MEGA-CC (COMPUTE CORE) AND MEGA-PROTO

Quick Start Tutorial

OVERVIEW…

- MEGA-CC (Molecular Evolutionary Genetics Analysis Computational Core) is an integrated suite of tools for statistics-based comparative analysis of molecular sequence data based on evolutionary principles (Tamura et al. 2011, Kumar et al. 2012). MEGA is used by biologists for reconstructing the evolutionary histories of species and inferring the extent and nature of selective forces shaping the evolution of genes and species.

…OVERVIEW CONTINUED

- MEGA-CC has 2 components
  - MEGA-Proto, an analysis prototyper that is used for generating analysis options files which tell megacc which analysis to run and which options to use. On Windows it is launched from the start menu. On Linux it is launched from a terminal using the *megaproto* command. On Mac it is launched from the *Applications* folder.
  - A command-line executable that performs all calculations. This executable is launched from a terminal using the *megacc* command.

DOCS AND EXAMPLE DATA FILES

- The installers for MEGA-CC copy doc files and example data files to OS-specific locations
  - For Windows users - *%HOMEPATH%\Documents\megacc*
  - For Linux users - */usr/share/megacc*
  - For Mac users - *~/Documents/megacc*
- For Linux and Mac users a man page is included with MEGA-CC. From a terminal:
  - man megacc
- The doc files and example data files are also available from the mega website:
  - http://www.megasoftware.net/webhelp/helpfile.htm
  - http://www.megasoftware.net/examples.php

MEGA-CC INPUT FILES

- MEGA Analysis Options file
  - Specifies the calculation and desired settings.
  - Created using MEGA-Proto.
  - Has a *.mao* file extension.
- Data file (one of the following)
  - Multiple sequence alignment in MEGA or Fasta format.
  - Distance matrix in MEGA format.
  - Unaligned sequences in Fasta format (for alignment only).
- Newick tree file (required for some analyses)
- Calibrations file (for timetree analysis – but it's optional)
- Groups file (optional)

MEGA-CC OUTPUT FILES

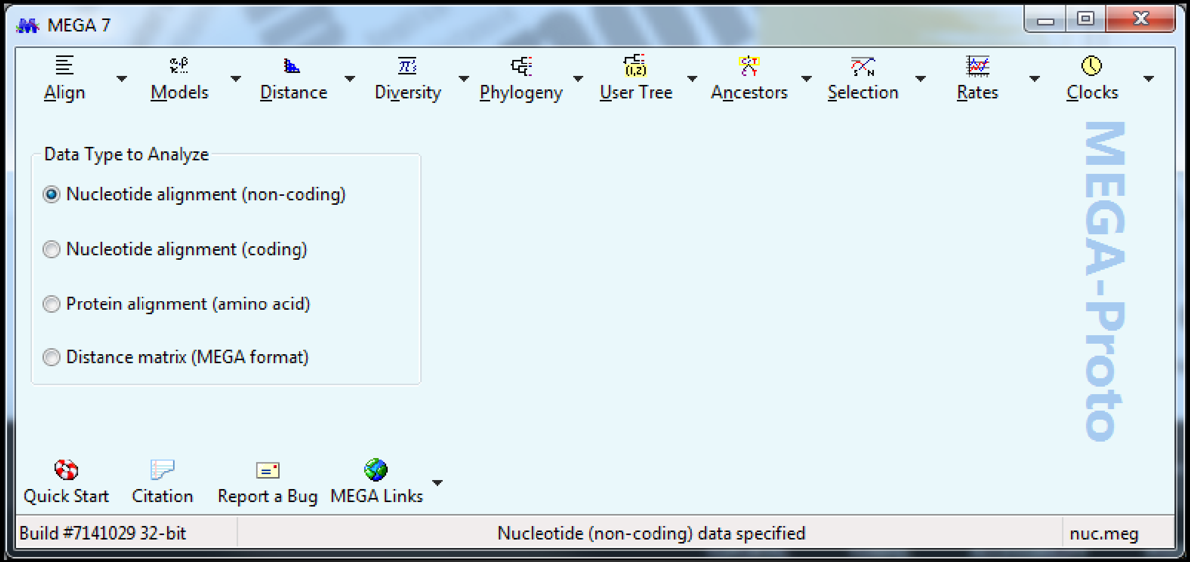
- In general, two output files are produced
  1. Calculation-specific results file (Newick file, distance matrix,…).
  2. A summary file with additional info (likelihood, SBL,…).
  - Some analyses produce additional output (bootstrap consensus tree, csv files, etc…).
- Output directory/filename
  - Default is the same location as the input data file.
  - Specify an output directory and/or file name using *-o* option.
  - If no output filename is specified, MEGA-CC will assign a unique name.
- Errors/warnings
  - If MEGA-CC produces any errors or warnings, they will be logged in the the summary file.

RUNNING MEGA-CC

- Easiest to run using command-line or batch scripts:
  - `megacc –a settings.mao –d alignment.meg –o outFile`
- Can also be run using custom scripts (Perl, Python, …):
  - `exec(‘megacc –a options.mao –d alignment.meg –o outFile’);`
- Integrated *File Iterator* system can process multiple files without the need for using scripts (see Demo2 below)
- In addition, other applications can launch MEGA-CC:
  - `status = CreateProcess(“path/to/megacc…”);`
- To see a list of available command options, call megacc from a command-line prompt with the –h flag (Unix users can view the man page).

MEGA-PROTO (ANALYSIS PROTOTYPER)

- Has the same look and feel as the GUI edition of MEGA.
- Produces MEGA Analysis Options files.
- Has no computational capabilities.



Data Type to Analyze

● Nucleotide alignment (non-coding)

○ Nucleotide alignment (coding)

○ Protein alignment (amino acid)

○ Distance matrix (MEGA format)

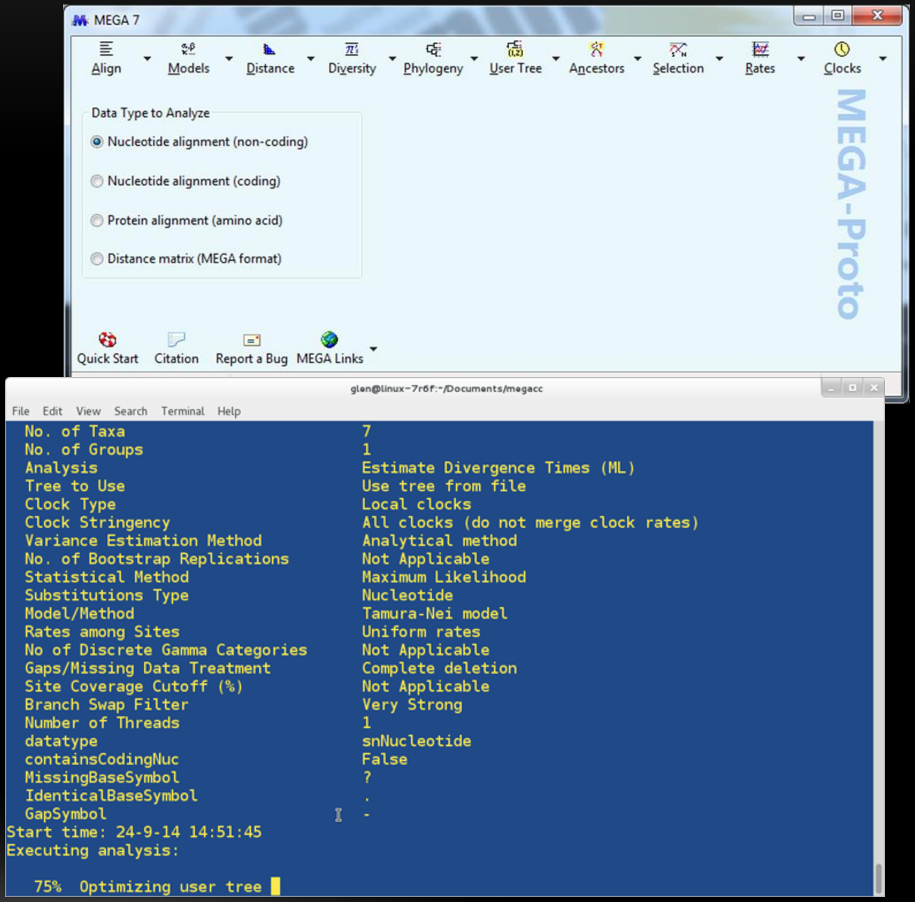
Build #7141029 32-bit | Nucleotide (non-coding) data specified | nuc.meg

