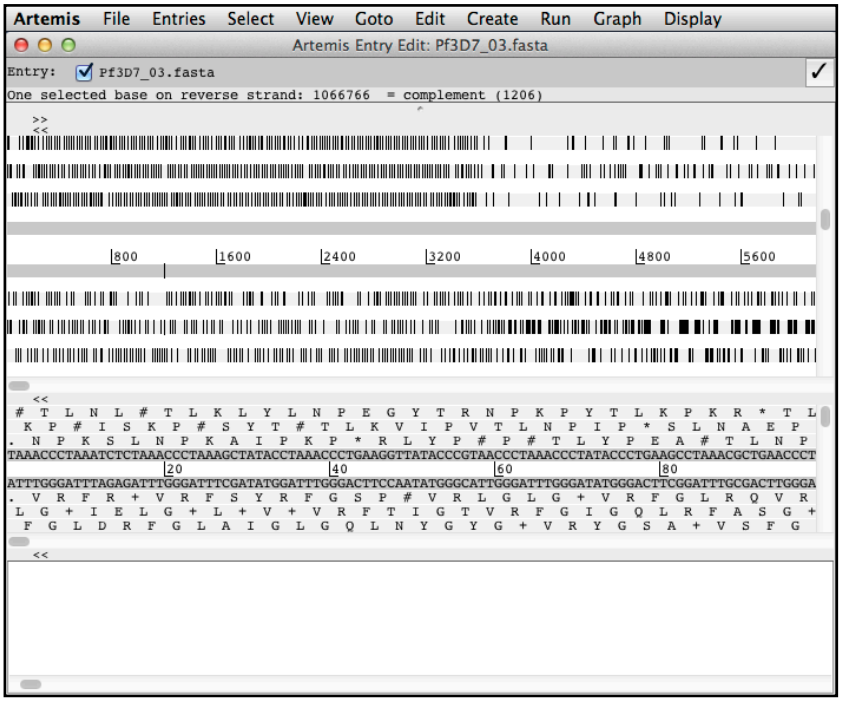
3 Single click to select the DNA file

4 Single click to open file in Artemis then wait

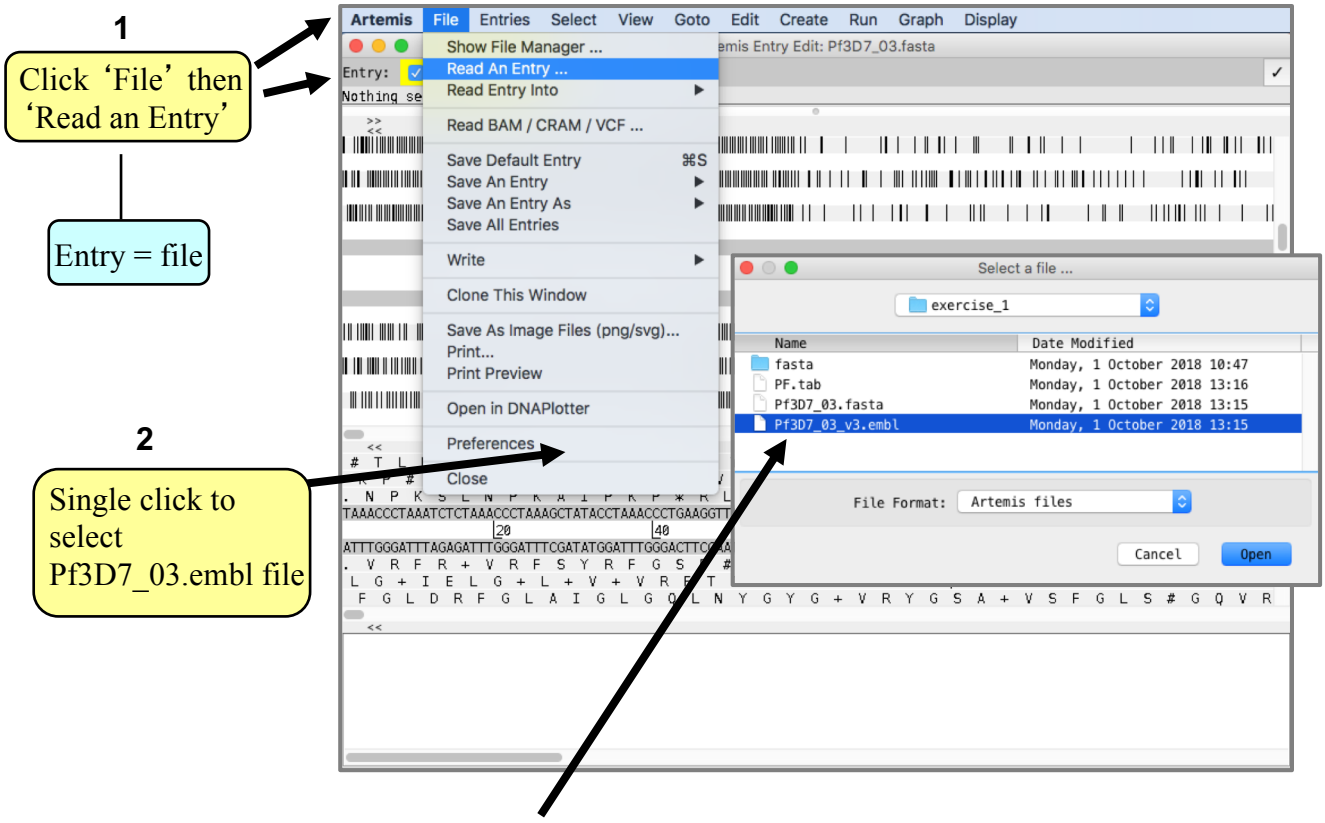
DNA sequence files will have the suffix '.fasta'. Annotation files end with '.embl', or '.tab'. Use this feature to select the type of file displayed in this panel.

2. Loading annotation files (entries) into Artemis

Hopefully you will now have an Artemis window like this! If not, ask a demonstrator for assistance.



Now follow the numbers to load up the annotation file for *Plasmodium falciparum* 3D7 chromosome 3.



3 Single click to open file in Artemis then wait

What's an "Entry"? It's a file of DNA and/or features which can be overlaid onto the sequence information displayed in the main Artemis view panel.

3. The basics of Artemis

Now you have an Artemis window open let's look at what's in there.

1. Drop-down menus. There's lots in there so don't worry about them right now.
2. Shows what entries are currently loaded (bottom line) and gives details regarding the feature selected in the window below; in this case an acyl-CoA synthetase (selected line).
3. This is the main sequence view panel. The central 2 grey lines represent the forward (top) and reverse (bottom) DNA strands. Above and below those are the 3 forward and 3 reverse reading frames. Stop codons are marked as black vertical bars. Genes and other features (eg. Pfam matches) are displayed as coloured boxes. We will refer to genes as coding sequences or CDSs from now on.
4. This panel has a similar layout to the main panel but is zoomed in to show nucleotides and amino acids. Double click on a gene in the main view to see the zoomed view of the start of that gene. Note that both this and the main panel can be scrolled left and right (7, below) zoomed in and out (6, below).
5. This panel lists the various features in the order that they occur on the DNA with the selected gene highlighted. The list can be scrolled (8, below).
6. Sliders for zooming view panels.
7. Sliders for scrolling along the DNA.
8. Slider for scrolling feature list.

4. Getting around in Artemis

The 3 main ways of getting to where you want to be in Artemis are the 'Goto' drop-down menu, the Navigator and the Feature Selector. The best method depends on what you're trying to do and knowing which one to use comes with practice.

4.1 The 'Goto' menu

The functions on this menu (ignore the Navigator for now) are shortcuts for getting to locations within a selected feature or for jumping to the start or end of the DNA sequence. Most are self-explanatory, so feel free to try any of them.

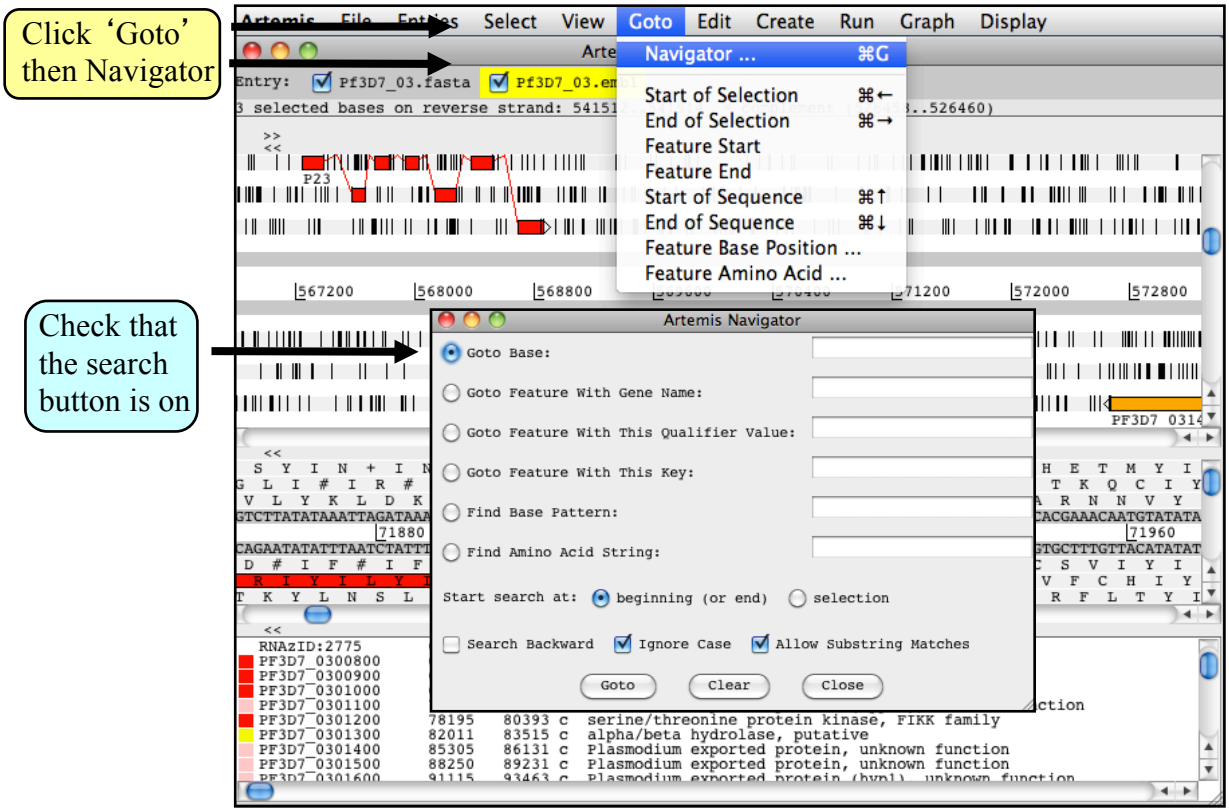
It may seem that 'Goto' 'Start of Selection' and 'Goto' 'Feature Start' do the same thing. Well they do if you have a feature selected but 'Goto' 'Start of Selection' will also work for a region which you have highlighted by click-dragging in the main window. So yes, give it a try! This is a very commonly used feature, so it is worth memorizing the keyboard short-cuts for these, ctrl<left arrow> and ctrl <right arrow> respectively.

Suggested tasks:

1. Zoom out, highlight a large region of sequence by clicking the left hand button and dragging the cursor, then go to the start and end of the highlighted region.
2. Select a gene then go to the start and end.
3. Go to the start and end of the genome sequence.
4. Select a gene. Within it, go to a base (nucleotide) and/or amino acid of your choice.

4.2 Navigator

The Navigator panel is fairly intuitive so open it up and give it a try.



