Introduction to Walk through MEGA

This section provides a MEGA tutorial. The data files for these examples can be found in the **EXAMPLES** folder, located in the MEGA installation directory (example in **C:\Program Files\MEGA\Examples)**. In these example files, data are deliberately written in different input formats. We recommend that you study the examples in the order presented because the techniques explained in the initial examples are used again in the subsequent ones.

In the following write-up, **highlighted** words indicate the keys you must press on the keyboard. If you must press two keys simultaneously, they are shown with a + sign between them (*e.g.*, **Alt** + **F3** means that the **Alt** and **F3** keys should be pressed simultaneously). Italicized letters are used to mark the commands found in menus, submenus, and other locations as they appear on the computer screen. In every example, we discuss many procedures introducing analytical techniques. For ease of reference in later examples, these procedures are numbered in the Ex *u.v.w* format, where *u* is the example number, *v* is the procedure number, and *w* is the step number. For instance, Ex 1.3.2 refers to the 2nd step of the 3rd procedure in example 1.

A list of tutorials is as follows:

1. Aligning Sequences
2. Estimating Evolutionary Distances
3. Building Trees
4. Testing Tree Reliability
5. Marking Genes/Domains
6. Testing for Selection
7. Grouping Sequences
8. Computing Sequence Statistics
9. Trees from Distance Data

Creating Multiple Sequence Alignments

In this example, we will create an alignment from protein sequence data that will be imported into the alignment editor using different methods.

Ex 1.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 1.0.2: Launch the Alignment Explorer by selecting the *Alignment*|*Alignment Explorer/CLUSTAL* menu command.

In order to align sequences contained in a Sequence Data File, do the following:

Ex 1.1.1: Add unaligned sequences from the **hsp20.fas** example file into the Alignment Explorer by clicking selecting the *Data*|*Open*|*Retrieve* Sequences from File menu command.

Ex 1.1.2: Select the *Edit*|*Select All* menu command to select every site for all sequences in the alignment.

Ex 1.1.3: Select the *Alignment*|*Align by ClustalW* menu command to align the selected sequences data using the ClustalW algorithm.

Ex 1.1.4: Save the current alignment session by selecting the *Data*|*Save Session* menu item. This will allow the current alignment session to be restored for future editing.

Ex 1.1.5: Exit the Alignment Explorer by selecting the Data|Exit Alignment Explorer menu item. A message will appear asking if you would like to save the data to a MEGA file. Choose "YES," and then a "Save As" dialog box will appear. Enter hsp20_aligned.meg as the file name, and click the "Save" button. An input box will appear asking for a title for the data. Enter "HSP 20 Aligned by MEGA" as the title, and click the "OK" button. Another dialog box will appear asking you if the sequence data is protein coding. In this case, click "Yes." A final dialog box will appear asking you if you would like to open the data file in MEGA. Click "Yes."

Now, we will examine how to send sequence data from the Internet (Web Explorer) to the Alignment Explorer.

Ex 1.2.1: If the Alignment Explorer already contains sequence data, select the *Data*| *Create new* menu command to create a new alignment from Alignment Explorer window. Choose "YES" on the dialog box that appears to indicate that you are creating a DNA sequence.

Ex 1.2.2: Activate the Web Explorer tab by selecting *Web*|*Query Gene Banks* from the menu.

Ex 1.2.3: When the *NCBI Entrez* site is loaded, select either the nucleotide or protein database, enter a search term into the search box, and press the "GO" button.

Ex 1.2.4: When the search results are displayed, select the specific search item and choose "Sequence" from the menu bar. Press the "Add to Alignment" button located to the left of the address box. This will display the Web Fetch dialog window.

Ex 1.2.5: Click the box to the left of each accession number whose sequences' information you would like to fetch from the web. When you are done, select accessions by pressing the "Fetch" button.

Ex 1.2.6: When the status column indicates that all sequences are fetched, press the "Send to Alignment" button to send the fetched sequence data to the Alignment Explorer.