USING MEGA-PROTO

1. Select input data type.
    - Nucleotide (non-coding)
    - Nucleotide (coding)
    - Protein (amino-acid)
    - Distance matrix (MEGA format)
2. Select analysis from menu.
3. Adjust analysis settings.
4. Click *Save Settings*… and save the MEGA Analysis Options (*.mao) file to your computer.

DEMO 1

- The following example demonstrates how to create a timetree using MEGA-Proto and MEGA-CC



Data Type to Analyze

● Nucleotide alignment (non-coding)
○ Nucleotide alignment (coding)
○ Protein alignment (amino acid)
○ Distance matrix (MEGA format)

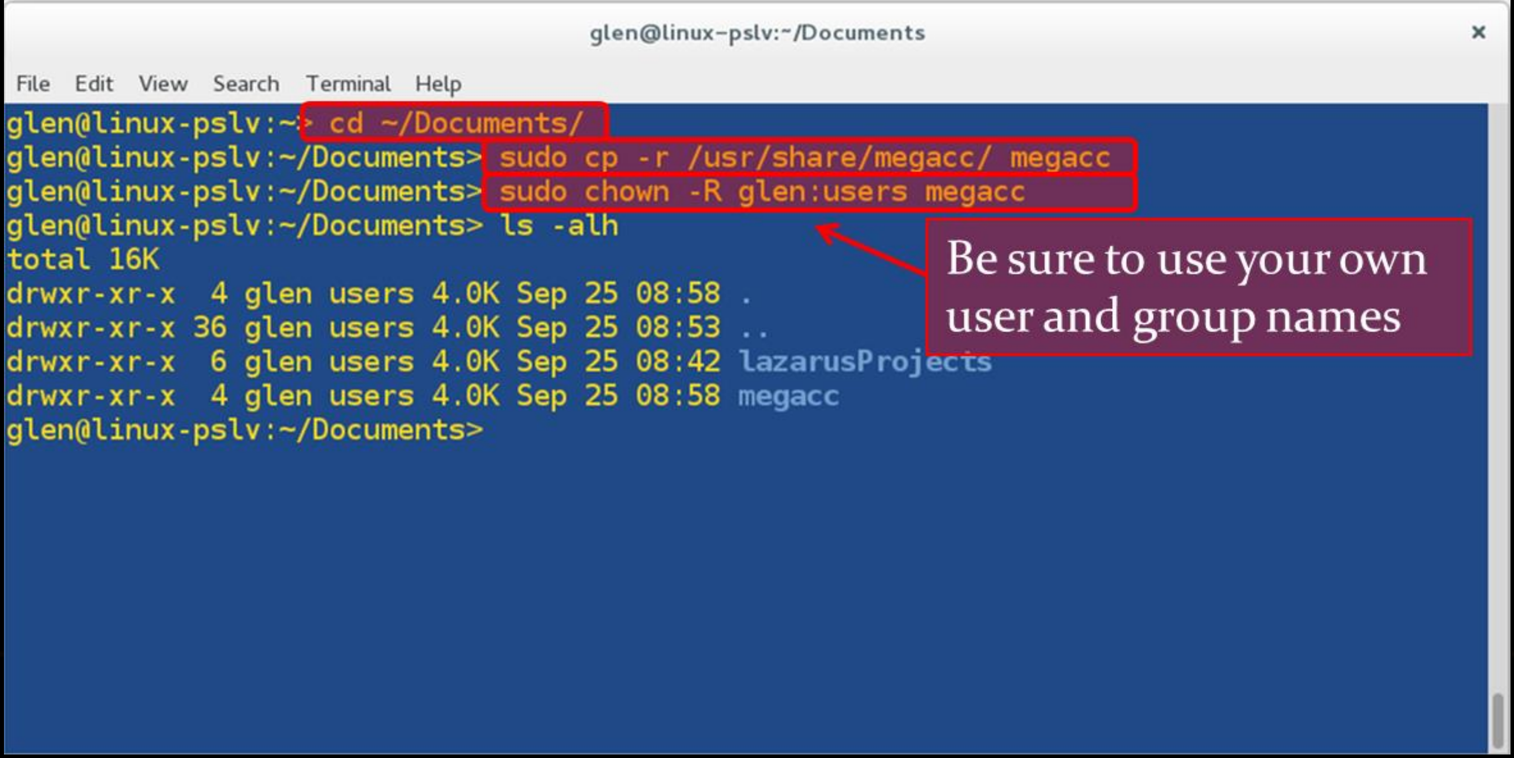
```
  No. of Taxa                           7
  No. of Groups                         1
  Analysis                              Estimate Divergence Times (ML)
  Tree to Use                           Use tree from file
  Clock Type                            Local clocks
  Clock Stringency                      All clocks (do not merge clock rates)
  Variance Estimation Method            Analytical method
  No. of Bootstrap Replications         Not Applicable
  Statistical Method                    Maximum Likelihood
  Substitutions Type                    Nucleotide
  Model/Method                          Tamura-Nei model
  Rates among Sites                     Uniform rates
  No of Discrete Gamma Categories       Not Applicable
  Gaps/Missing Data Treatment           Complete deletion
  Site Coverage Cutoff (%)              Not Applicable
  Branch Swap Filter                    Very Strong
  Number of Threads                     1
  datatype                              snNucleotide
  containsCodingNuc                     False
  MissingBaseSymbol                     ?
  IdenticalBaseSymbol                   .
  GapSymbol                             -
Start time: 24-9-14 14:51:45
Executing analysis:

   75%  Optimizing user tree
```

DEMO 1 DATA FILES

- For this demo, we will be using some of the example data files that were copied to your computer by the installer
- For Windows users, the files are located in your %HOMEPATH%\Documents\megacc directory
- For Linux users, the files are located in your */usr/share/megacc* directory
- For Mac users, the files are located in your *~/Documents/megacc* directory

DEMO 1 SETUP (LINUX ONLY)

- If you are using Linux (Windows and Mac users can skip this), we want to move the example data files to a more accessible location and change ownership (currently owned by root). Execute the following commands to move the files into your Documents directory

