#### **First Time User**

Thank you for choosing to use MEGA in your research. This manual provides comprehensive documentation for the MEGA software application. New users of MEGA may wish to read and follow along with our <u>walkthrough tutorial</u> which attempts to touch on every major part of MEGA which you may find useful. You may also wish to check out the <u>newest</u> features in MEGA.

Quick Start (useful for more technical users)

#### **MEGA User Mode**

MEGA can be used with either a graphical user interface (useful for visual exploration of data and results) or a <u>command-line interface</u> (useful for batch or scripted execution).

The graphical user interface (GUI) is run in one of two modes. The first mode is the *Analyze* mode in which all GUI tools in MEGA are enabled and visual results explorers are available for tasks such as editing sequence alignments and viewing phylogenies. This is the mode that most MEGA users are familiar with. The second mode is the *Prototype* mode which is used solely for generating <u>MEGA Analysis Options</u> (.mao) files that specify analysis settings when using MEGA from a command shell

The command-line interface of MEGA is accessed by opening a command shell and executing the **megacc** command. The **megacc** command requires several options, including the path to a .mao file and paths to input data file(s) to be analyzed

# **Aligning Sequences (using GUI)**

- 1. MEGA supports sequence alignment using both the ClustalW and MUSCLE programs.
- 2. Alignment (or refinement) is done in the Analysis Explorer (*Alignment -> Open Alignment Explorer* from main menu).
- 3. We either can start with a blank alignment (if we are importing sequences from *NCBI*, or don't have a compatible sequence file) or from a compatible sequence file.
- 4. With our sequences in the Alignment Explorer (AE), we select Alignment from the menu, then either *ClustalW* or Muscle.
- 5. Set the alignment parameters to the values you wish or leave the options alone to use the defaults. Click Compute/OK.
- 6. Depending on the length and number of sequences you may see a progress bar while the alignment is running.
- 7. The aligned sequences will replace the previously unaligned sequences in the Alignment Explorer. You may now export them to MEGA or Fasta format for analysis.

## **Running an Analysis (using GUI)**

(Note: Sequences MUST be aligned before analysis can proceed.)

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- 1. Select the analysis you wish to run from the top toolbar in the main window.
- 2. You are shown a list of options for this analysis. You can only change the options which are drawn in a white box. Click Compute.
- 3. Depending on the length of the analysis you may see a progress bar while the analysis is running.
- 4. Your output will appear as either a Tree, Matrix, Text, etc.
- 5. In most results there will be the option to save your analysis. This usually resides in the File or Data menus of the results window.

### **Executing Analyses From a Command Shell**

- 1. When using MEGA's command-line interface, all calculations are launched in the same way.
- 2. In the MEGA main form, click the *Prototype* button and then specify the type of input data that will be used for analysis.
- 3. Select the analysis you wish to run from the top toolbar in the main window.
- 4. When the options dialog is displayed, select the desired options or use the default ones. Click *Save Settings* to save the options to a .mao file.
- 5. From a command shell, execute the **megacc** command with the .mao file and input data file(s) as parameters.

See also

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