Suggestions of where to go:

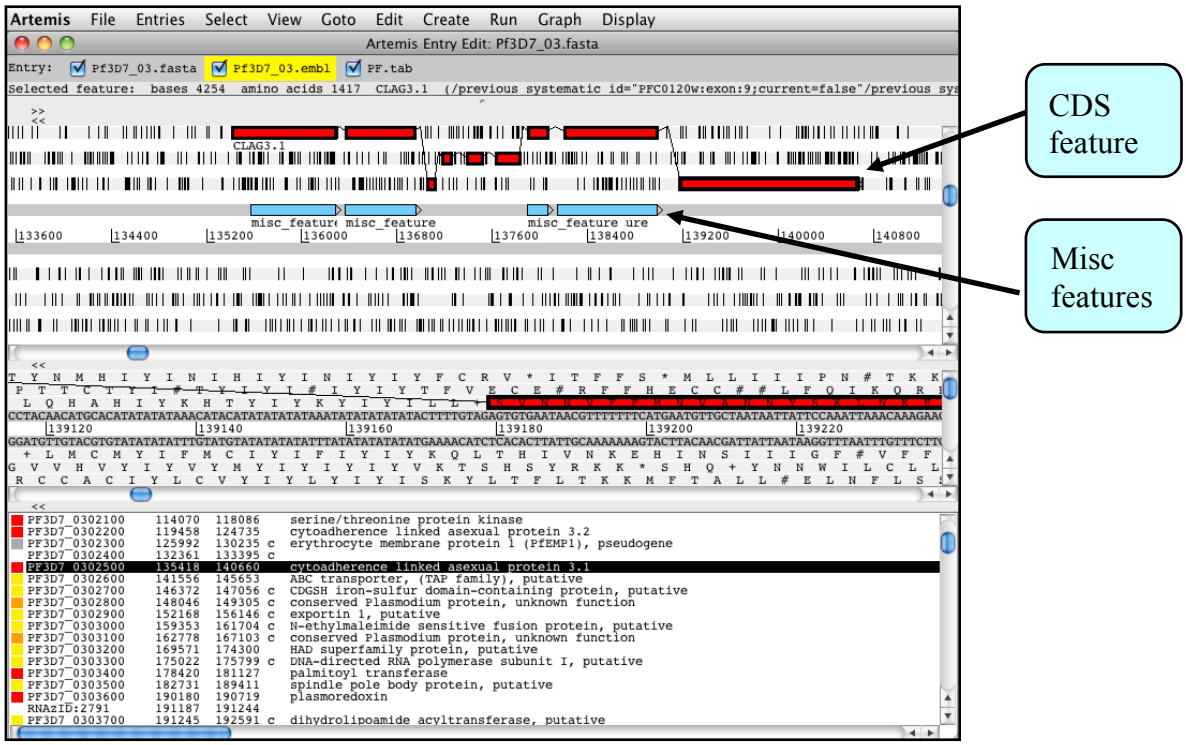
1. Think of a number between 1 and 1067971 and go to that base (notice how the cursors on the horizontal sliders move with you).
2. Your favourite gene name (it may not be there so you could try 'VAR').
3. Use 'Goto Feature With This Qualifier value' to search the contents of all qualifiers for a particular term. For example using the word 'pseudogene' will take you to the next feature with the word 'pseudogene' in any of its qualifiers. Note how repeated clicking of the 'Goto' button takes you through the pseudogenes as they occur on the chromosome.
4. tRNA genes. Type 'tRNA' in the 'Goto Feature With This Key'.
5. Amino acid consensus sequences (real or made up!). You can use 'X's. Note that it searches all six reading frames regardless of whether the amino acids are encoded or not.

What are Keys and Qualifiers? See **Appendix IV**

Clearly there are many more features in Artemis which we will not have time to explain in detail. Before getting on with this next section it might be worth browsing the menus. Hopefully you will find most of them easy to understand.

Artemis Exercise 1 Part II

This part of the exercise uses the files and data you already have loaded into Artemis from Part I. By a method of your choice go to the region located between bases 134000 to 141000 on the DNA sequence. This region encodes the *CLAG3.1* gene which codes for cytoadherence linked asexual protein. You can use either the Navigator, Feature Selector or Goto functions discussed previously to get there. The region you arrive at should look similar to that shown below.



Once you have found this region have a look at some of the information that is available to you:

Information to view:

Annotation

If you click on a particular feature you can view the annotation attached to it:
select a CDS feature (or any other feature) and click on the 'Edit' menu and select 'Selected Feature in Editor', or simply push 'E'. A window will appear containing all the annotation that is associated with that CDS.

Viewing amino acid or protein sequence

Click on the view menu and you will see various options for viewing the bases or amino acids of the feature you have selected, in two formats i.e. EMBL or FASTA. This can be very useful when using other programs that are not integrated into Artemis e.g. those available on the Web that require you to cut and paste sequence into them.

Plots/Graphs

Feature plots can be displayed by selecting a CDS feature then clicking 'View' and 'Feature Plots'. The window which appears shows plots predicting hydrophobicity, hydrophilicity and coiled-coil regions for the protein product of the selected CDS.

Load additional files

The results from the Pfam protein motif searches are not shown, but can be viewed by loading the appropriate file. Click on 'File' then 'Read an Entry' and select the file PF.tab. Each Pfam match will appear as a coloured blue feature in the main display panel on the grey DNA lines. To see the details click the feature then click 'View' then 'Selection' or click 'Edit' then 'Selected Features in Editor'. You can also run Pfam by going to the Run menu and selecting ' Pfam search '. For this you need to select one CDS.

Viewing the results of database searches

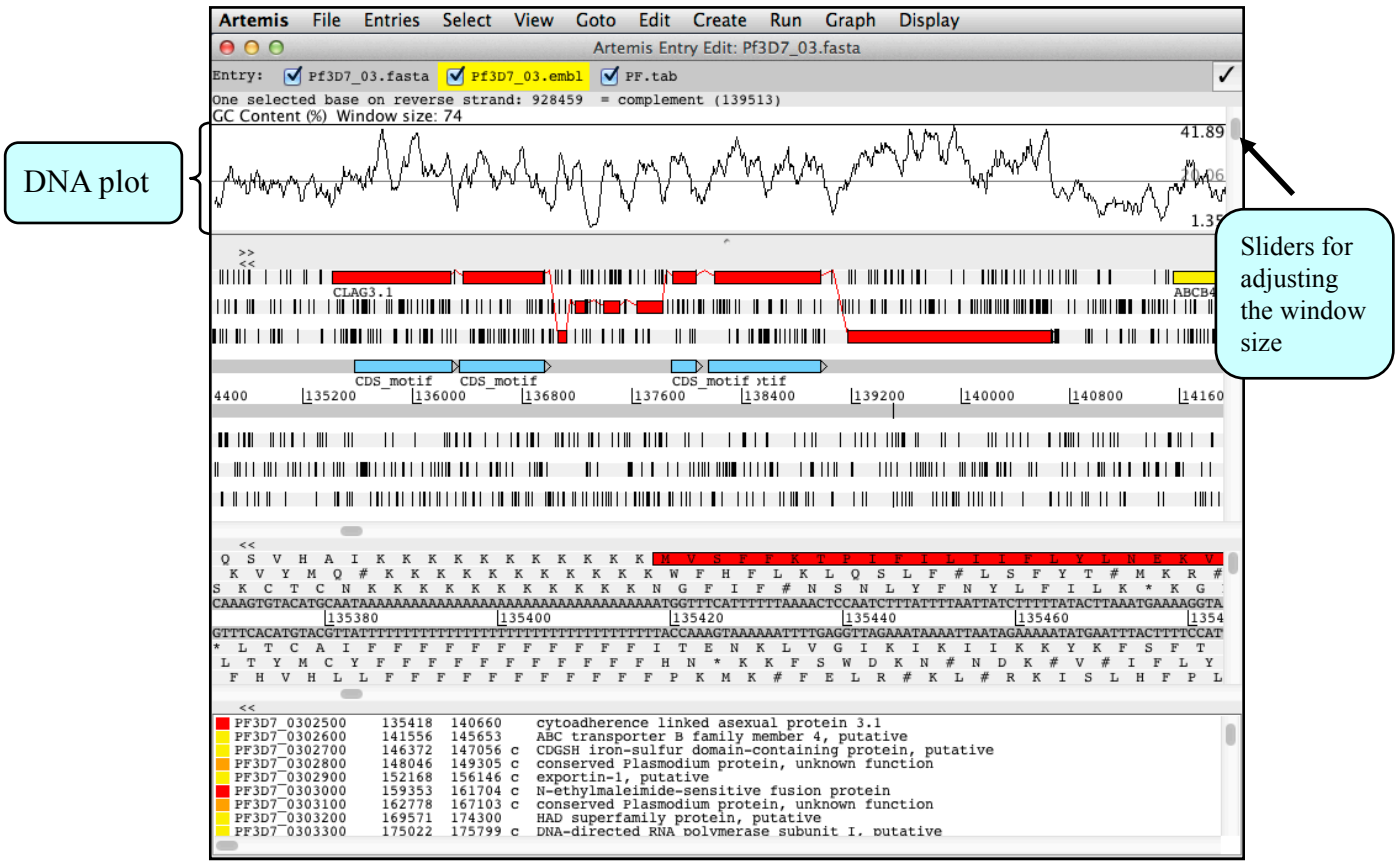
Click the 'View' menu, then select 'Search Results' and then 'Fasta results'. The results of the database search will appear in a scrollable window.

Further information on specific Pfam entries can be found on the web at
http://pfam.xfam.org/

In addition to looking at the fine details of the annotated features it is also possible to look at the characteristics of the DNA covering the region displayed. This can be done by adding to the display various plots showing different characteristics of the DNA.

To view the graphs:

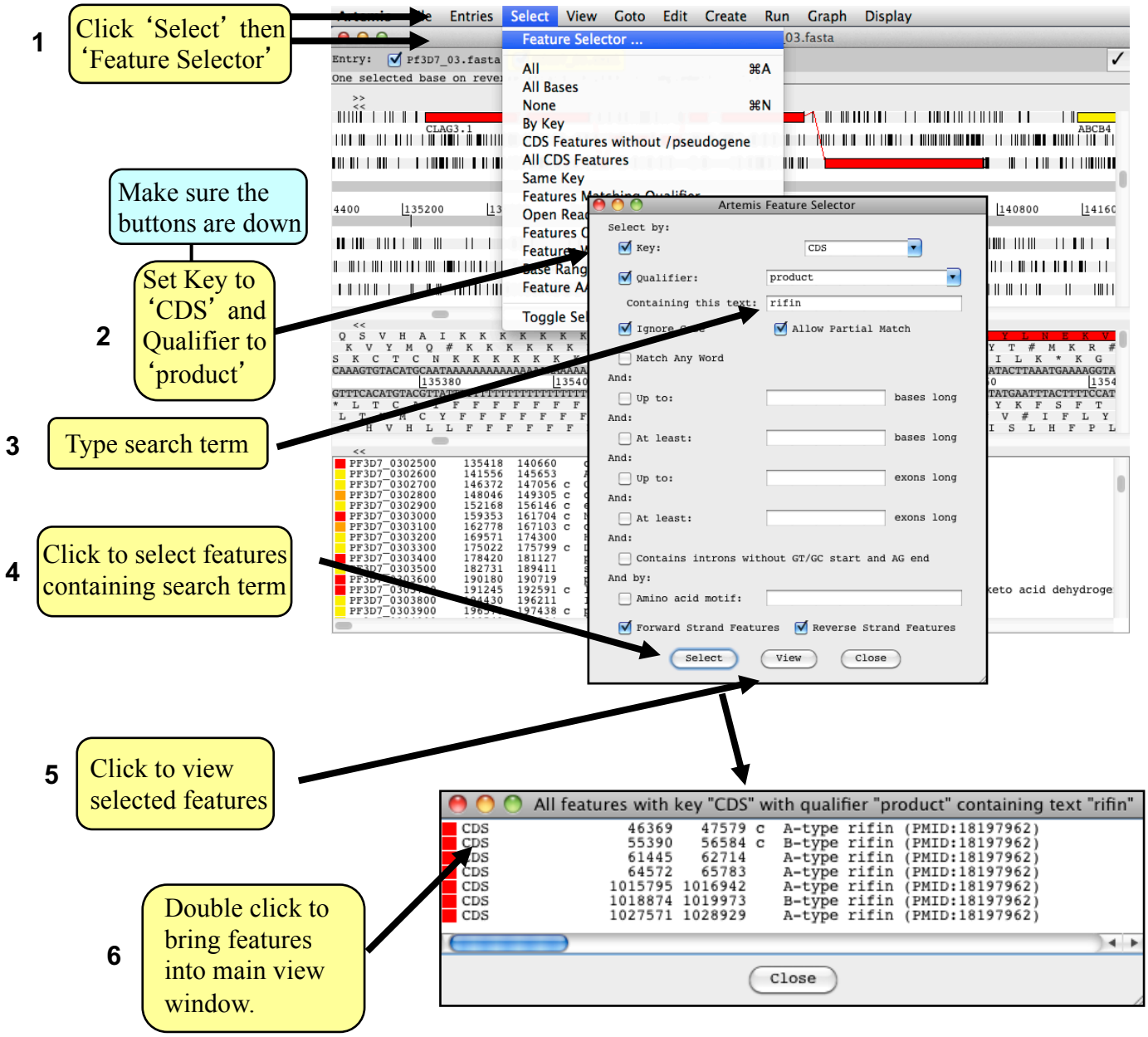
Click on the 'Graph' menu to see all those available. Some of the most useful plots for *P. falciparum* is the 'GC Content (%)' as shown below. G+C content is a very good indicator of coding capacity in Malaria. On average, the coding regions are ~23% G+C and the non-coding regions are ~19%. Have a look at the G+C content for this region by selecting the appropriate graph. Left click within the graph window and then select by clicking on the exons to see how this relates to the G+C peaks on the graph.



Artemis Exercise 1 Part III

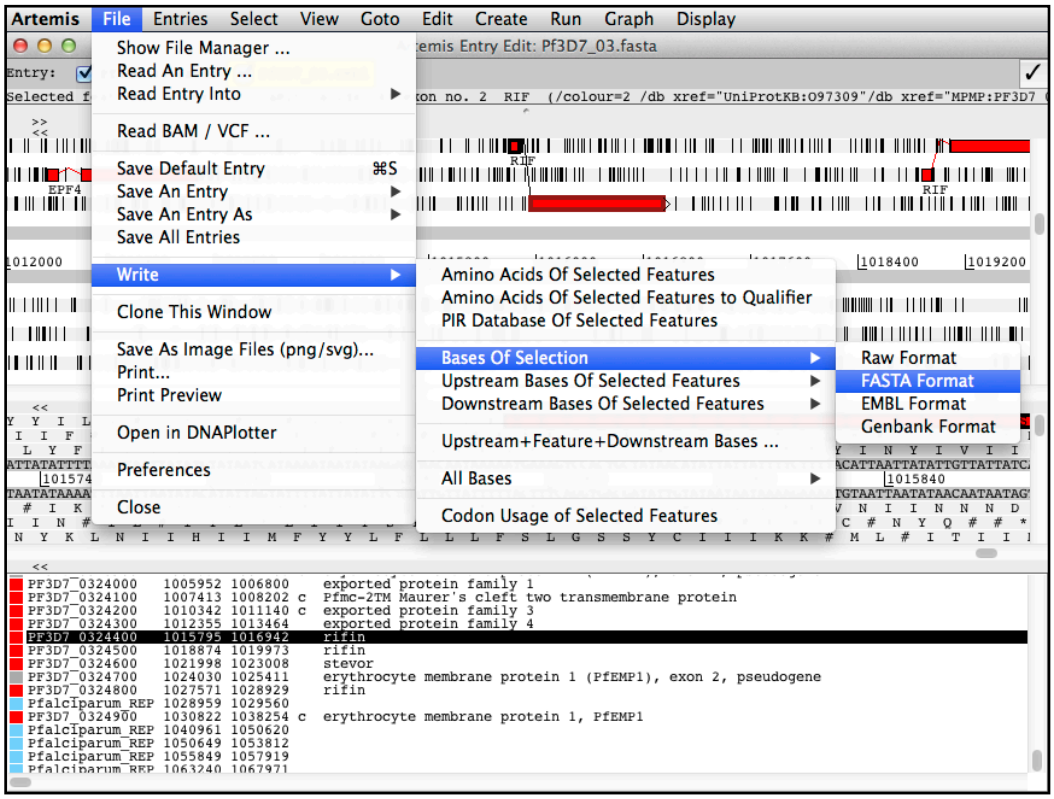
In this part of the Module we will be looking at methods of selecting and extracting features. We are going to extract different genes and regions and perform some more detailed analysis on it. We will aim to write and save new EMBL format files which will include just the annotation and DNA for this region.

In Artemis you can select genes fitting different search criteria. One possibility is to look for a specific product, for example *rifin*, as shown below.



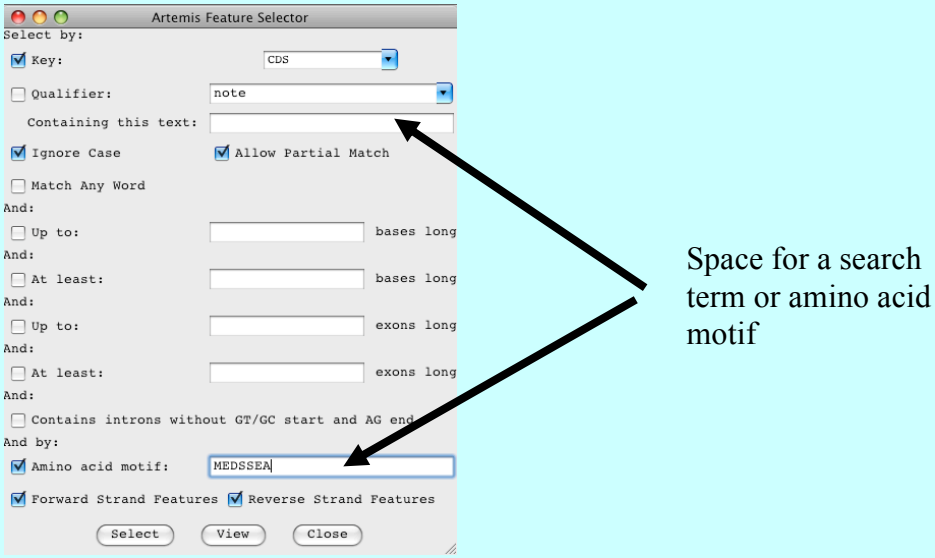
The genes listed in **6** (on the previous page) are only those fitting your selection criterion. They can be copied or moved in to a new entry so they can be viewed in isolation from the rest of the information within Pf3D7_03.embl. To create a new entry go to 'Create' and choose 'New Entry'.

In the next step of the exercise choose one of the selected genes and write out a fasta-file of the sequence.



Click 'File' then 'Write 'Bases of Selection' 'FASTA Format'

Additional methods of selecting/extracting features using the Feature Selector
It is worth noting that the Feature Selector can be used in many other ways to select and extract subsets of features from the genome such as text or amino acid searches.



In the next part of the exercise we will be looking at the region containing the *rifin* genes in more detail. They are located at the end of the chromosomes, in the subtelomeric region. We are going to extract this region from the whole chromosome sequence. Then we will aim to write and save new EMBL format files which will include just the annotation and DNA for this region.

3

Click 'Subsequence (and Features)'

2

Click 'Edit'

1

Select the region containing rifins by clicking with the left mouse button and dragging.

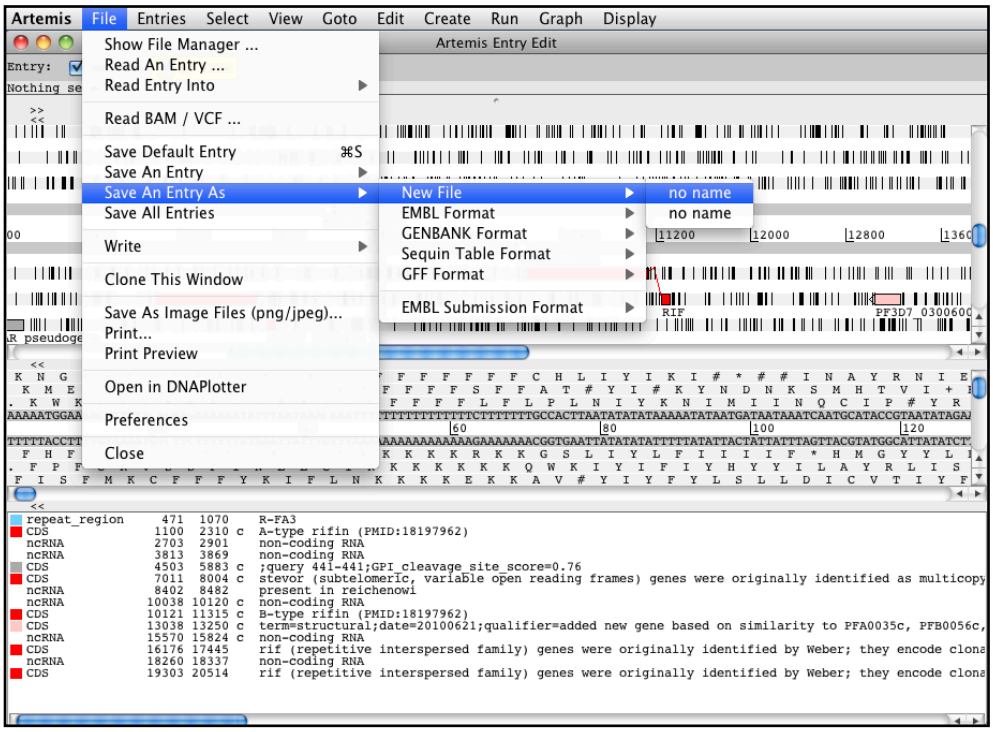
4

A new Artemis window will appear displaying only the region that you have highlighted.

Note the entry names have changed

Note the bases have been renumbered from the first base you selected.

Note that the two entries on the grey Entry line are now denoted ‘no name’, they represent the same information in the same order as the original Artemis window but simply have no assigned name. So click on the File menu then ‘Save an entry as’ and then ‘New file’. Another menu will ask you to choose one of the entries listed. At this point they will both be called ‘no name’. Left click on the top entry in the list. A window will appear asking you to give this file a name. The new files can be saved in different formats.



Once you have finished this exercise remember to close this Artemis session down completely before starting the next exercise.