Ex 1.2.7: Align the fetched data using the steps detailed in Ex 1.1.2 – Ex 1.1.5.

You may also open a trace file in the *Trace Data Viewer/Editor* and send it directly to the Alignment Explorer.

Estimating Evolutionary Distances from Nucleotide Sequences

In this example, we will compute various distances for the *Adh* sequences from 11 *Drosophila* species. We will use the data from the previous example to study various sequence statistics. In addition, we will see how these distances can be written in a file in various formats through options for page size, precision, and relative placement of distances and their standard errors.

Ex 2.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Activate the data file **Drosophila_Adh.meg** using the instructions given in **Ex 2.2.1** – **Ex 2.2.3**.
We will begin by computing the proportion of nucleotide differences between each pair of *Adh* sequences.

Ex 2.1.1: Select the *Distance*|*Compute Pairwise* command (**F7**) to display the distance analysis preferences dialog box.

Ex 2.1.2: In the *Options Summary* tab, click the *Model* preference pull-down and then select the *Nucleotide*|*p-distance* option.

Ex 2.1.3: You may look around at the other options, but at this moment, we will be using the defaults for the remaining options. Click "Compute" to begin the computation.

Ex 2.1.4: A progress indicator will appear briefly, and then the distance computation results will be displayed in grid form in a new window.

We will now compute distances and compare them using other methods.

Ex 2.2.1: Select the *Distance*|*Compute Pairwise* command. Use the *Models* pull-down to select the *Nucleotide*|*Jukes-Cantor* method. Now click "Compute" to begin the computation.

Ex 2.2.2: Follow the steps in Ex. 2.1.1- Ex 2.1.3 and compute the *Tamura-Nei Distance*.

Ex 2.2.3: You should now have open results windows containing the distances estimated by three different methods, which you can now compare.

Ex 2.2.4: After you've compared the results, select the *File*|*Quit Viewer* option for each result window.

Summary: we have computed nucleotide distances from the nucleotide sequence data in the file Drosophila_Adh.meg.

Let us now compute the proportion of amino acid differences. Note that MEGA will automatically translate the nucleotide sequences into amino acid sequences using the selected genetic code table.

Ex 2.3.1: Select the *Distance*|*Compute Pairwise* command (**F7**) to display the distance analysis preferences dialog box.

Ex 2.3.2: In the *Options Summary* tab, click the *Models* pulldown and then select the *Amino Acid*|*p-distance* option.

Ex 2.3.3: Click the "Compute" button to accept the default values for the rest of the options and begin the computation.

Ex 2.3.4: A progress dialog box will appear briefly. As with the previous nucleotide estimation, a results viewer window will be displayed, showing the distances in a grid format.

Ex 2.3.5: After you have inspected the results, use the *File*|*Quit Viewer* command to close the results viewer. To shut down MEGA, select the *File*|*Exit* menu command from the main MEGA application window and indicate that you would like to close the data file.

Constructing Trees and Selecting OTUs from Nucleotide Sequences

The Crab_rRNA.meg file contains nucleotide sequences for the large subunit mitochondrial rRNA gene from different crab species (Cunningham *et al*. 1992). Since the rRNA gene is transcribed, but not translated, it falls in the category of non-coding genes. Let us use this data file to illustrate the procedures of building trees and in-memory sequence data editing, using the commands present in the *Data and Phylogeny* menus.

Ex 3.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 3.1.1: Activate the data file Crab_rRNA.meg using the instructions given in Ex 2.1.1 - Ex 2.1.3.

Let us start by building a neighbor-joining tree.

Ex 3.2.1: Select the *Phylogeny*|*Construct Phylogeny*|*Neighbor-Joining* command to display the analysis preferences dialog box.

Ex 3.2.2: In the Options Summary tab, click the *Models* pull-down (found in the Substitution Model section), and then select the *Nucleotide*|*p-distance* option.