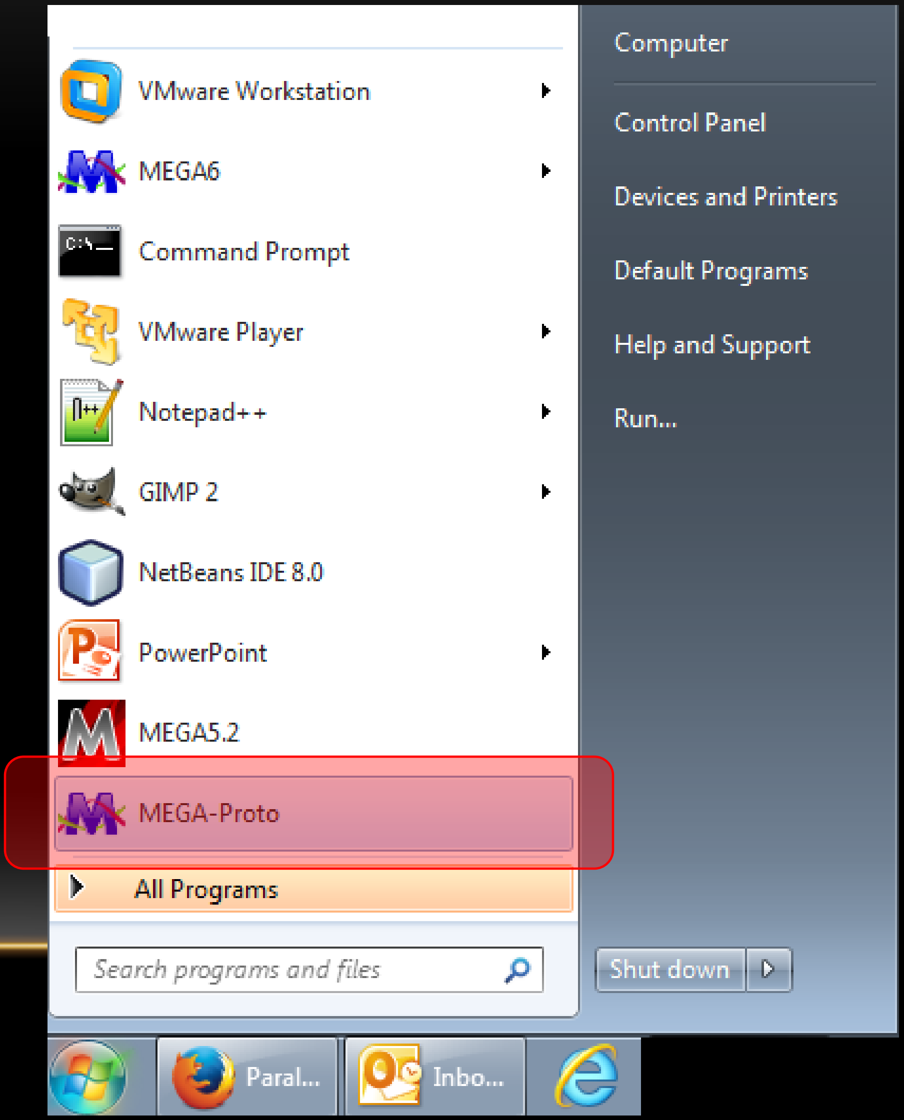


```
glen@linux-pslv:~> cd ~/Documents/
glen@linux-pslv:~/Documents> sudo cp -r /usr/share/megacc/ megacc
glen@linux-pslv:~/Documents> sudo chown -R glen:users megacc
glen@linux-pslv:~/Documents> ls -alh
total 16K
drwxr-xr-x  4 glen users 4.0K Sep 25 08:58 .
drwxr-xr-x 36 glen users 4.0K Sep 25 08:53 ..
drwxr-xr-x  6 glen users 4.0K Sep 25 08:42 lazarusProjects
drwxr-xr-x  4 glen users 4.0K Sep 25 08:58 megacc
glen@linux-pslv:~/Documents>
```

Be sure to use your own user and group names

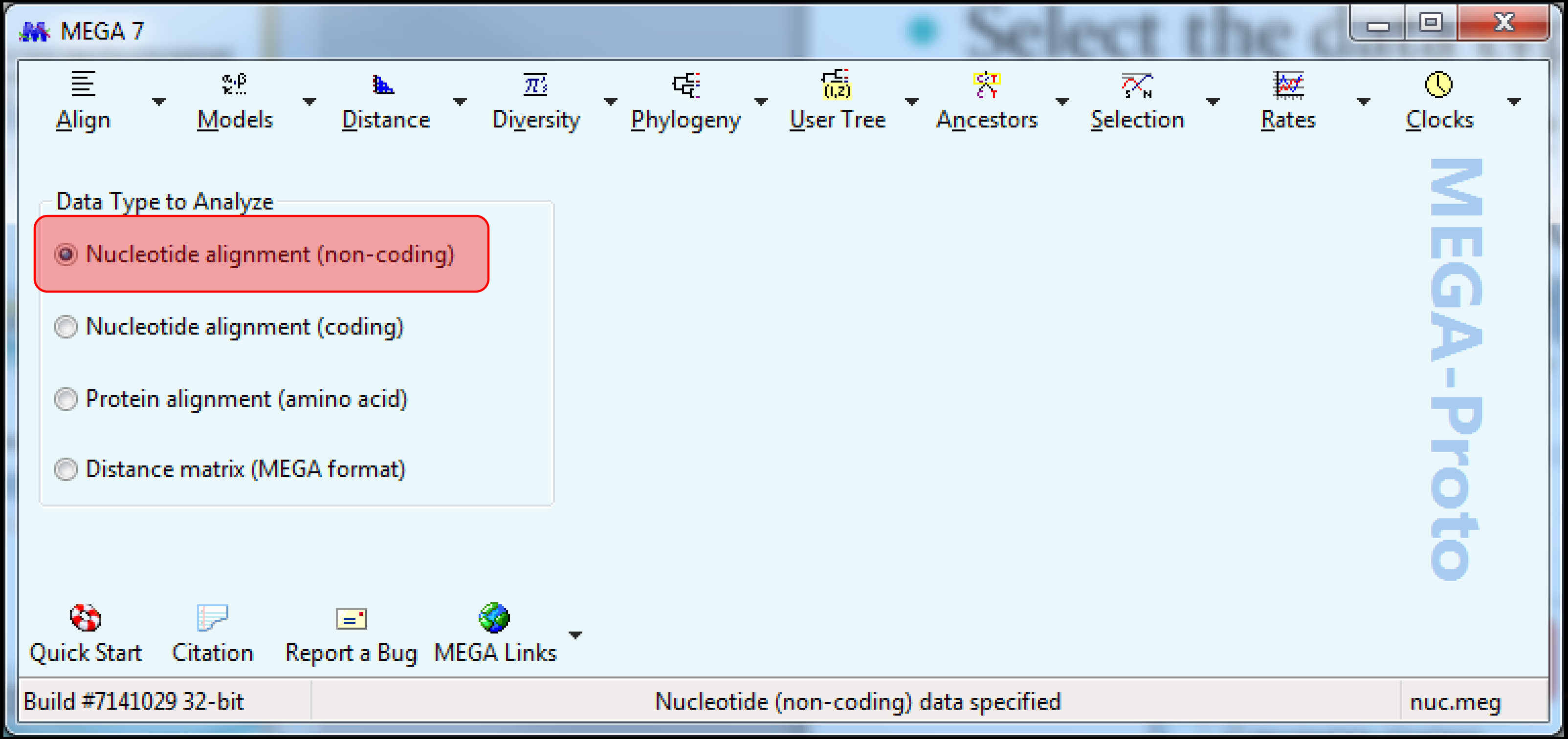
STEP 1

- Windows - Open MEGA-Proto by selecting MEGA-Proto from the Start Menu.
- Linux – Open MEGA-Proto by entering the megaproto command in a terminal window.
- Mac – Open MEGA-Proto by double-clikcing it in your Applications folder.