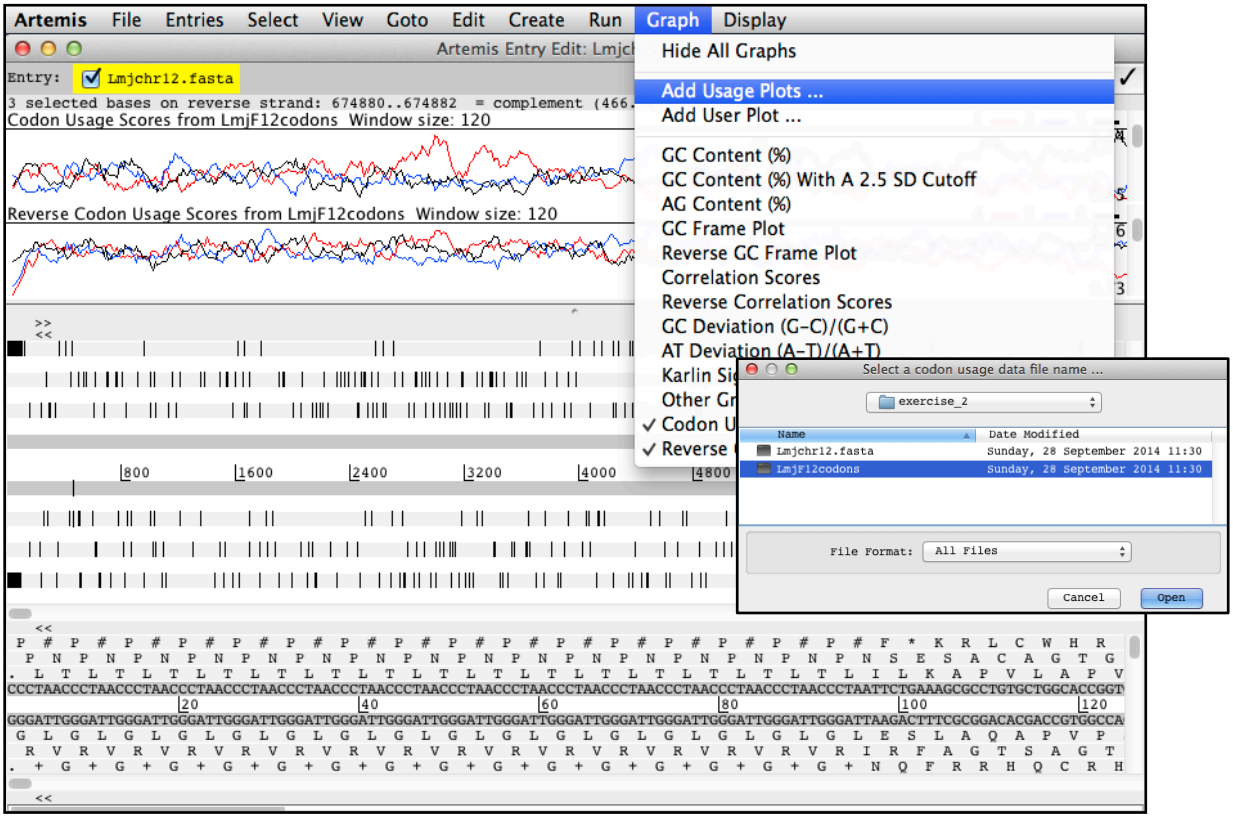
Artemis Exercise 2

We are now switching to a different organism. The following exercise demonstrates how to use Artemis as a tool for structural annotation. Given a length of chromosome with no existing annotation Artemis can mark up ORFs above a given size. This also shows how codon usage plots can be exploited in gene model prediction.

If you haven't already closed the previous session of Artemis, do so now. Double click the ARTEMIS Icon on your Desktop and navigate to the directory Module_1_Artemis, exercise_2 and open the sequence file Lmjchr12.fasta.

Next, open the codon usage table file LmjF12codons by selecting 'Add Usage Plots' from the Graph menu. Codon usage is a very good indicator of coding capacity in *Leishmania* genomes where there is a much more prominent codon bias for some amino acids.

Note, we will cover the use of RNAseq data in gene prediction later on during the course.



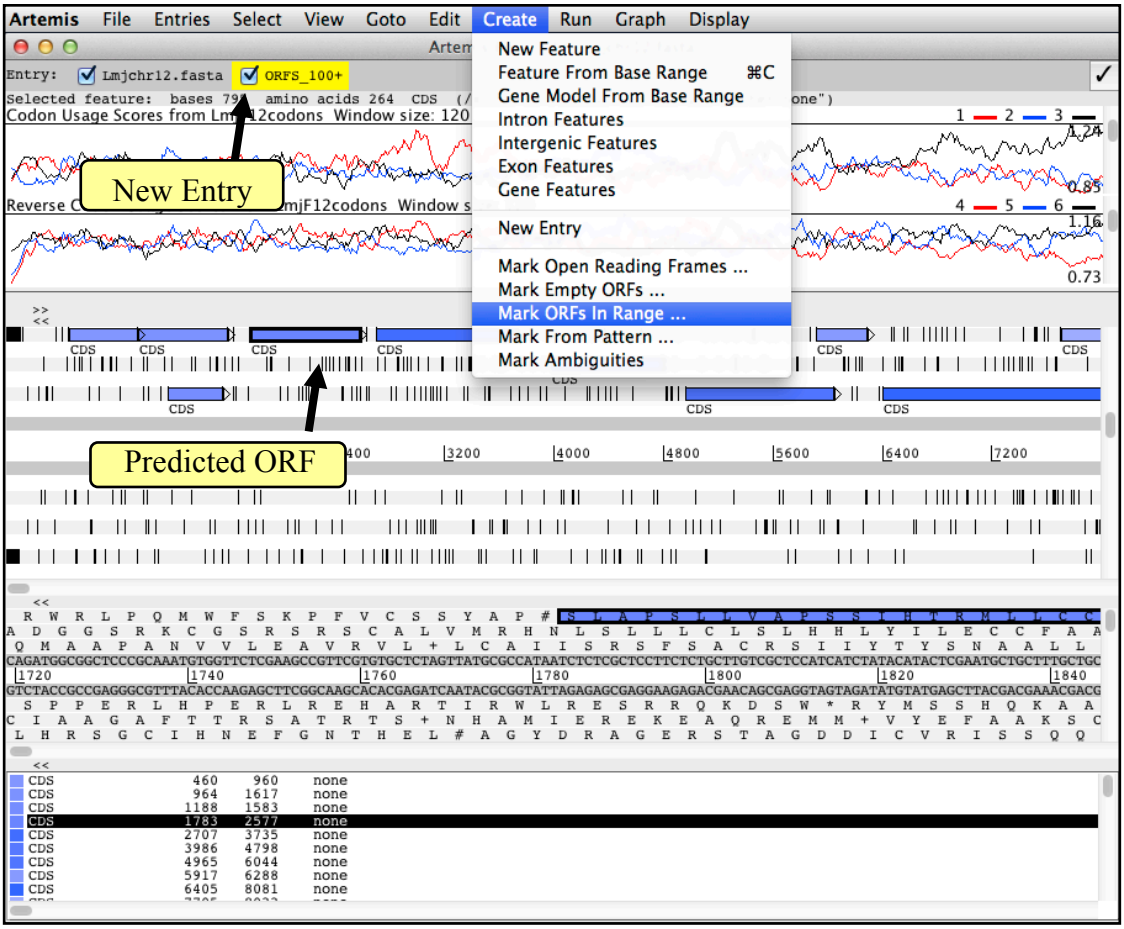
Select the first 100 kbs of sequence on the positive strand either by highlighting the sequence in the sequence window (use shift and click to select the final base) or choose the 'Base Range' option in the select menu and enter '1..100000'.

With this region selected, select 'Mark ORFs in Range' from the Create menu. When prompted for minimum ORF size enter 100. Note that this results in the creation of a new entry called 'ORFS_100+'. You can experiment with a range of ORF sizes by de-selecting this entry and repeating the first steps in this process.

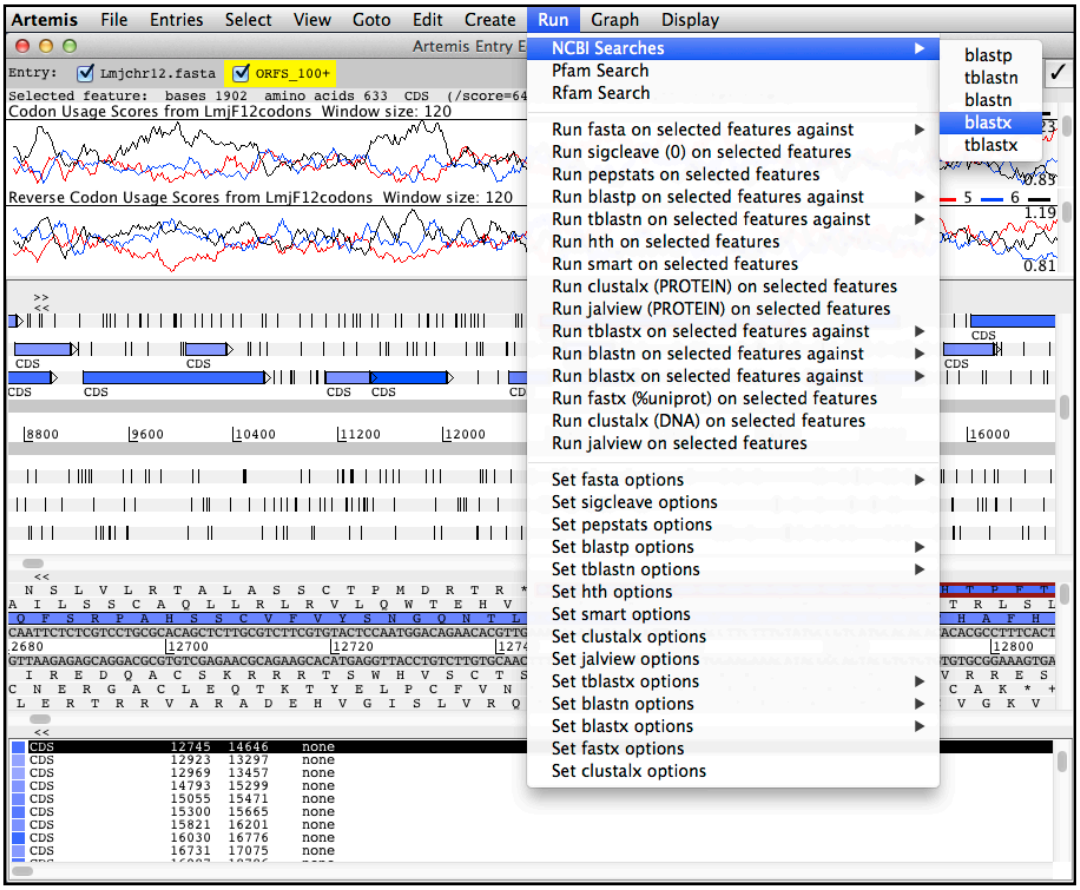
Note that the marked up ORFs vary in colour from pale to navy blue. This colouring reflects the codon usage support for this model with darker blue being highly supported by codon usage.

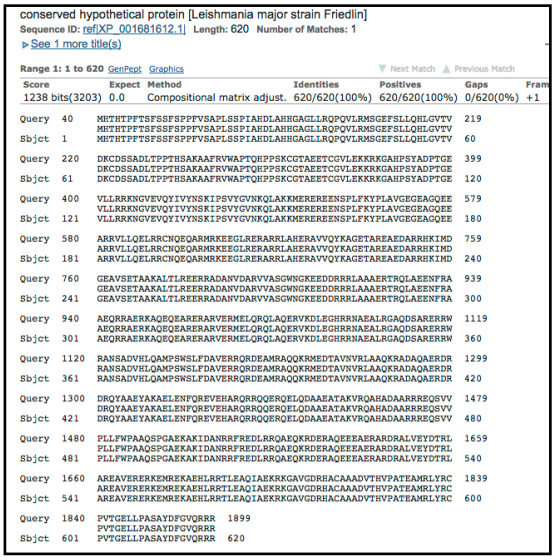
Try selecting some of the newly created features in the gene window. Double clicking on one of these will bring up the predicted peptide sequence in the bottom window. You can rapdily move to the N- or C-terminus of the predicted peptide by holding down ctrl, and then left or right arrow respectively.

Note that we have chosen only to generate ORFs for the positive strand for this example. In a genome not organized into transcription units we would normally do likewise for the reverse strand as well.