Ex 3.2.3: Click "Compute" to accept the defaults for the rest of the options and begin the computations. A progress indicator will appear briefly before the tree displays in the Tree Explorer.

Ex 3.2.4: To select a branch, click on it with the left mouse button. If you click on a branch with the right mouse button, you will get a small options menu that will let you flip the branch and perform various other operations on it. To edit the OTU labels, double click on them.

Ex 3.2.5: Change the branch style by using the *View*|*Tree/Branch Style* command from the Tree Explorer menu.

Ex 3.2.6: Press the Up arrow key (?) just once to move the cursor upwards to the next branch.

Ex 3.2.7: Select the *View*|*Topology Only* command from the Tree Explorer menu to display the branching pattern (without actual branch lengths on the screen.

Ex 3.2.8: Press F1 to examine the help for tree editor. Use this feature to become familiar with the many operations that Tree Explorer is capable of performing.

Ex 3.2.9: DO NOT remove the tree from the screen. We shall use it for illustrating how a tree can be printed.

Now, you will print the NJ tree that you have on your screen in MEGA.

Ex 3.3.1: Select the *File*|*Print* command from the Tree Explorer menu to bring up a standard Windows print dialog.

Ex 3.3.2: To restrict the size of the printed tree to a single sheet of paper, choose the *File*|*Print in a Sheet* command from the Tree Explorer menu.

Ex 3.3.3: Select the *File*|*Exit* Tree Explorer (Ctrl-Q) command to exit the Tree Explorer. A warning box will inform you that your tree data has not been saved. Click the "OK" button to close Tree Explorer without saving the tree session.

In MEGA, you can also construct Maximum Parsimony (MP) trees. Let us construct a Maximum Parsimony tree(s) by using the *branch-&-bound search* option.

Ex 3.4.1: Select the *Phylogeny* |*Construct Phylogeny* | *Maximum Parsimony* command. In the *Analysis Preference* window, choose the Max-Mini Branch-&-Bound Search option in the MP Tree Search Options tab.

Ex 3.4.2: Click the "Compute" button to accept the defaults for the other options and begin the calculation. A progress window will appear briefly, and the tree will be displayed in Tree Explorer.

Ex 3.4.3: Now print this tree (See Ex 3.3.1 - 3.3.2). You do not have to specify the printer name again, because *MEGA* remembers your selection.

Ex 3.4.4: Select the *File*|*Exit* Tree Explorer (Ctrl-Q) command to exit the Tree Explorer. A warning box will inform you that your tree data has not been saved. Click "OK" to close Tree Explorer without saving the tree session.

Ex 3.4.5: Compare the NJ and MP trees. For this data set, the branching pattern of these two trees is identical.

As an exercise, use the Heuristic Search for finding the MP tree. In this example, you will find the same tree as that obtained by the branch-and-bound method if you use the default option (search factor equal to 2 for all steps of OTU addition). However, the computational time will be much shorter. Actually, in this example, even a search factor equal to 0 will recover the MP tree.

We will now examine how some data editing features work in MEGA. For noncoding sequence data, OTUs as well as sites can be selected for analysis. Let us remove the first OTU from the current data set.

Ex 3.5.1: Select the *Data*|*Setup/Select Taxa & Groups* command. A dialog box is displayed.

Ex 3.5.2: All the OTU labels are checked ( ) in the left box. This indicates that all OTUs are included in the current active data subset. To remove the first OTU from the data, uncheck the checkbox next to the OTU name in the left pane.

Ex 3.5.3: Now, from this data set, construct a neighbor-joining tree (Ex 3.2.1) that contains 12 OTUs instead of 13. To inactivate the operational data set and end the current session of MEGA, press the hot-key Alt + X.

Tests of the Reliability of a Tree Obtained

In this example, we will conduct two different tests using protein-coding genes from the chloroplast genomes of nine different species.

Ex 4.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 4.0.2: Activate the data in the Chloroplast_Martin.meg file by using the *File*|*Open* command.