STEP 2

- Select the data type of the input data file to be analyzed. For this demo, we will accept the default setting - Nucleotide (non-coding).



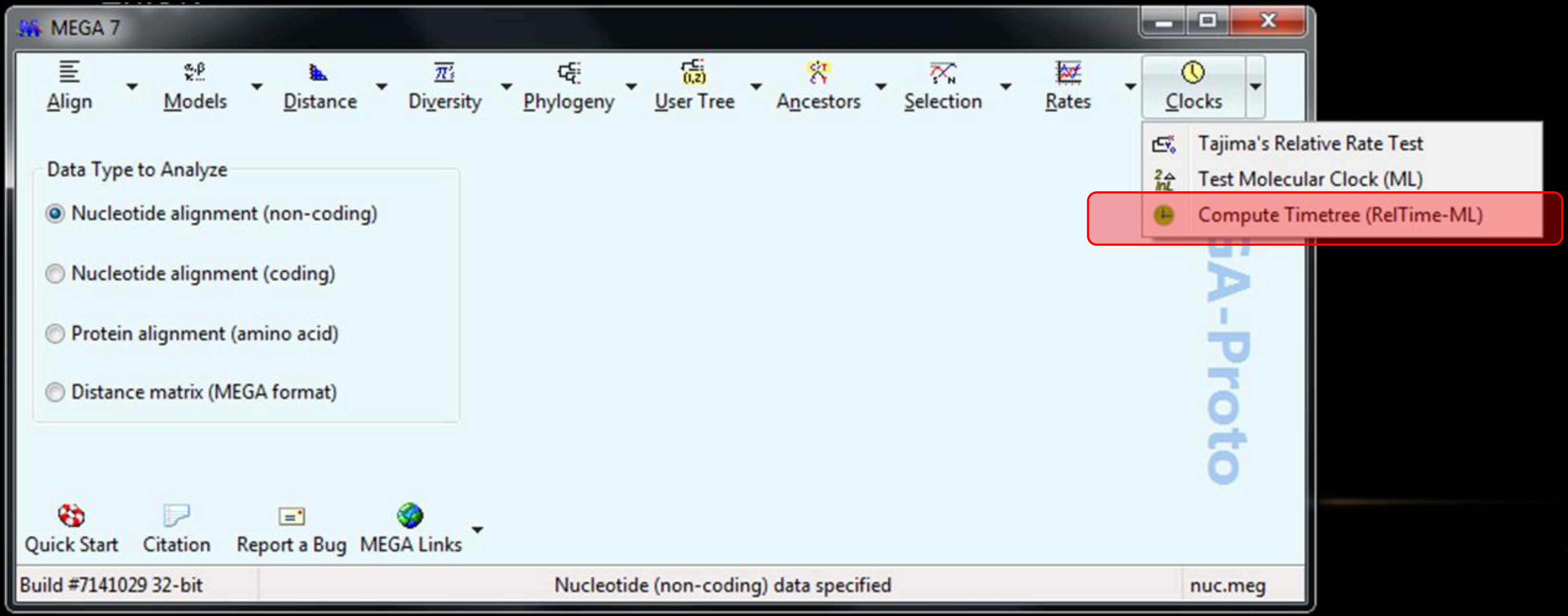
Data Type to Analyze

● Nucleotide alignment (non-coding)
○ Nucleotide alignment (coding)
○ Protein alignment (amino acid)
○ Distance matrix (MEGA format)

Build #7141029 32-bit | Nucleotide (non-coding) data specified | nuc.meg

STEP 3

- Select *Compute Timetree (Reltime ML)* from the *Clocks* menu.



Data Type to Analyze

● Nucleotide alignment (non-coding)

○ Nucleotide alignment (coding)

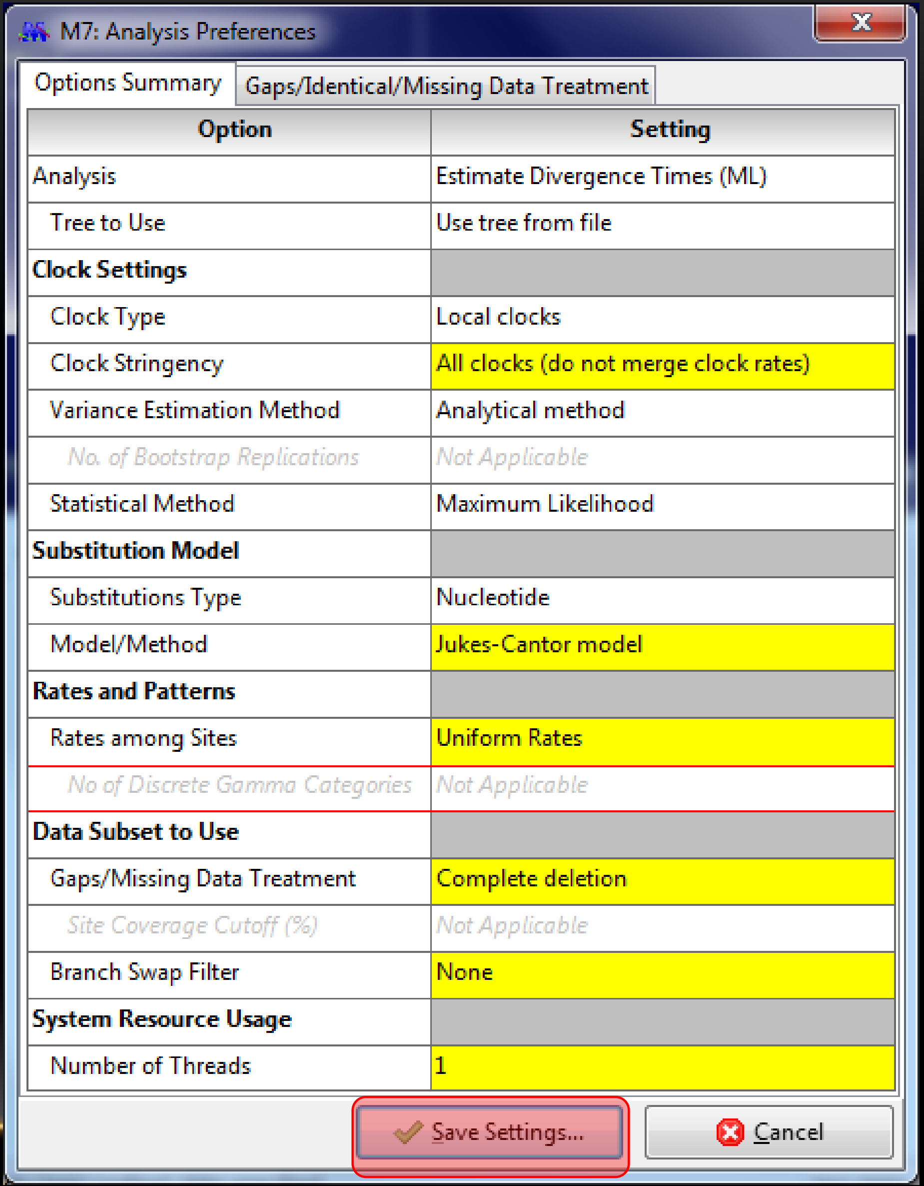
○ Protein alignment (amino acid)

○ Distance matrix (MEGA format)