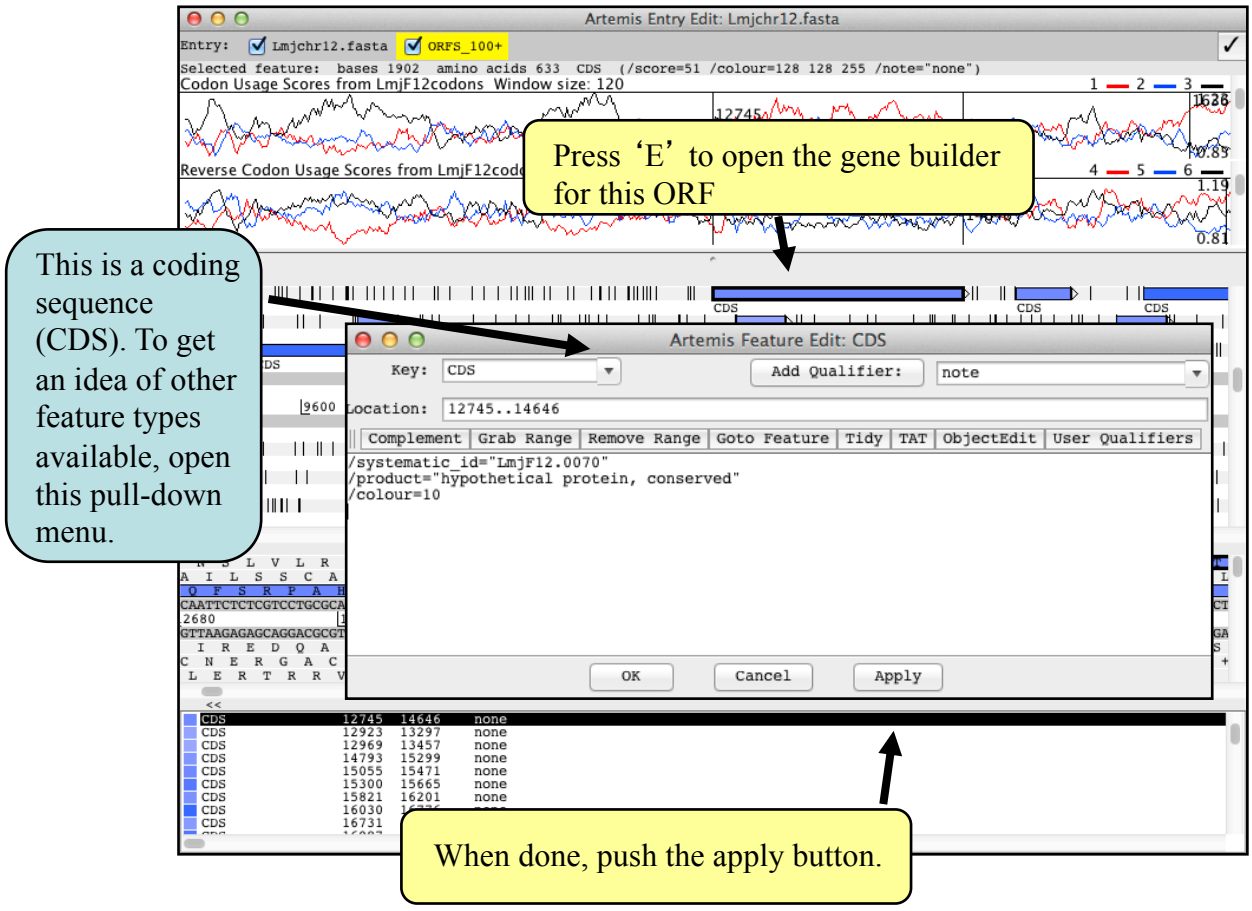
Although some of these predictions are likely to be correct, there is considerable overlap between predicted ORFs, and many are small and unsupported by codon usage. To validate/negate our predicted models we need to do further sequence comparison. This can be done with a tool such as ACT (to be discussed later in the Comparative Genomics Module), or with one of the integrated Blast options in Artemis. Select the ORF at position 12745, click on it, then select RUN>NCBI Searches>blastx. This will open a browser window with NCBI results.





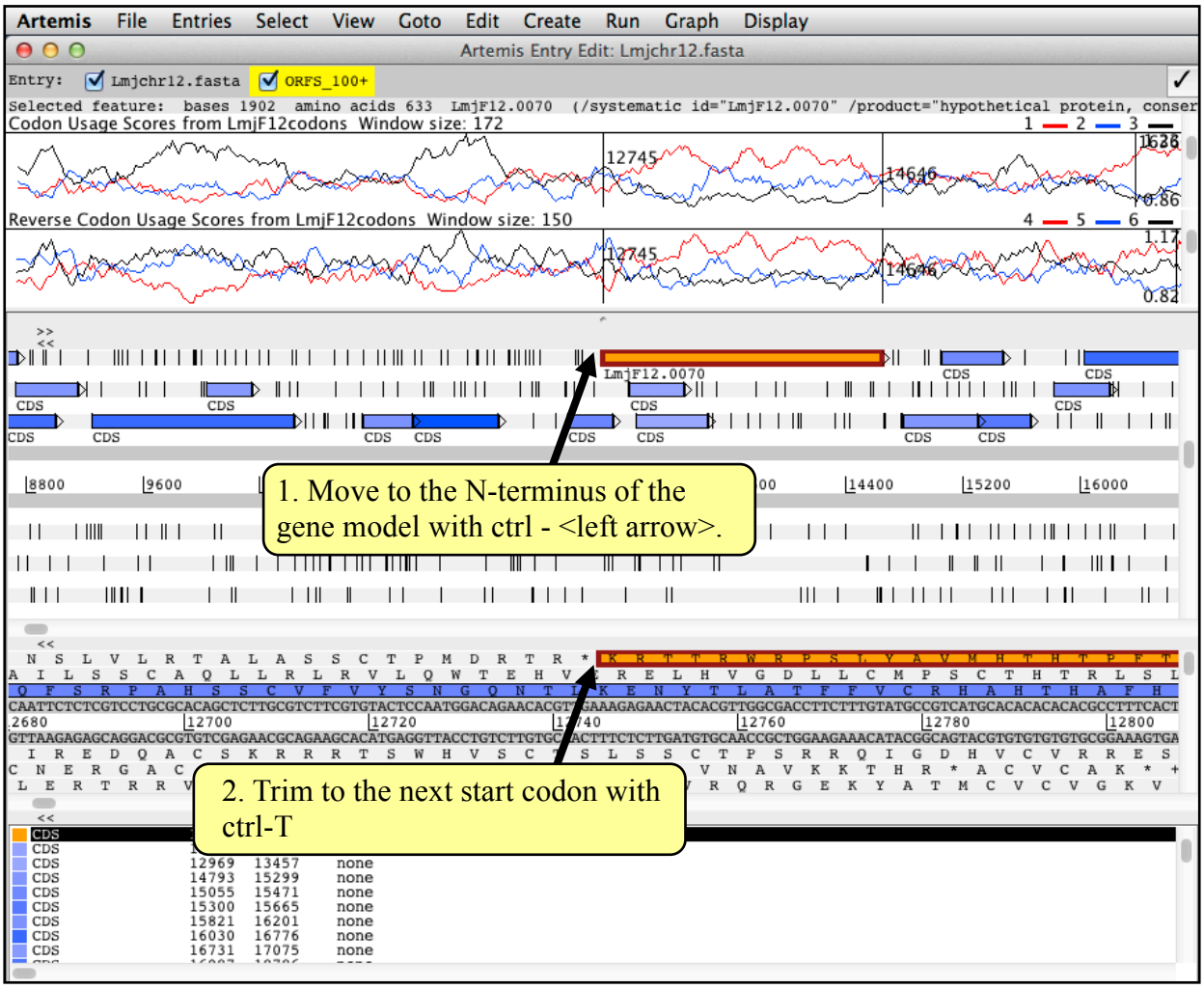
Not surprisingly, the top hit is to a gene on chromosome 12 in *L. major,* a hypothetical protein.

Now that we know that this is a real gene we can make a few adjustments. First, open the gene builder window by selecting the ORF and pressing E. This will open a text window where we can add annotations on the gene. Start by deleting the current 'automatic' annotations in this window. Try entering the text in the gene builder shown below to record gene ID, predicted product and a colour code that will distinguish this gene from the automatically generated ORFs.



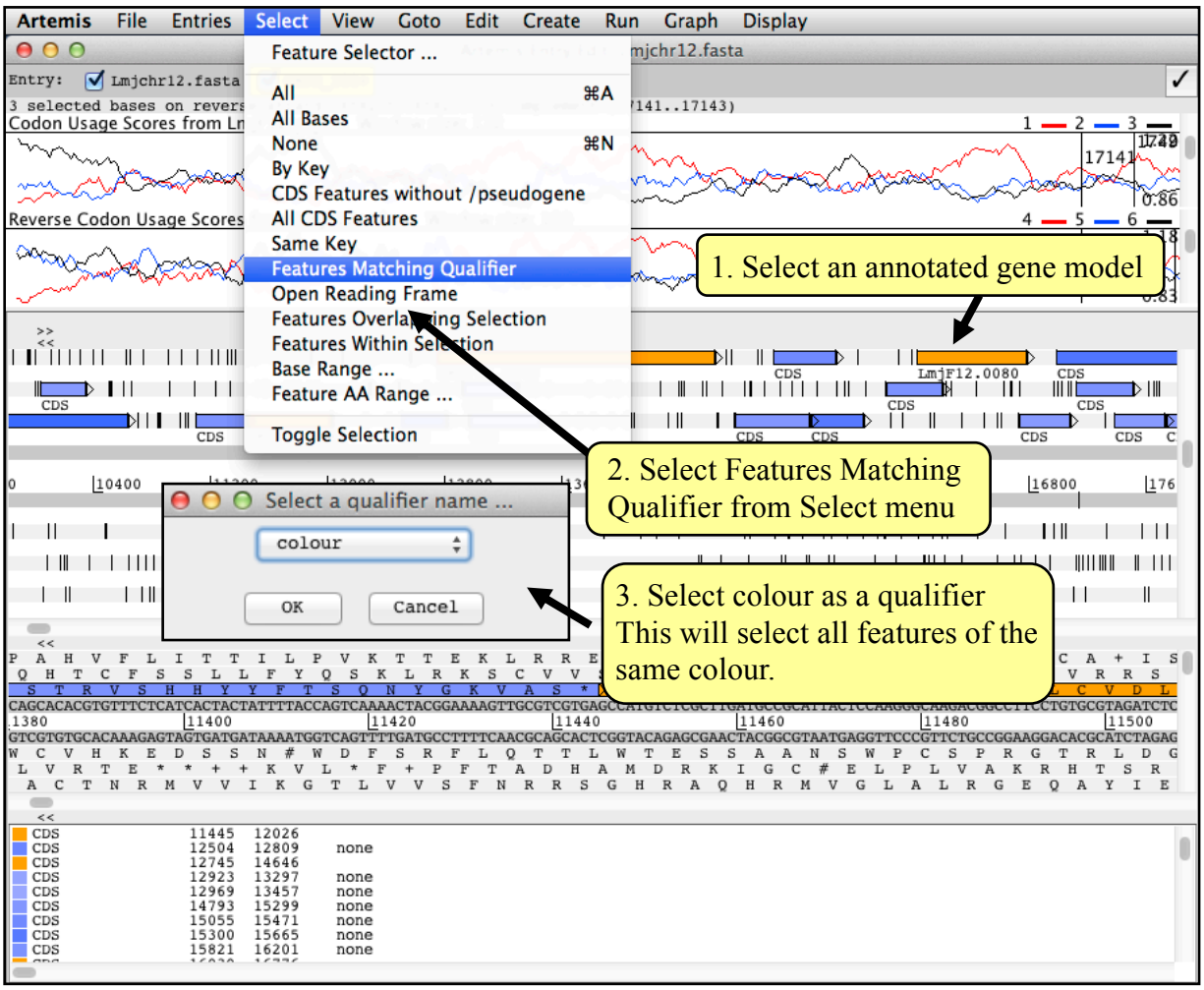
Based on the NCBI blast results we can adjust the N-terminus of this model to the correct start codon. To automatically position the sequence window at the N-terminus of the gene model push ctrl-<left arrow>.