We will begin with the bootstrap test for the neighbor-joining tree.

Ex 4.1.1: Select the *Phylogeny* |*Bootstrap Test of Phylogeny*|*Neighbor-Joining Tree* command from the main application menu.

Ex 4.1.2: An analysis preferences dialog box appears. Use the *Models* pull-down to ensure that the *Amino Acid*|*p-distance* model is selected. Note that only the *Amino Acid* submenu is available.

Ex 4.1.3: Click "Compute" to accept the default values for the rest of the options. A progress indicator provides the progress of the test as well as the details of your analysis preferences.

Ex 4.1.4: Once the computation is complete, the Tree Explorer appears and displays two tree tabs. The first tab is the original Neighbor-Joining tree, and the second is the Bootstrap consensus tree.

Ex 4.1.5: To produce a condensed tree, use the *Compute*|*Condensed Tree menu* command from the Tree Explorer menu. This tree shows all the branches that are supported at the default cutoff value of *BCL* ≥ 50.

Ex 4.1.6: To change this value, select the *View*|*Options* menu command and click the *cutoff values* tab. Select the *Compute*|*Condensed Tree* menu command and the NJ tree will reappear.

Ex 4.1.7: Print this tree. (see Ex 3.3.1 - Ex 3.3.2)

Ex 4.1.8: Select the *File*|*Exit Tree Explorer* (**Ctrl-Q**) command to exit the Tree Explorer. A warning box will inform you that your tree data has not been saved. Click "OK" to close Tree Explorer without saving the tree session.

For neighbor-joining trees, you may conduct the standard error test for every interior branch by using the *Phylogeny*|*Neighbor-Joining* command. In MEGA, this test is available for the *p*-distance, Poisson Correction, and Gamma distance for amino acid sequences. Since we did the above analysis for the p-distance, we will use the same distance estimation method to compare the results from the bootstrap and standard error tests.

Ex 4.2.1: Go to the *Phylogeny* menu and select the *Construct Phylogeny*|*Neighbor-Joining* command to produce an analysis preferences dialog box. In the *Models preference* pull-down, be sure that p-distance is the model chosen. Click on the *Test of Phylogeny* tab to reveal the test options. Under the *Test of Inferred Phylogeny* option group, check the *Interior Branch Test* option.

Ex 4.2.2: Click "Compute" to begin the computation. A progress indicator will appear briefly. The neighbor-joining tree with confidence probabilities (*CP*) from the standard error test of branch lengths is displayed on the screen.

Ex 4.2.3: Compare the *CP* values on this tree with the *BCL* values of the tree that you printed in the previous procedure.

Ex 4.2.4: Now exit MEGA using the **Alt + X** command.

Working With Genes and Domains

Ex 5.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 5.0.2: Activate the data present in the Contigs.meg file by using the *File*|*Open* command.

We will now examine how to define and edit gene and domain definitions

Ex 5.1.1: Select the *Data*|*Setup/Select Genes & Domains* menu command.

Ex 5.1.2: Delete the Data domain by right clicking on it and selecting *Delete Gene/Domain* from the popup menu.

Ex 5.1.3: Right-click on the Genes/Domains item in the Names column, and select *Add New Domain*. Right-click on the new domain and select Edit Name from the popup menu and set the name to "Exon1."

Ex 5.1.4: Select the ellipsis button next to the question mark in the **From** column to set the first site of the domain. When the site selection window appears, select site number 1 and push the "OK" button.

Ex 5.1.5: Select the ellipsis button in the **To** column to set the last site of the domain. When the site selection window appears, select site number 3918 and push the "OK" button.

Ex 5.1.6: Check the box in the **Coding** column to indicate that this domain is protein coding.

Ex 5.1.7: Add two more domains to the Genes/Domains item. One of these domains will be named "Intron1" and will begin at site 3919 and end at site 5191. The other will be named "Exon2" and will begin at site 5192 and end at site 8421. Be sure to check the checkbox in the **Coding** column for "Exon2" to indicate a protein-coding domain.