Build #7141029 32-bit | Nucleotide (non-coding) data specified | nuc.meg

STEP 4

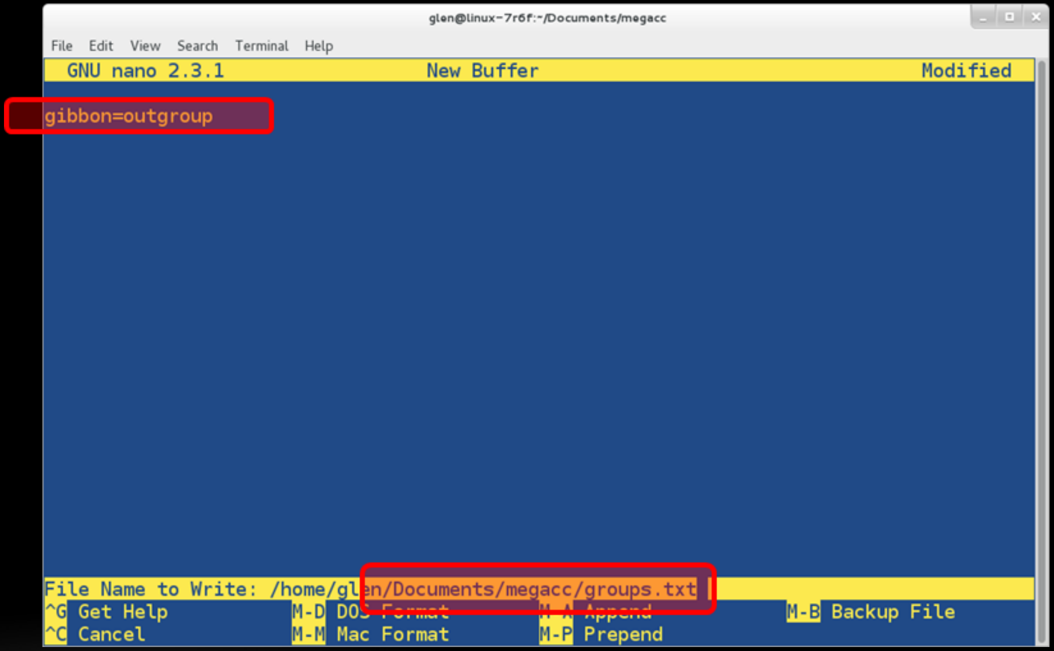
- Adjust the analysis preferences to match those shown.
- Click the *Save Settings…* button and save the analysis options file as *demoSettings.mao* in the megacc\examples directory.



Option	Setting
Analysis	Estimate Divergence Times (ML)
Tree to Use	Use tree from file
Clock Settings	
Clock Type	Local clocks
Clock Stringency	All clocks (do not merge clock rates)
Variance Estimation Method	Analytical method
No. of Bootstrap Replications	*Not Applicable*
Statistical Method	Maximum Likelihood
Substitution Model	
Substitutions Type	Nucleotide
Model/Method	Jukes-Cantor model
Rates and Patterns	
Rates among Sites	Uniform Rates
No of Discrete Gamma Categories	*Not Applicable*
Data Subset to Use	
Gaps/Missing Data Treatment	Complete deletion
Site Coverage Cutoff (%)	*Not Applicable*
Branch Swap Filter	None
System Resource Usage	
Number of Threads	1

STEP 5

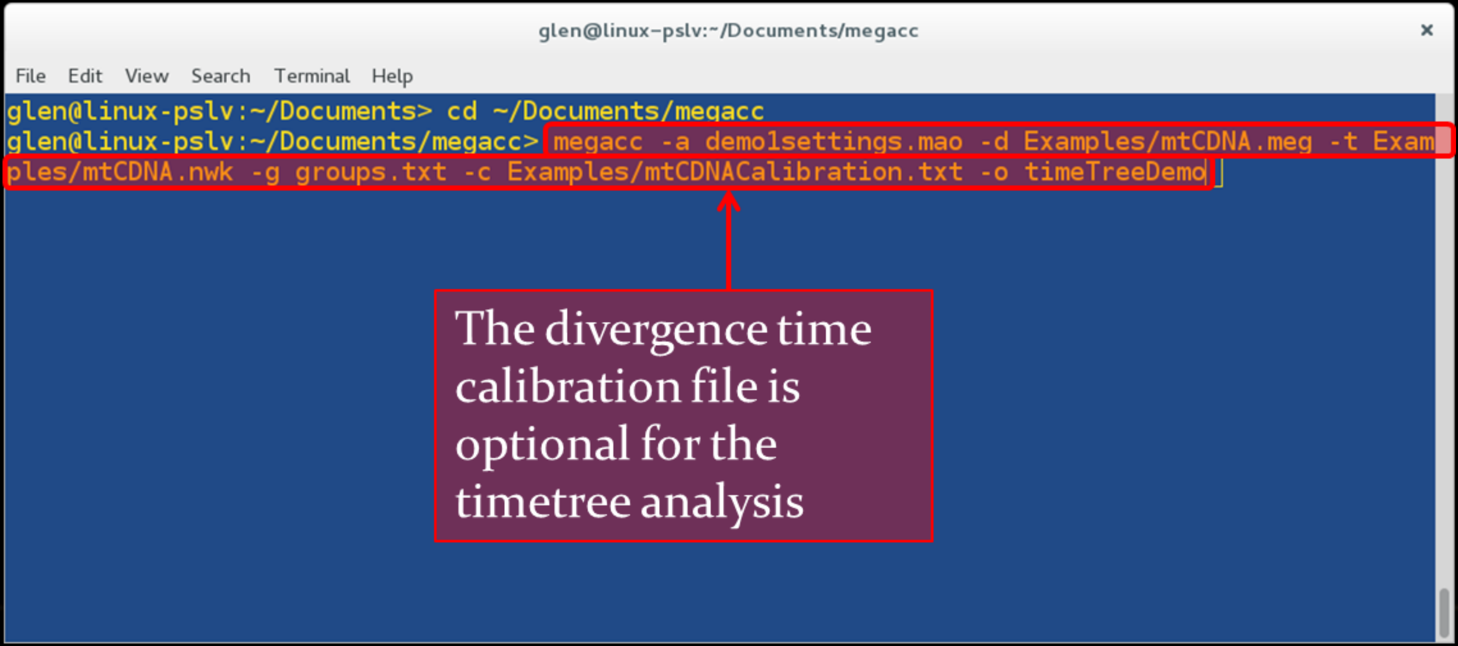
- The timetree analysis requires that we specify an outgroup. To do so, create a text file and add the line ‘gibbon=outgroup’. Save this file as *groups.txt* in the *megacc* directory.



```
  GNU nano 2.3.1                  New Buffer                          Modified

gibbon=outgroup

File Name to Write: /home/glen/Documents/megacc/groups.txt
^G Get Help          M-D DOS Format        M-A Append           M-B Backup File
^C Cancel            M-M Mac Format        M-P Prepend
```

STEP 6

- Open a command terminal and navigate to ~/Documents/megacc using the cd command
- Execute the analysis by calling megacc as follows:

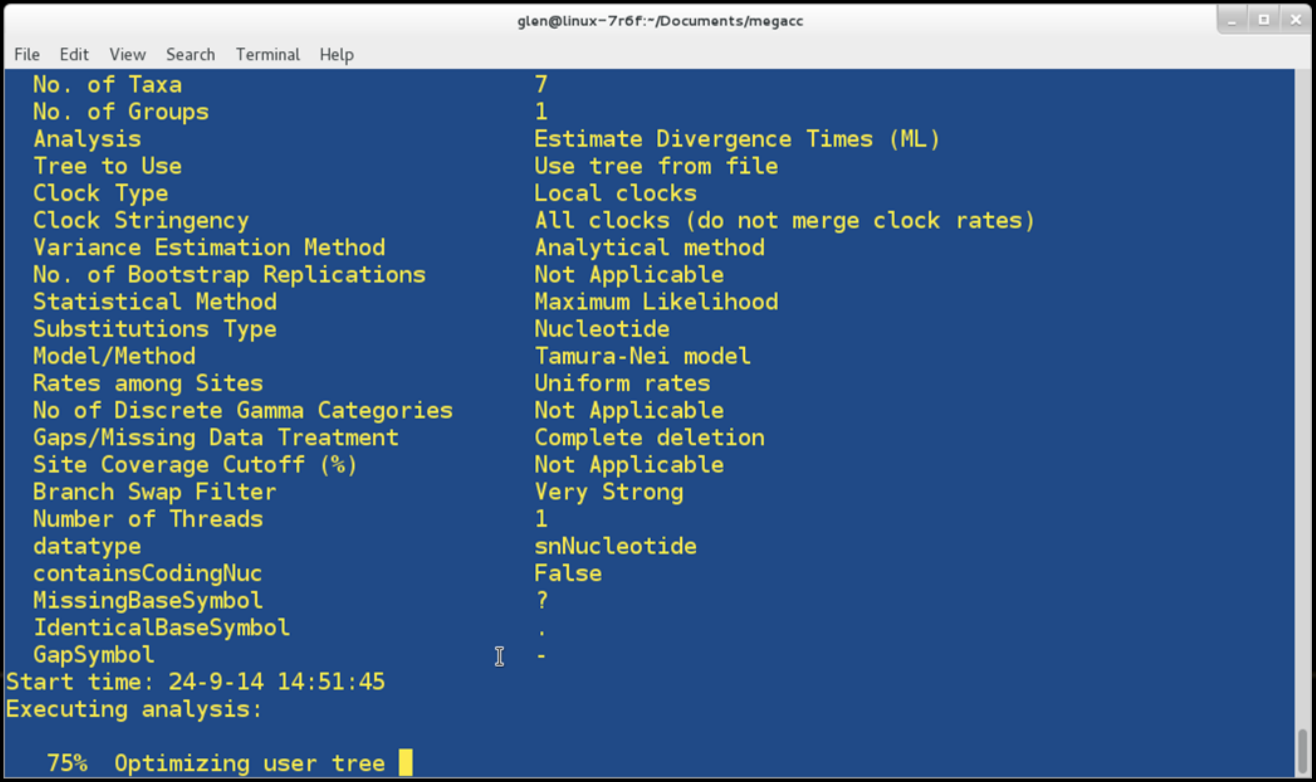


```
glen@linux-pslv:~/Documents> cd ~/Documents/megacc
glen@linux-pslv:~/Documents/megacc> megacc -a demo1settings.mao -d Examples/mtCDNA.meg -t Exam
ples/mtCDNA.nwk -g groups.txt -c Examples/mtCDNACalibration.txt -o timeTreeDemo
```

The divergence time calibration file is optional for the timetree analysis

STEP 7

- The analysis will be launched and progress updates will be displayed in the command prompt window.



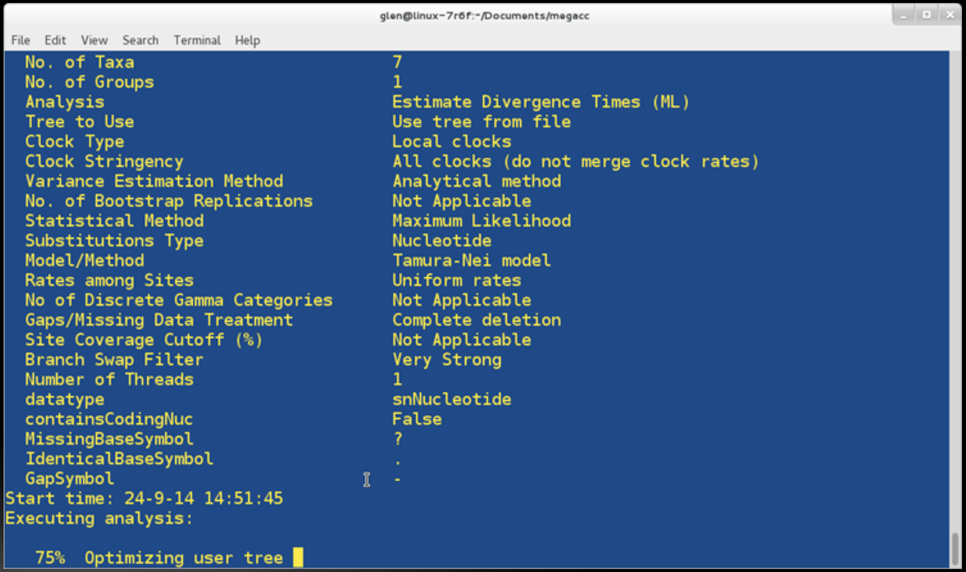
```
  No. of Taxa                         7
  No. of Groups                       1
  Analysis                            Estimate Divergence Times (ML)
  Tree to Use                         Use tree from file
  Clock Type                          Local clocks
  Clock Stringency                    All clocks (do not merge clock rates)
  Variance Estimation Method          Analytical method
  No. of Bootstrap Replications       Not Applicable
  Statistical Method                  Maximum Likelihood
  Substitutions Type                  Nucleotide
  Model/Method                        Tamura-Nei model
  Rates among Sites                   Uniform rates
  No of Discrete Gamma Categories     Not Applicable
  Gaps/Missing Data Treatment         Complete deletion
  Site Coverage Cutoff (%)            Not Applicable
  Branch Swap Filter                  Very Strong
  Number of Threads                   1
  datatype                            snNucleotide
  containsCodingNuc                   False
  MissingBaseSymbol                   ?
  IdenticalBaseSymbol                 .
  GapSymbol                           -
Start time: 24-9-14 14:51:45
Executing analysis:

   75%  Optimizing user tree
```

FINALLY

- The analysis will produce several output files in the directory megacc\examples\M7CC_Out
  - mtCDNA-xxxx_exactTimes.nwk
    - This Newick file gives the timetree scaled according to the estimated divergence times.
  - mtCDNA-xxxx_relTimes.nwk
    - This Newick file gives the timetree scaled according to the estimated relative divergence times.
  - mtCDNA-xxxx.txt
    - This text file gives a more detailed representation of the timetree, including relative times, exact times, evolutionary rates, and divergence time std errors.
  - mtCDNA-xxxx_summary.txt
    - This file gives analysis information such as the log likelihood value of the Maximum Likelihood tree, ts/tv ratio, etc…