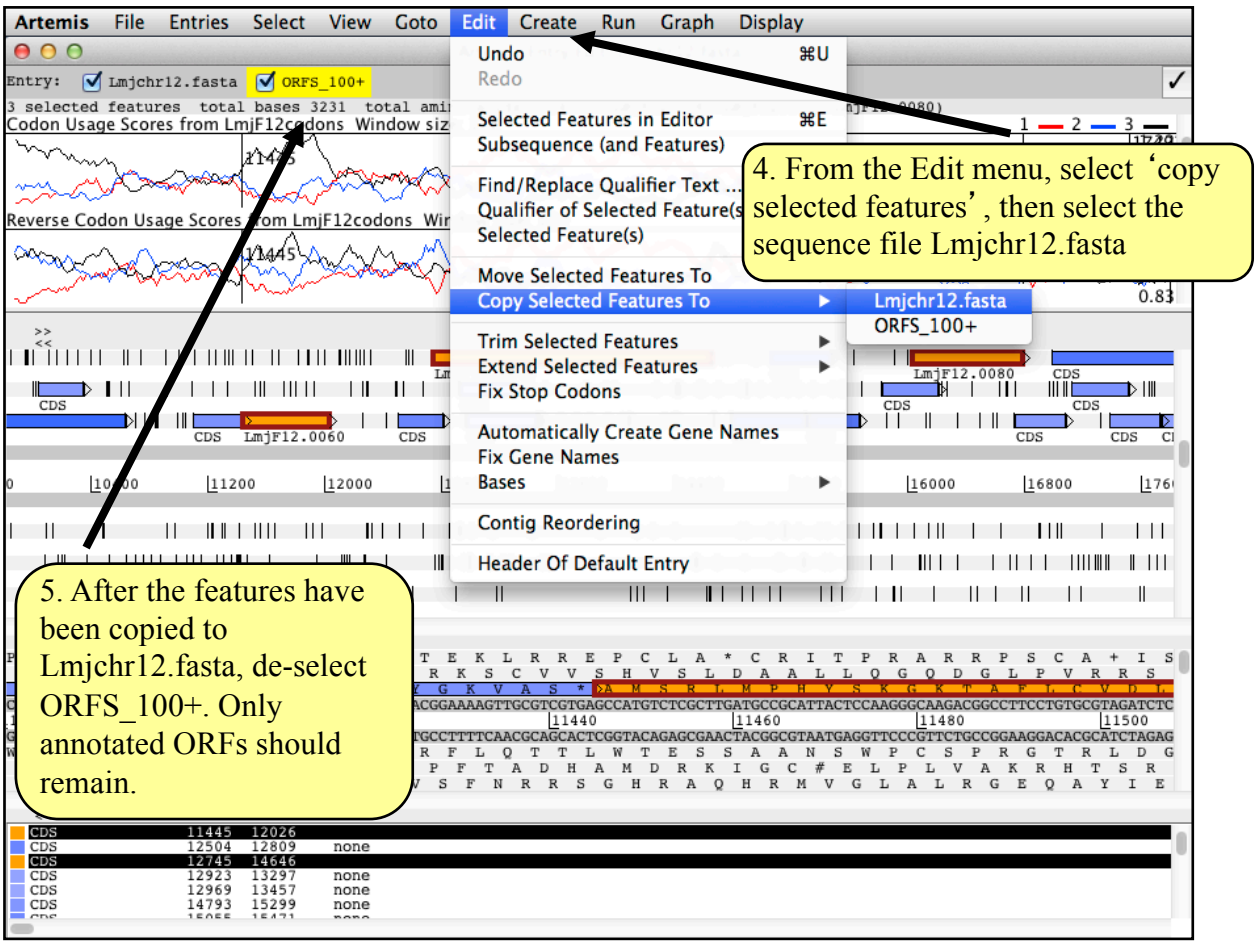
Go to Edit>Trim Selected Feature>To Next Met (or ctrl-T), then reposition the sequence window at the new start as described above. Continue until the start resembles the NCBI blast results. If trimmed passed the desired start codon the model can be reset through Edit>Extend Selected Feature>To Previous Stop Codon, or ctrl-Q.

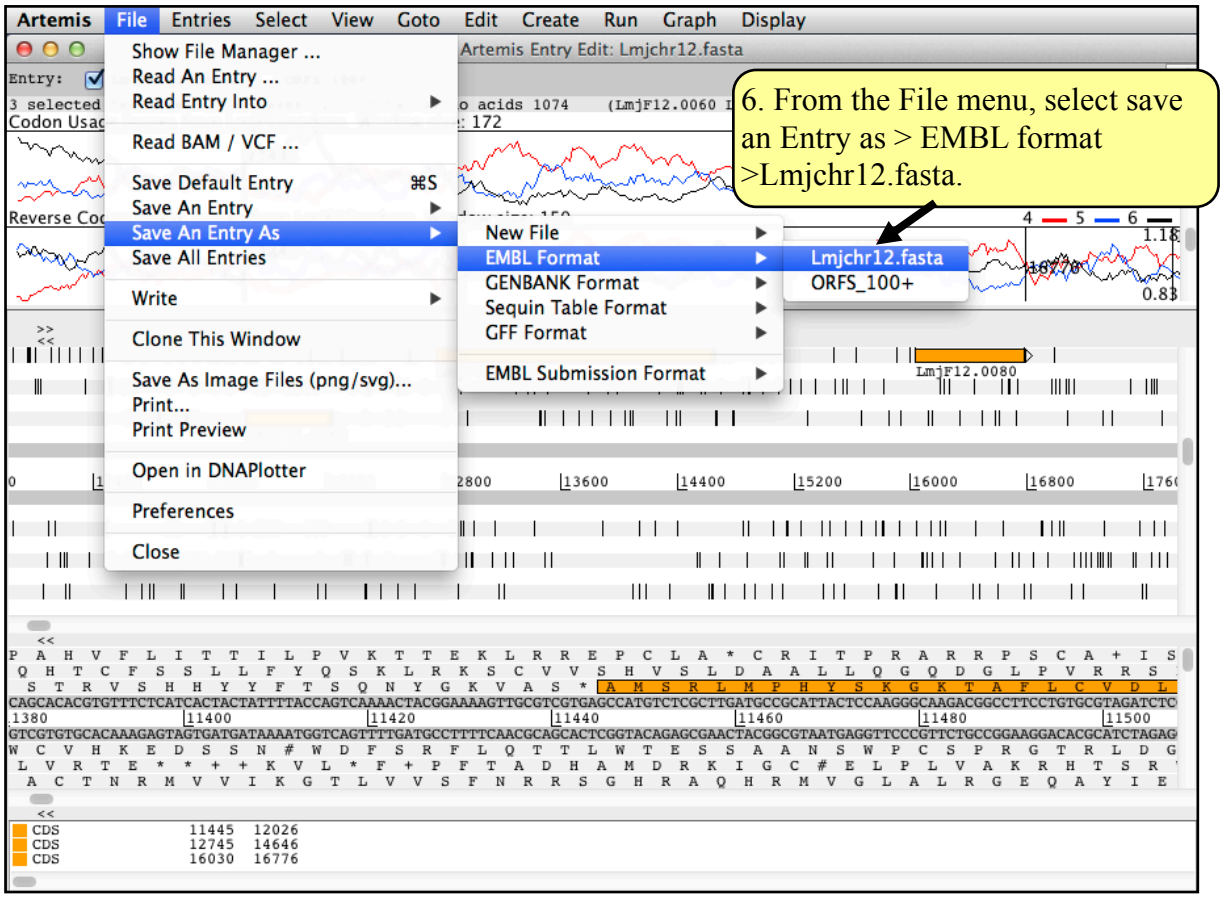


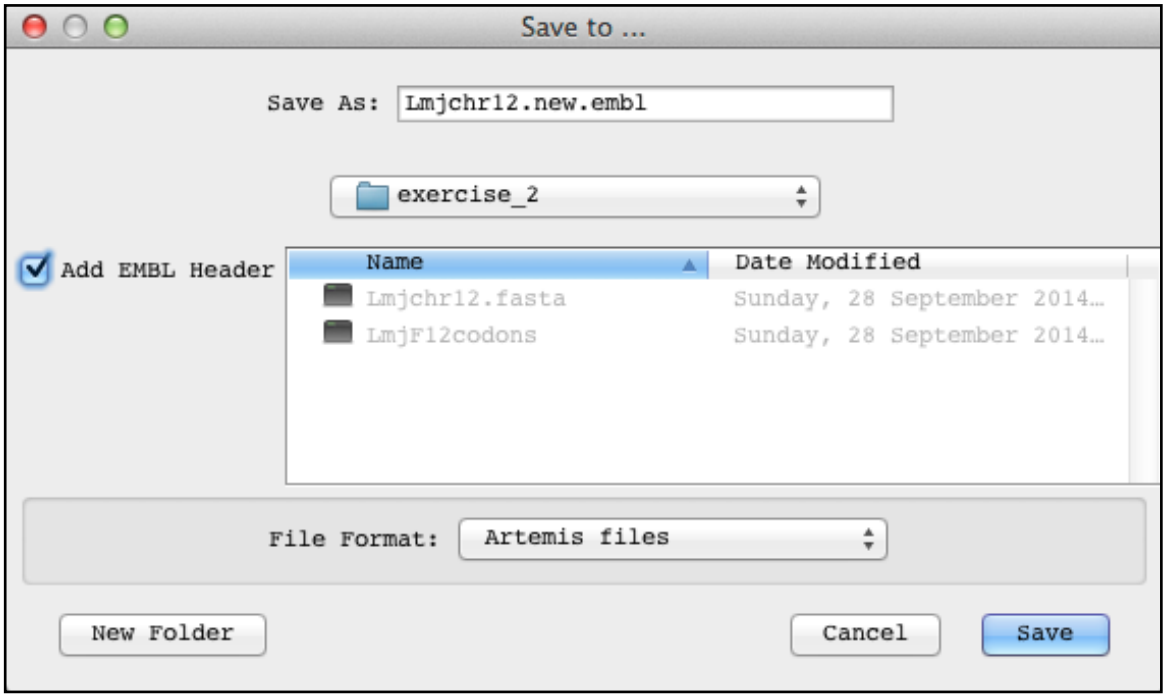
There are more than 20 protein coding genes in the first 100 kbs of chromosome 12. See how many of these you can find by repeating the steps in the past slides.

IMPORTANT!! Any changes made to the predicted ORFs will be written to an entry file called ORFS_100+. When you're done with gene predictions follow the steps below to save these entries to the sequence file instead. Make sure all of the annotated features have a /colour=10 in their gene builder window.




Artemis File Entries Select View Goto Edit Create Run Graph Display
Undo ⌘U
Redo
Selected Features in Editor ⌘E
Subsequence (and Features)
Find/Replace Qualifier Text ...
Qualifier of Selected Feature(s
Selected Feature(s)
Move Selected Features To
Copy Selected Features To
Lmjchr12.fasta
ORFS_100+
Trim Selected Features
Extend Selected Features
Fix Stop Codons
Automatically Create Gene Names
Fix Gene Names
Bases
Contig Reordering
Header Of Default Entry
4. From the Edit menu, select 'copy selected features', then select the sequence file Lmjchr12.fasta
5. After the features have been copied to Lmjchr12.fasta, de-select ORFS_100+. Only annotated ORFs should remain.