Ex 5.1.8: Right-click on the Genes/Domains item and select Insert New Gene from the popup menu. Change the name of this gene to "Predicted Gene," and click-and-drag all of the domains to this new gene such that they are displayed as children of the "Predicted Gene" node in the display tree.

Ex 5.1.9: Press the "Close" button at the bottom of the window to exit the Gene/Domain manager.

We will now use these domain definitions in computing pairwise distances.

Ex 5.2.1: Select the *Distances*|*Compute Pairwise* menu item from the main menu.

Ex 5.2.2: On the *Include Sites* tab, make sure that the "Noncoding sites" option does not have a checkmark next to it. Go back to the main menu and press the "Compute" button to begin the analysis.

Ex 5.2.3: When the computation is complete the Distance Explorer will display the pairwise distance computed using only the sequence data from exonic domains of the "Predicted Gene."

Test of Positive Selection

In this example, we present various analyses of protein-coding nucleotide sequences for five alleles from the human HLA-A locus (Nei and Hughes 1991).

Ex 6.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 6.1.1: Activate the data present in the **HLA_3Seq.meg** file by using the *File*|*Open* command.

Ex 6.1.2: Now that the data file is active, note that various details about the data file are displayed at the bottom of the main application window, and more menu items have become available on the main menu.

Let us compute the synonymous and nonsynonymous distances appropriate for studying positive Darwinian selection in this set of antigen recognition codons.

Ex 6.2.1: Select the *Selection*|*Codon-based Z-Tests* from the menu command. An analysis preferences dialog appears. Use the *Models* pull-down in the *Options Summary tab* to select *Syn-Nonsysnonymous*|*Nei-Gojobori Method*|*p-distance* model. In the *Test Hypothesis (HA: alternative)* tab, select *Positive Selection (HA: dN > dS)* from the pull-down, and select the *Overall Average* from the *Analysis Scope* tab. Click the *GAPS/Missing Data* tab and make sure that the *Pairwise Deletion* option is selected.

Ex 6.2.2: Click on "Compute" to accept the default values for the remaining options. A progress indicator appears briefly; the computation results are displayed in a results window in grid format.

Ex 6.2.3: The *Prob* column contains the probability computed (must be <0.05 for hypothesis rejection at 5% level), and the *Stat* column contains the statistic used to compute the probability. The difference in synonymous and nonsynonymous substitutions should be significant at the 5% level.

Ex 6.2.4: Exit MEGA and deactivate the active data file using the **Alt** + **X** command.

Managing Taxa with Groups

Ex 7.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 7.0.2: Activate the data present in the **Crab_rRNA.meg** file by using the *File*|*Open* command.

We will now examine how to define and edit groups of taxa.

Ex 7.1.2: Select the *Data*|*Setup/Select Taxa & Groups* menu command.

Ex 7.1.3: Press the "New Group" button found below the Taxa/Groups pane to add a new group to the data. Name this new group "Pagurus."

Ex 7.1.4: While holding the Control button on the keyboard, click on all of the Pagurus species in the Ungrouped Taxa pane to highlight them. When they are all highlighted, press the left-facing arrow button found on the vertical toolbar between the two windowpanes.

Ex 7.1.5: Select the "All" group in the Taxa/Groups pane and press the "**New Group**" button to add a second group. Name this group "Non-Pagurus." Add the remaining unassigned taxa to this group and press the "**Close**" button at the bottom of the window to exit this view.

Ex 7.1.6: Now that groups have been defined, the *Compute Within Group Mean*, *Compute Between Group Means*, and *Compute Net Between Group Means* menu commands from the *Distance* menu item may be used to analyze the data.

Computing Statistical Quantities for Nucleotide Sequences

In this exercise, we illustrate the use of the *Data Explorer* for computing various statistical quantities of nucleotide sequences. In addition, we explain shortcuts for obtaining frequently used commands, methods of accessing on-line help, and the distinction between enabled and disabled commands.