DEMO 2

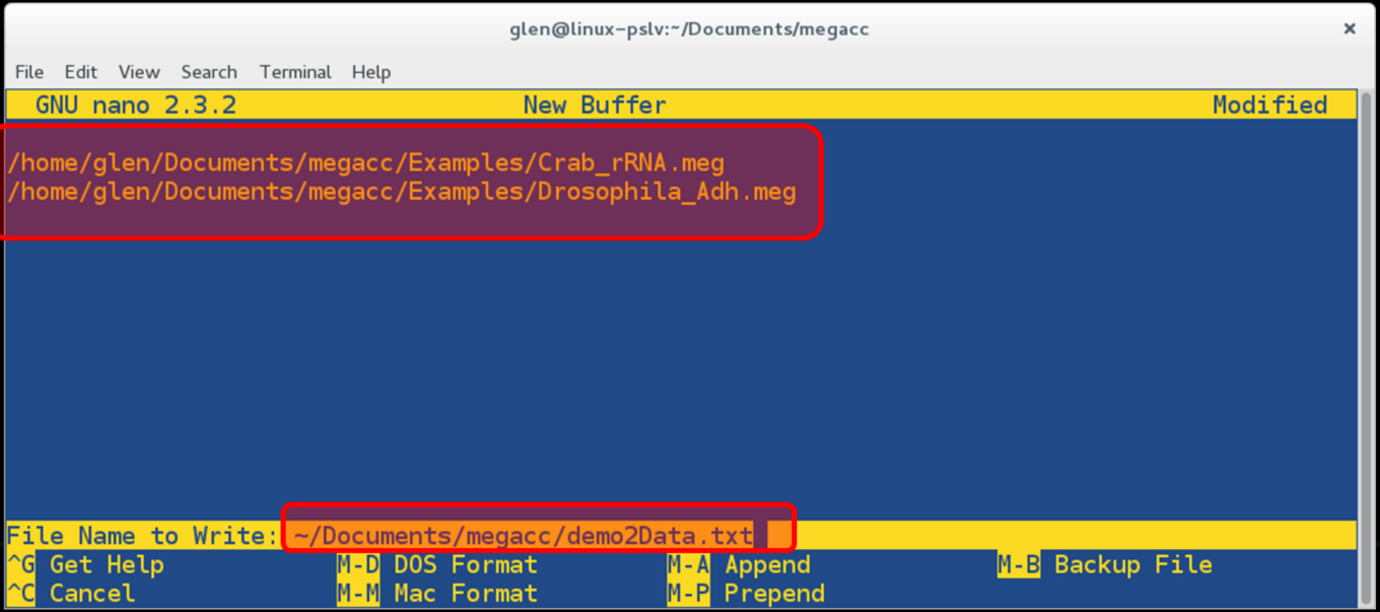
- The following example demonstrates how to use the File Iterator system in MEGA-CC to process multiple input data files using a single analysis options file.



```
 No. of Taxa                          7
 No. of Groups                        1
 Analysis                             Estimate Divergence Times (ML)
 Tree to Use                          Use tree from file
 Clock Type                           Local clocks
 Clock Stringency                     All clocks (do not merge clock rates)
 Variance Estimation Method           Analytical method
 No. of Bootstrap Replications        Not Applicable
 Statistical Method                   Maximum Likelihood
 Substitutions Type                   Nucleotide
 Model/Method                         Tamura-Nei model
 Rates among Sites                    Uniform rates
 No of Discrete Gamma Categories      Not Applicable
 Gaps/Missing Data Treatment          Complete deletion
 Site Coverage Cutoff (%)             Not Applicable
 Branch Swap Filter                   Very Strong
 Number of Threads                    1
 datatype                             snNucleotide
 containsCodingNuc                    False
 MissingBaseSymbol                    ?
 IdenticalBaseSymbol                  .
 GapSymbol                            -
Start time: 24-9-14 14:51:45
Executing analysis:

   75%  Optimizing user tree
```

STEP 1

- Create a text file named demo2Data.txt which we will use to specify multiple alignment files for ML phylogeny inference. Save this file in the *megacc* directory.
- In this file, add the full paths to the Crab_rRNA.meg and Drosophila_Adh.meg example files.

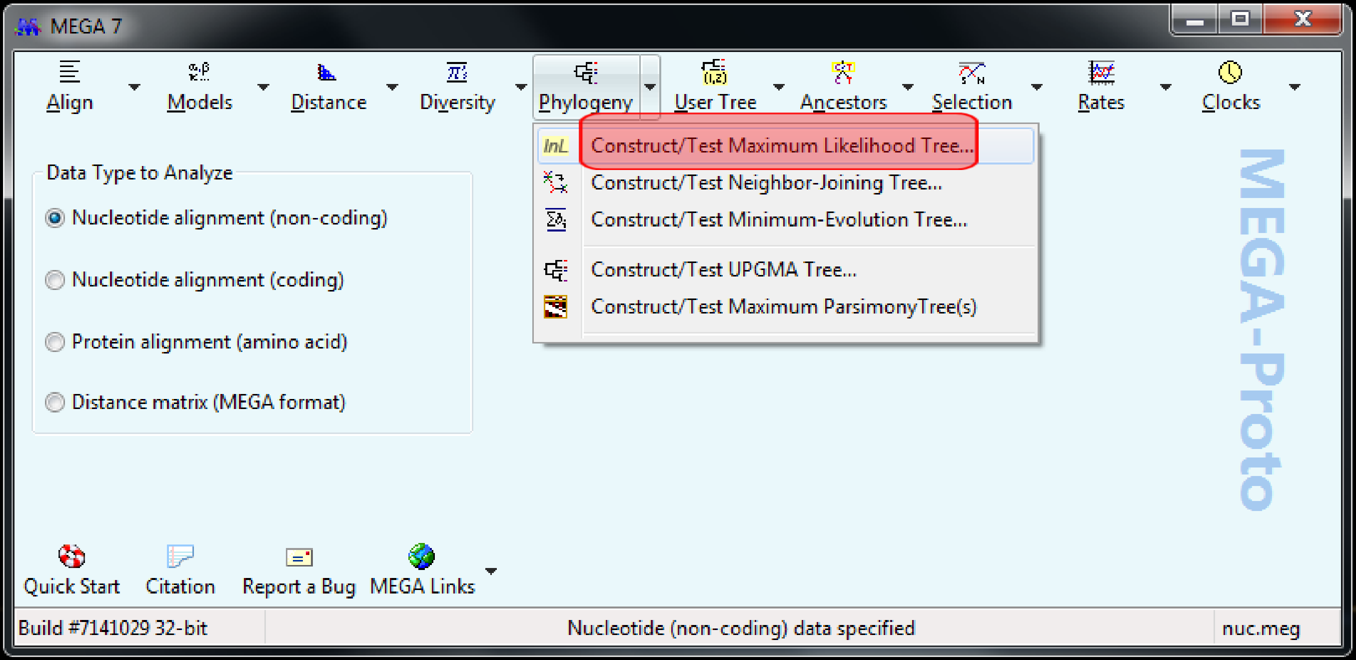


```
/home/glen/Documents/megacc/Examples/Crab_rRNA.meg
/home/glen/Documents/megacc/Examples/Drosophila_Adh.meg
```

```
File Name to Write: ~/Documents/megacc/demo2Data.txt
```

STEP 2

- Using MEGA-Proto, create a .mao file for ML phylogeny construction with the default settings and save the file to ~/Documents/megacc/demo2settings.mao



Data Type to Analyze

● Nucleotide alignment (non-coding)

○ Nucleotide alignment (coding)