Artemis File Entries Select View Goto Edit Create Run Graph Display
Artemis Entry Edit: Lmjchr12.fasta
Show File Manager ...
Read An Entry ...
Read Entry Into
Read BAM / VCF ...
Save Default Entry ⌘S
Save An Entry
Save An Entry As
Save All Entries
Write
Clone This Window
Save As Image Files (png/svg)...
Print...
Print Preview
Open in DNAPlotter
Preferences
Close
New File
EMBL Format
GENBANK Format
Sequin Table Format
GFF Format
EMBL Submission Format
Lmjchr12.fasta
ORFS_100+
6. From the File menu, select save an Entry as > EMBL format >Lmjchr12.fasta.



Save the sequence file as Lmjchr12.new.embl.

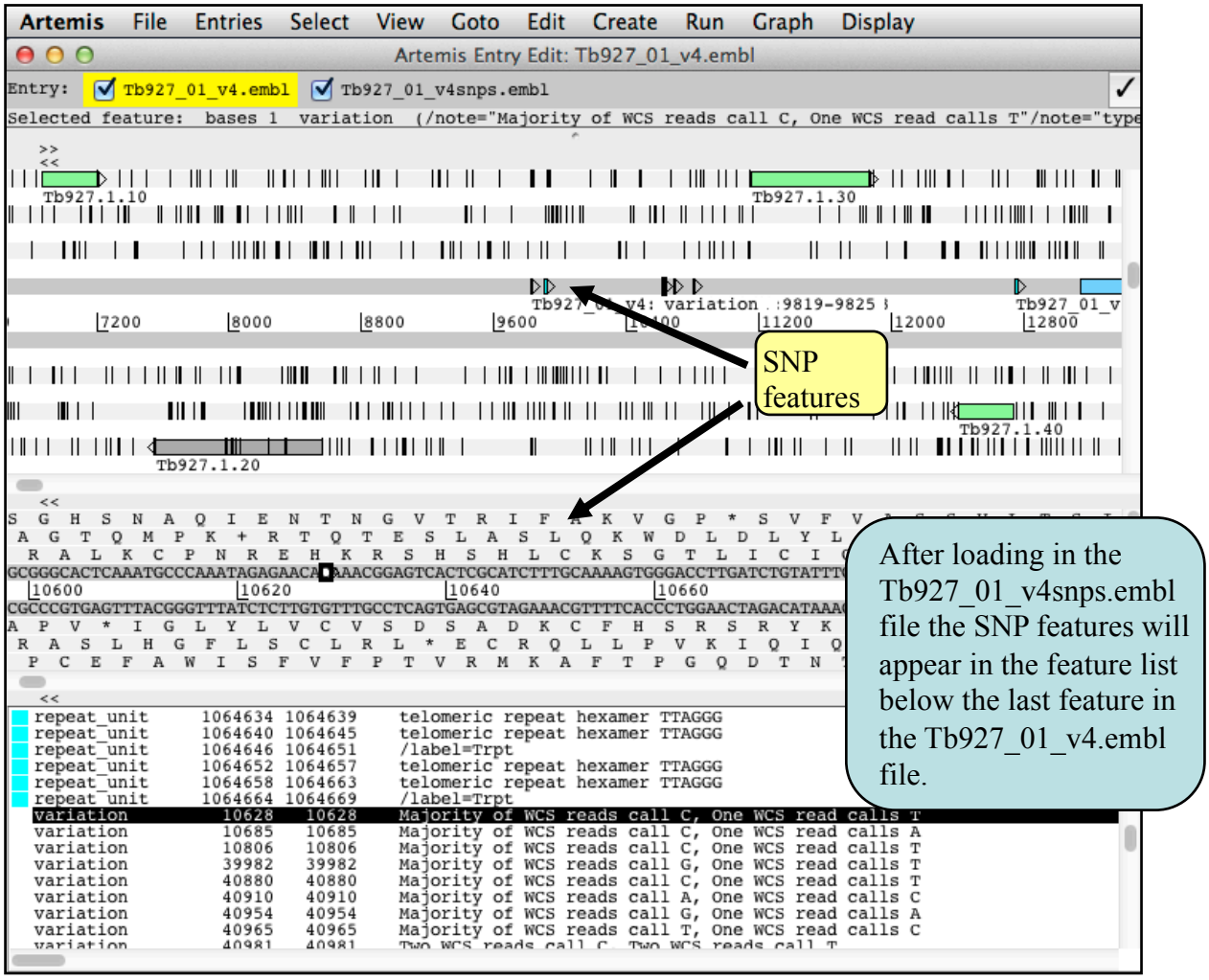
Optional exercise

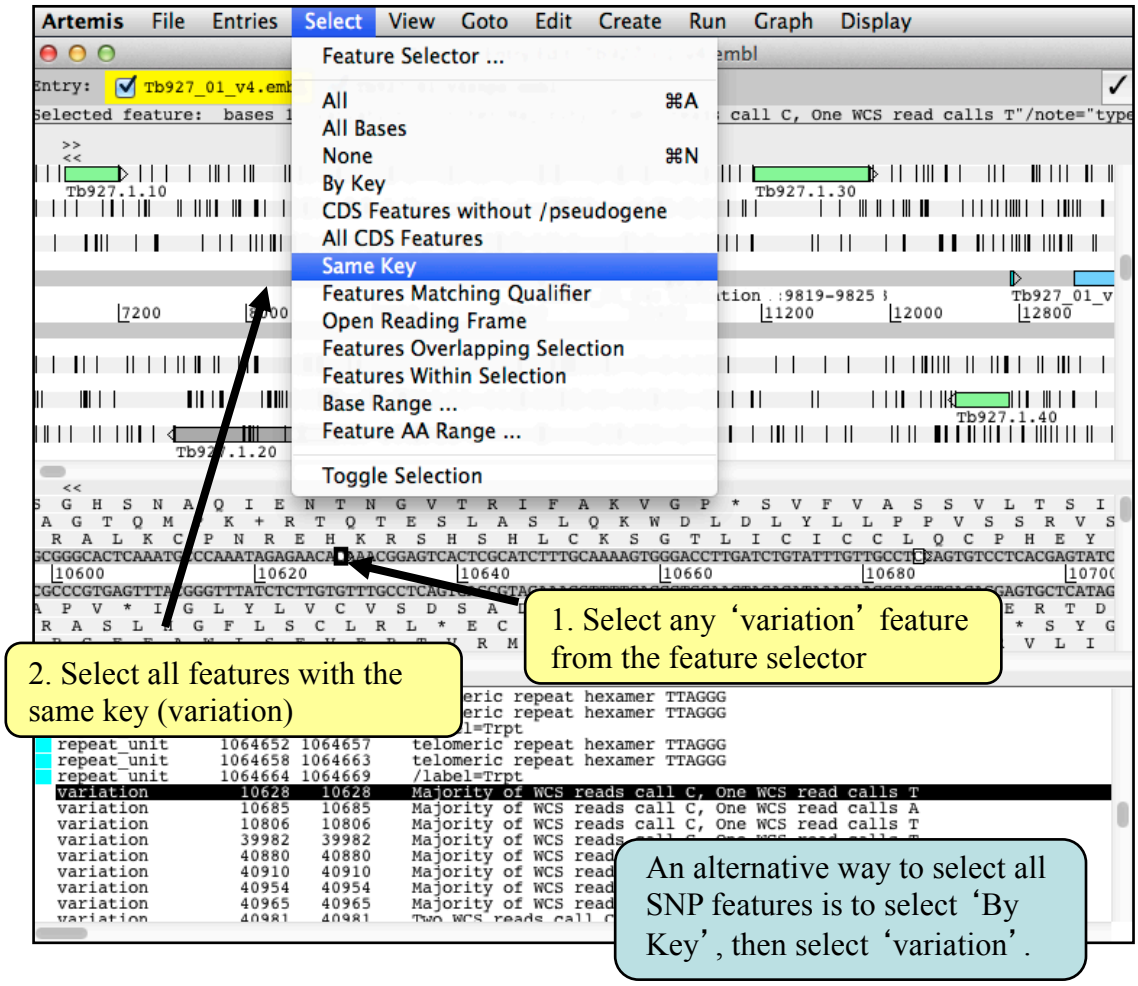
This optional exercise demonstrates how to use Artemis to construct queries for features within features. In the exercise below we will load a file containing SNP data, then retrieve a list of all CDS that overlap with SNP features.

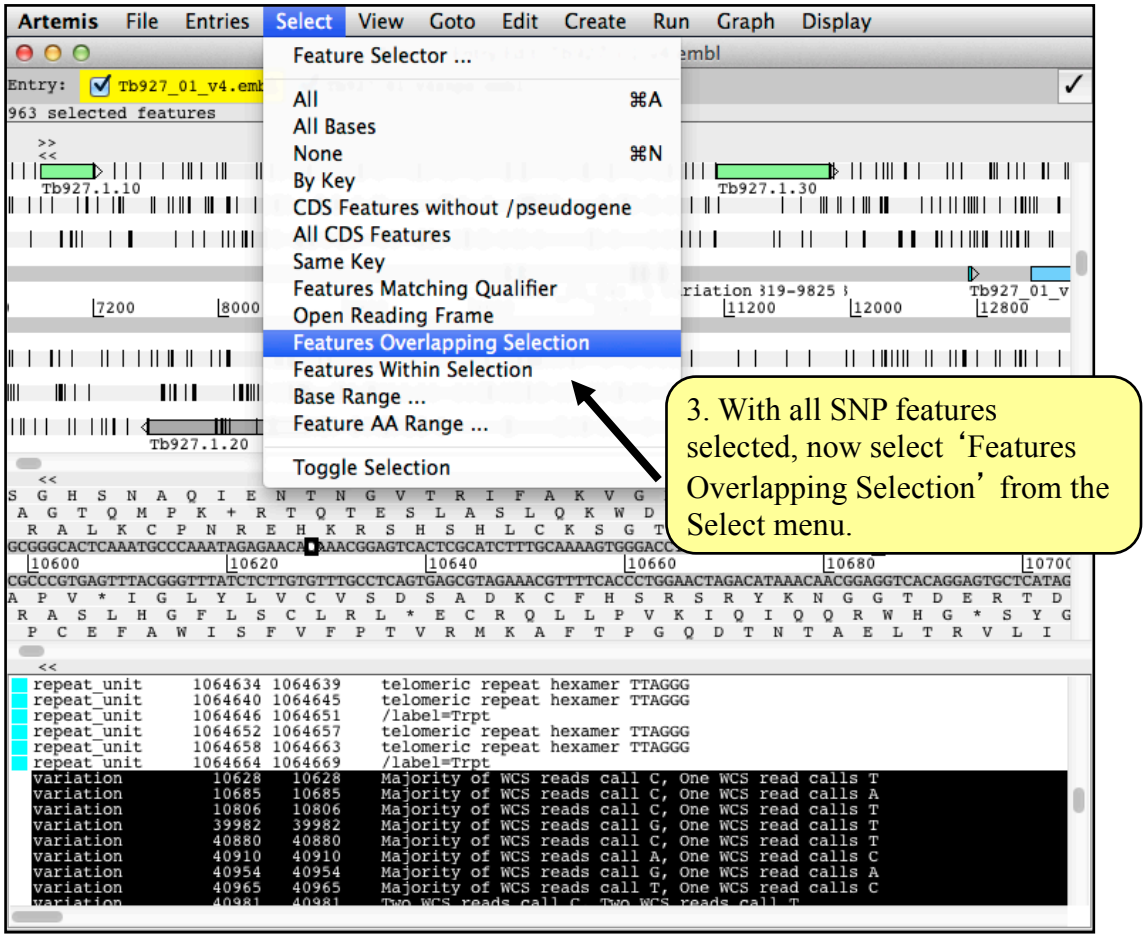
Files required:

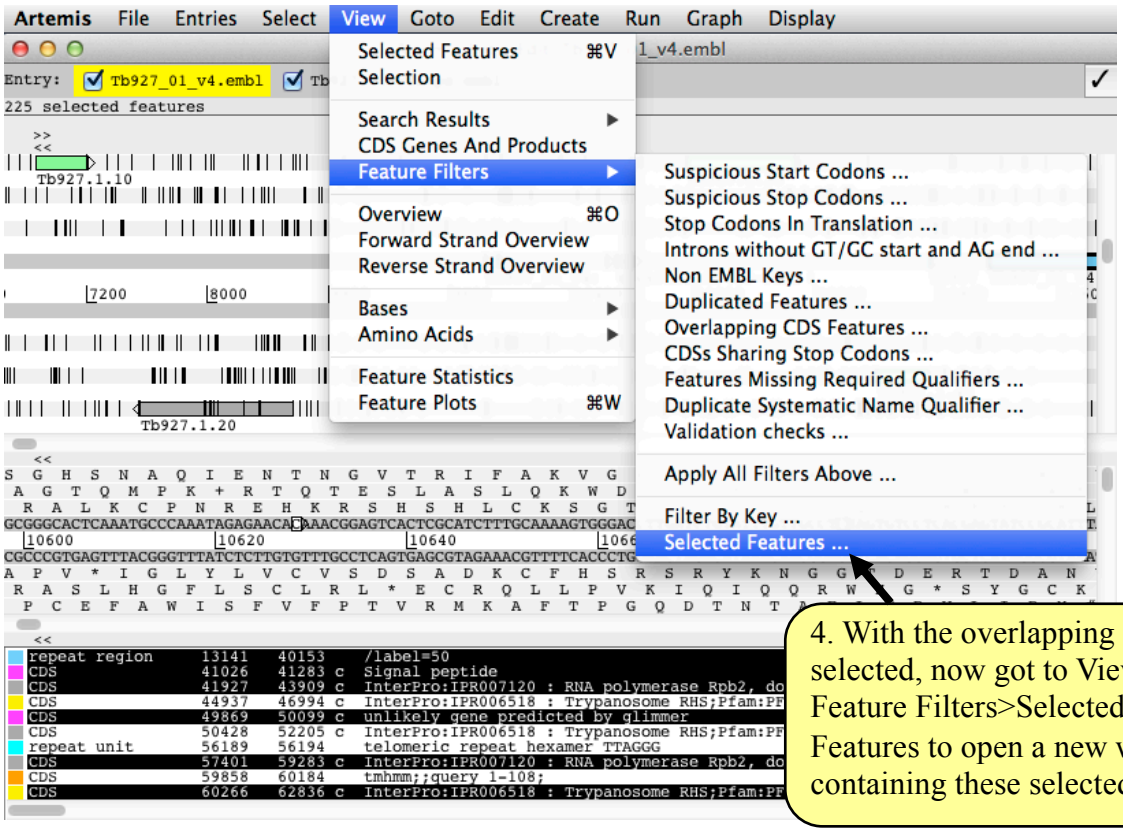
Tb927_01_v4.embl - Contains sequence and annotation for *T. brucei* chromosome 1
Tb927_01_v4snps.embl - Contains SNP features for *T. brucei* chromosome 1

Navigate to the directory Module_1_Artemis, optional_exercise.
Use the file manager to open Tb927_01_v4.embl, then as shown in previous exercises select File>Read Entry >Tb927_01_v4snps.embl.



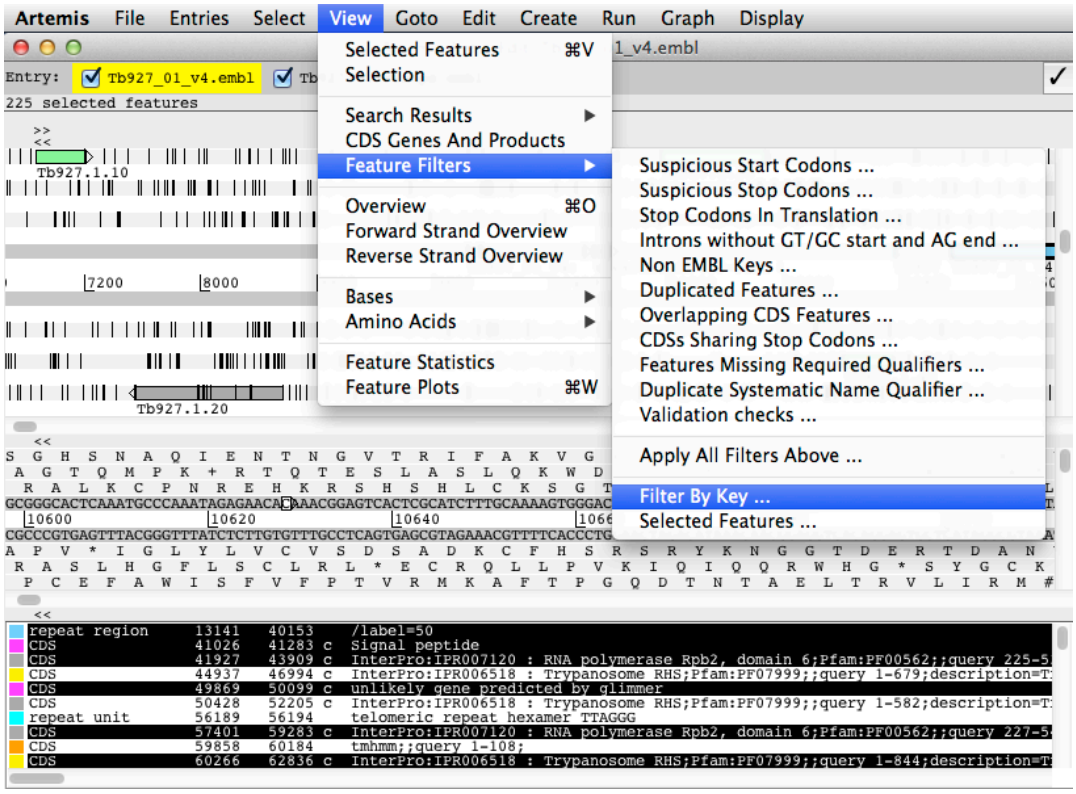

Artemis File Entries Select View Goto Edit Create Run Graph Display
Feature Selector ...
All ⌘A
All Bases
None ⌘N
By Key
CDS Features without /pseudogene
All CDS Features
Same Key
Features Matching Qualifier
Open Reading Frame
Features Overlapping Selection
Features Within Selection
Base Range ...
Feature AA Range ...
Toggle Selection
1. Select any 'variation' feature from the feature selector
2. Select all features with the same key (variation)
An alternative way to select all SNP features is to select 'By Key', then select 'variation'.

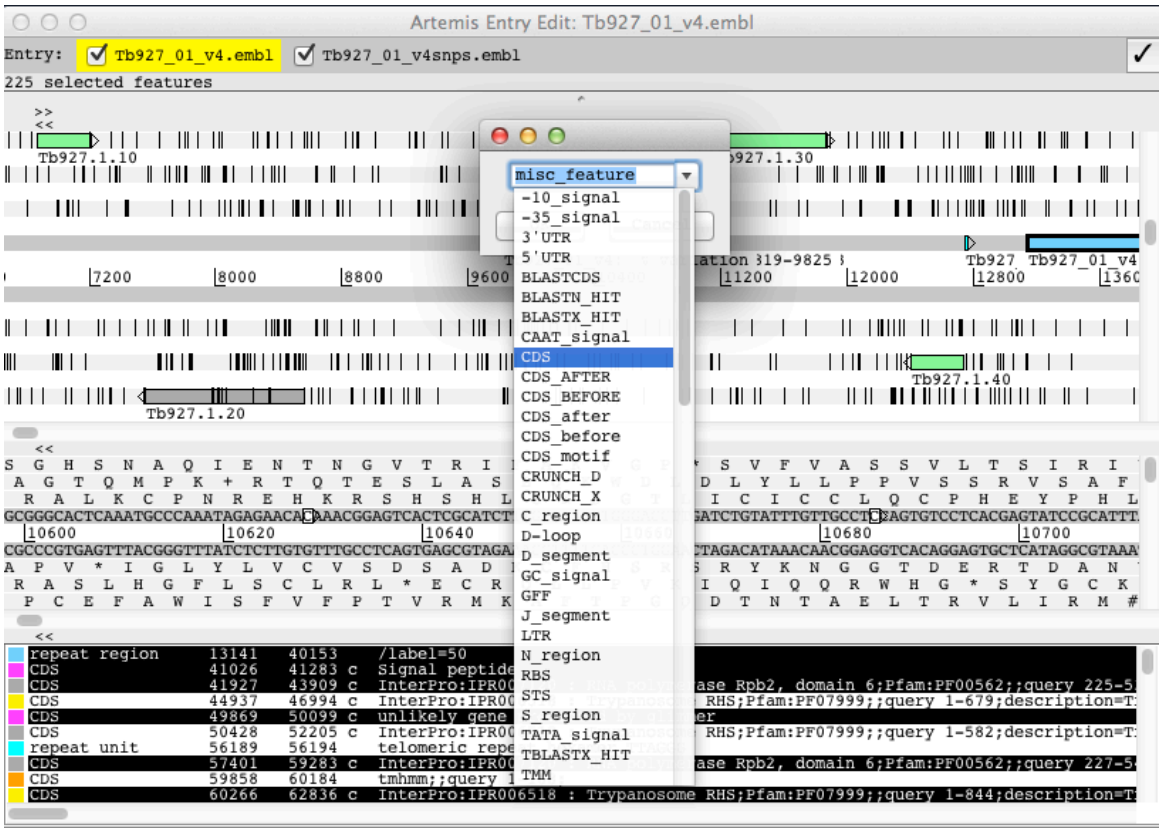

Artemis File Entries Select View Goto Edit Create Run Graph Display
Feature Selector ...
All ⌘A
All Bases
None ⌘N
By Key
CDS Features without /pseudogene
All CDS Features
Same Key
Features Matching Qualifier
Open Reading Frame
Features Overlapping Selection
Features Within Selection
Base Range ...
Feature AA Range ...
Toggle Selection
3. With all SNP features selected, now select 'Features Overlapping Selection' from the Select menu.



4. With the overlapping features selected, now got to View select Feature Filters>Selected Features to open a new window containing these selected results.

All the features overlapping with the SNP features should now appear in a new window. Note that this window contains not only CDS features, but features such as 5' UTRs, repeat regions and other miscellaneous features that overlap with SNPs. To see only CDS features we need to apply a second filter. With the non-overlapping features still selected, select View>Feature Filters>Filter by Key. Select CDS for the Key, and only CDS containing SNPs should appear in the filter window.





Other Queries to Try:

1. Try performing the ‘reverse’ query, select all SNPs that overlap with CDS features.
2. Save a list of features to a file by right clicking on the feature filter window and Selecting ‘Save List to File’
3. Use the Select Menu to select all features with the same ‘key’
4. Use the Filter menu to look for suspicious gene models - missing start codons, missing stop codons, stop codons in translation and duplicated features.
5. Search for a qualifier value (try ‘hypothetical protein’), in the Edit menu, select ‘Find/Replace Qualifier Text’. Try doing a boolean search in the same way (try ‘hypothetical AND conserved, or ‘hypothetical AND unlikely’).
6. Using the same option, find features containing duplicate qualifiers (more than one qualifier with the same value)