Ex 8.0.1: Start *MEGA* by double-clicking on the *MEGA* desktop icon, or by using the Windows start-menu to click on the *MEGA* icon located in the programs folder.

We now will examine the contents of the file Drosophila_Adh.meg by using the built-in *Text Editor*.

Ex 8.1.1: Click on the *File* menu item to expand the menu options. To activate the text editor, either click *File* |*Text Editor* or press the **F3** key on your keyboard. In the text editor, use the *File*|*Open* command to open the **Drosophila_Adh.meg** file.

Ex 8.1.2: Examine the **Drosophila_Adh.meg** file. Take note of the #mega format specifier, title, OTU names, and the interleaved sequence data.

Ex 8.1.3: We advise that you exit the text editor before proceeding with data analysis. Select the *File* menu item from the text editor's menu, and click the *Exit* option from the expanded menu. If the editor asks you if you would like to save the changes that you have made to the file, select *No*.

To study statistical quantities of the data in the file Drosophila_Adh.meg, we must first activate it.

Ex 8.2.1: You can activate a data file using the link titled "Click me to activate a data file" in the main application window, or select the *File* menu item from the main menu and click the *Open Data* option from the expanded menu. You may also press the **F5** key on your keyboard. All of these methods will display a standard Windows open file dialog box.

Ex 8.2.2: Open the **Drosophila_Adh.meg** data file under the **Examples** folder.

Ex 8.2.3: A progress dialog box will appear briefly. When the data file is active, details about it are displayed at the bottom of the main application window. More menu items now are available on the main menu.

Examine the main menu. Now that the data file is active, the menu items *Data, Distances, Pattern,* and *Selection* have become available.

We now will use Data Explorer to compute some basic statistics for these data.

Ex 8.3.1: Select the *Data*|*Data Explorer* command, or press the **F4** key if the *Sequence Data Viewer* is not available.

Ex 8.3.2: DNA sequences are displayed on the screen in a grid format. Use the left and right arrow keys (??) or the mouse to move from site to site; note a change in the bottom-left corner of the display. Use the up and down (??) arrow keys or the mouse to move between OTUs. The *Total Sites* view on the bottom-left panel displays the sequence length under the current site position, and the Highlighted *Sites* display "None" because no special site attributes are yet highlighted.

Ex 8.3.3: To highlight variable sites, select the *Highlight*|*Variable Sites* option, click the button labeled "V" from the shortcut bar below the menu, or press the **V** key. All sites that are variable are highlighted, and the number in the Highlighted *Sites* displays changes. When you press **V** again, the sites return to the normal color, and *Highlighted Sites* displays "None."

Ex 8.3.4: Now to highlight the parsimony-informative, press the **P** key, click on the button labeled "Pi" from the shortcut bar below the menu, or select the *Highlight/Parsim-info* sites menu command. To highlight 0, 2, and 4-fold degenerate sites, press the **0**, **2**, or **4** keys from the *Sequence Data Explorer*, respectively, click on the corresponding button from the shortcut bar below the menu, or select the corresponding command from the highlight menu.

Ex 8.3.5: To compute the nucleotide base frequencies, select the *Statistics*|*Nucleotide Composition* menu command. This will calculate the composition and display the results of the calculation in a text file using the built-in text editor.

Ex 8.3.6: To compute codon usage, select the *Statistics*|*Codon Usage* menu command. This will calculate the codon usage and display the results of the calculation in a text file using the built-in text editor.

Ex 8.3.7: To compute nucleotide pair frequencies, select the *Statistics*|*Nucleotide Pair Frequencies*|*Directional,* or the *Statistics*|*Nucleotide Pair Frequencies*|*Unidirectional* menu command. This will calculate the pair frequencies and display the results of the calculation in a text file using the built-in text editor.

Ex 8.3.8: To translate these protein-coding sequences into amino acid sequences and back, press the **T** key, or select the *Data*|*Translate/Untranslate* menu command from the Data Explorer menu.

Ex 8.3.9: Once the sequences are translated, calculate the amino acid composition by selecting the *Statistics*|*Amino Acid Composition* menu command from the *Data Explorer* Menu.

Ex 8.3.10: To shut down MEGA, select the *File*|*Exit* menu command from the main MEGA application window and close the data file.

Constructing Trees from Distance Data

This example introduces procedures for selecting options from menus, opening files in the read-only mode, activating a distance data file, and building trees from the distance data.

Ex 9.0.1: Start MEGA by double-clicking on the MEGA desktop icon, or by using the Windows start-menu to click on the MEGA icon located in the programs folder.

Ex 9.0.2: A *Splash screen* appears which displays the current version of MEGA.

Ex 9.0.3: The *Splash screen* automatically disappears, and the MEGA application becomes available.

In this example, we use the data in the Hum_Dist.meg file. Although we will not edit the file, we will use MEGA's built-in text editor to examine its contents before we proceed further.

Ex 9.1.1: Click on *File* menu to expand the menu options. Click on the menu item labeled *Text Editor,* or press the **F3** key to activate the built-in text editor.

Ex 9.1.2: Use the *Text Editor* to view the contents of the **Hum_Dist.meg** file. To open a file with the *Text Editor,* click on the folder icon below the main menu or on the *File* menu item, then choose *Open* from the expanded menu. You may also use the key combination **Ctrl+O** to open a file. All of these options will lead you to a standard Windows open file dialog box. Use this dialog box to locate the **Examples** folder found in the MEGA installation directory, and open the **Hum_Dist.meg** file. After you open the file with the dialog box, you will see the file contents displayed in the *Text Editor* window.

Ex 9.1.3: Examine the contents of the data file and then exit the *Text Editor* before proceeding with data analysis. Select the *File* menu item from the *Text Editor's* menu, and click *Exit* from the expanded menu. If the editor asks you if you would like to save your changes, select *No*. A data file must be activated before an analysis can be performed. (Note that opening a file for browsing or editing is different from activating it for analysis).

Now we will activate the Hum_Dist.meg data file.

Ex 9.2.1: You can activate a data file by using the link titled "Click me to activate a data file" in the main application window, or by selecting the *File* menu item from the main menu and clicking the *Open Data* option from the expanded menu. You can also press the **F5** key. All of these methods will display a standard Windows open file dialog box.

Ex 9.2.2: Use the open file dialog box to locate and open the **Hum_Dist.meg** file located in the **Examples** folder. After you have selected the file for opening, a progress indicator will appear briefly.

Ex 9.2.3: When the data file is active, the *Input Distance Data Viewer* is launched to display the contents of the data file.

We will now make a phylogenetic tree from the distance data.

Ex 9.3.1: Switch back to main MEGA application window. From the expanded menu in the *Phylogeny* menu, select the *Construct Phylogeny***|***Neighbor-Joining* command.

Ex 9.3.2: A confirmation *Analysis Preferences* window will appear, indicating that MEGA is ready to conduct the requested analysis. Click on the button labeled "Compute." A progress meter will appear briefly.

Ex 9.3.3: The *Tree Explorer* will display a neighbor-joining tree on the screen when the analysis completes. To exit the *Tree Explorer*, select the *File* menu item from the *Tree Explorer* menu and click the *Exit Tree Explorer* option from the expanded menu. The *Tree Explorer* will ask you if you would like to save the tree data. If you save the tree, you can use *Tree Explorer* to view and manipulate it in the future.

With this, let us end this session of MEGA.

Ex 9.4.1: Go to the *File* menu and click on the *Close Data* command. The program will inquire if you would like the active data to be closed. Select "Yes."

Ex 9.4.2: To exit MEGA, press **Alt** + **X,** or select the *Exit* command from the expanded *File* menu.