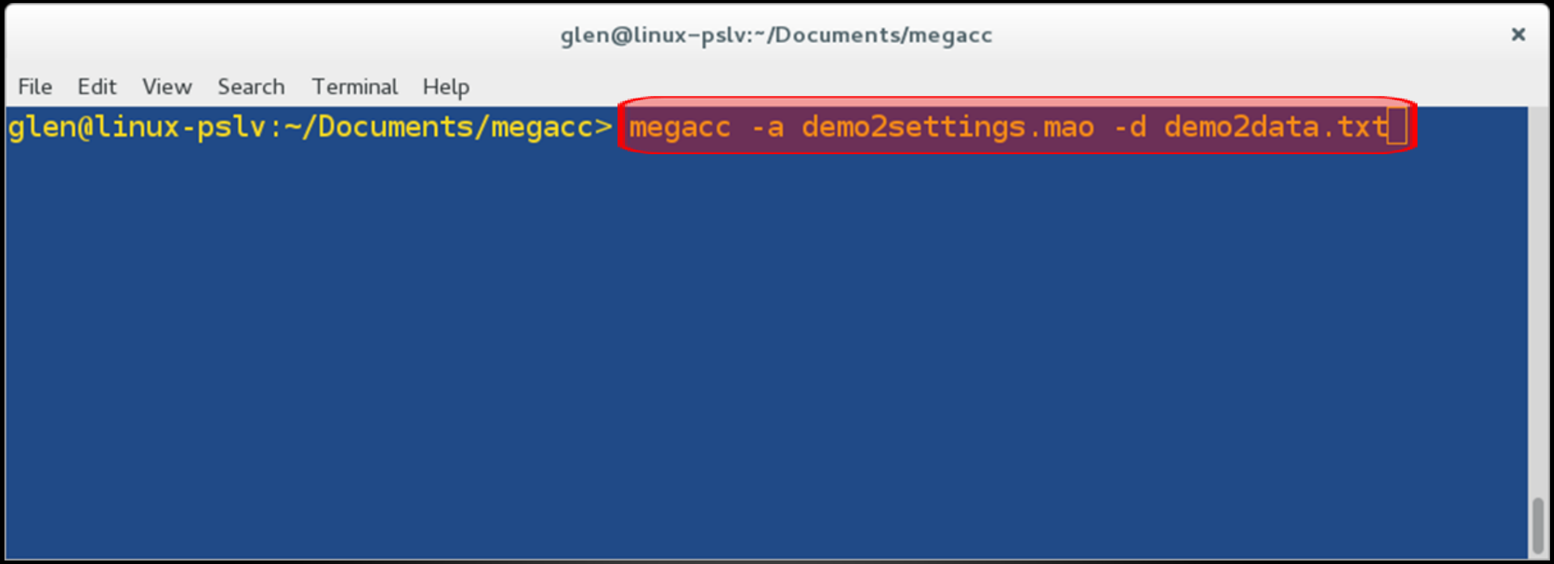
○ Protein alignment (amino acid)

○ Distance matrix (MEGA format)

Build #7141029 32-bit | Nucleotide (non-coding) data specified | nuc.meg

STEP 3

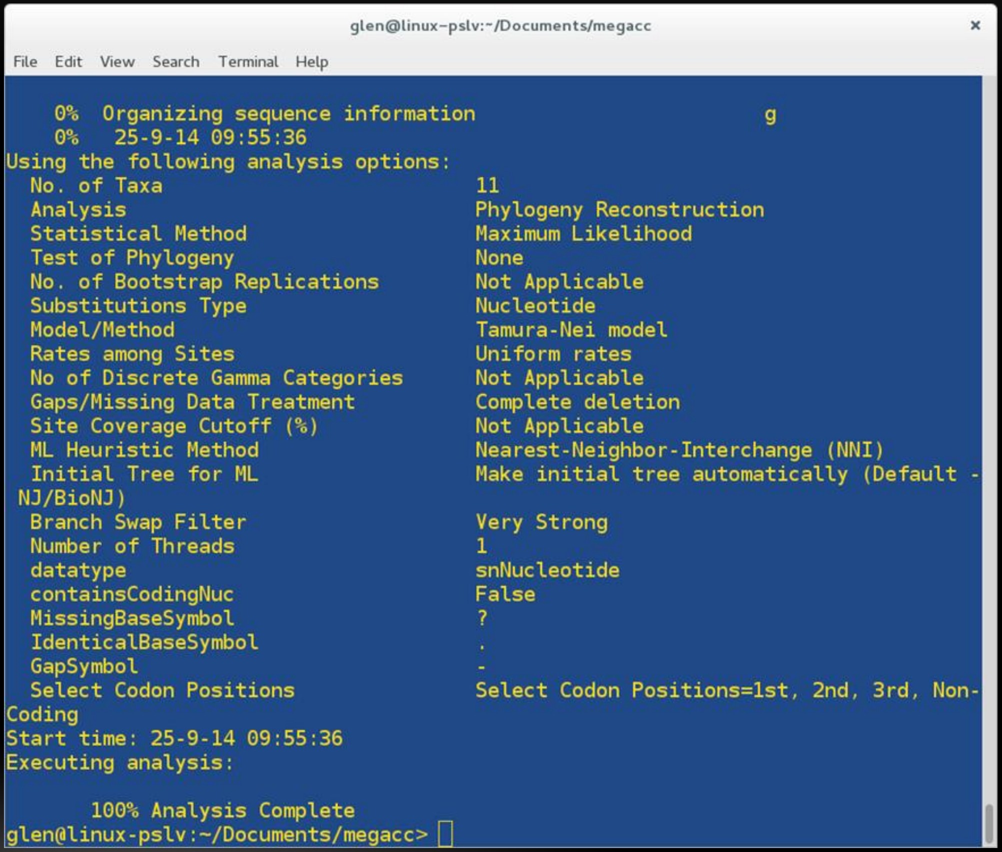
- From a command-line prompt, call MEGA-CC as below (note that we don’t specify an output name):



```
glen@linux-pslv:~/Documents/megacc> megacc -a demo2settings.mao -d demo2data.txt
```

STEP 4

- The analyses will be launched sequentially and progress updates will be displayed in the command prompt window.



```
    0%  Organizing sequence information                        g
    0%   25-9-14 09:55:36
Using the following analysis options:
  No. of Taxa                                    11
  Analysis                                       Phylogeny Reconstruction
  Statistical Method                             Maximum Likelihood
  Test of Phylogeny                              None
  No. of Bootstrap Replications                  Not Applicable
  Substitutions Type                             Nucleotide
  Model/Method                                   Tamura-Nei model
  Rates among Sites                              Uniform rates
  No of Discrete Gamma Categories                Not Applicable
  Gaps/Missing Data Treatment                    Complete deletion
  Site Coverage Cutoff (%)                       Not Applicable
  ML Heuristic Method                            Nearest-Neighbor-Interchange (NNI)
  Initial Tree for ML                            Make initial tree automatically (Default -
 NJ/BioNJ)
  Branch Swap Filter                             Very Strong
  Number of Threads                              1
  datatype                                       snNucleotide
  containsCodingNuc                              False
  MissingBaseSymbol                              ?
  IdenticalBaseSymbol                            .
  GapSymbol                                      -
  Select Codon Positions                         Select Codon Positions=1st, 2nd, 3rd, Non-
Coding
Start time: 25-9-14 09:55:36
Executing analysis:

       100% Analysis Complete
glen@linux-pslv:~/Documents/megacc> 
```

FINALLY

- The analysis will produce output files for each input data file
- In this example, the same analysis options were used for each alignment file
- Enjoy!

MEGA-CC DEVELOPMENT TEAM

Koichiro Tamura

- Research Center for Genomics and Bioinformatics, Tokyo Metropolitan University, Hachioji, Tokyo, Japan
- Department of Biological Sciences, Tokyo Metropolitan University, Hachioji, Tokyo, Japan

Glen Stecher

- Institute for Genomics and Evolutionary Medicine, Temple University
- Center for Evolutionary Medicine and Informatics, Arizona State University

Sudhir Kumar

- Institute for Genomics and Evolutionary Medicine, Temple University
- Department of Biology, Temple University
- Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia