Bioinformatics tools for phylogeny and visualization

Yanbin Yin

Homework assignment 5

PART 1

1. Take the MAFFT alignment http://cys.bios.niu.edu/yyin/teach/PBB/purdue.cellwall.list.lignin.fa.aln as input and use MEGA5 to build a phylogenetic tree
2. Try maximum likelihood (ML), neighbor-joining (NJ) and maximum parsimony (MP) algorithms with 100 bootstrap replications and compare the running time and the topology of the resulting trees. If encounter errors, try to use the HELP link to find out and solve it
3. Color the branches and leafs in the resulting ML tree graph using different colors for different gene subfamilies
4. Export the tree as a newick format file
5. Prepare a color definition file for different gene subfamilies (see step 3); upload the newick tree file and the color definition file to iTOL to display the tree

Homework assignment 5 Cont.

PART 2

1. Go to https://edu.isb-sib.ch/pluginfile.php/2878/mod_resource/content/4/couselab-html/content.html and finish all the quizzes in Chapter 1 and Chapter 2

Write a report (in word or ppt) to include all the operations and screen shots.

Due on Oct 17 (send by email, if there are 2+ files, put them in a zip file; include your last name in the file name)

Outline

- Introduction to phylogenetic analysis

- Hands on practice of MEGA 5 and iTOL

Phylogenetics is the science of estimating the evolutionary past, in the case of molecular phylogeny, based on the comparison of DNA or protein sequences:

- Study the evolution of genomes and gene families (duplication and transfer)
- Study the diversity of genes or fragments
- Cluster homologous sequences into subfamilies based on evolutionary history
- Infer functions for unknown genes

A simple case of horizontal gene transfer

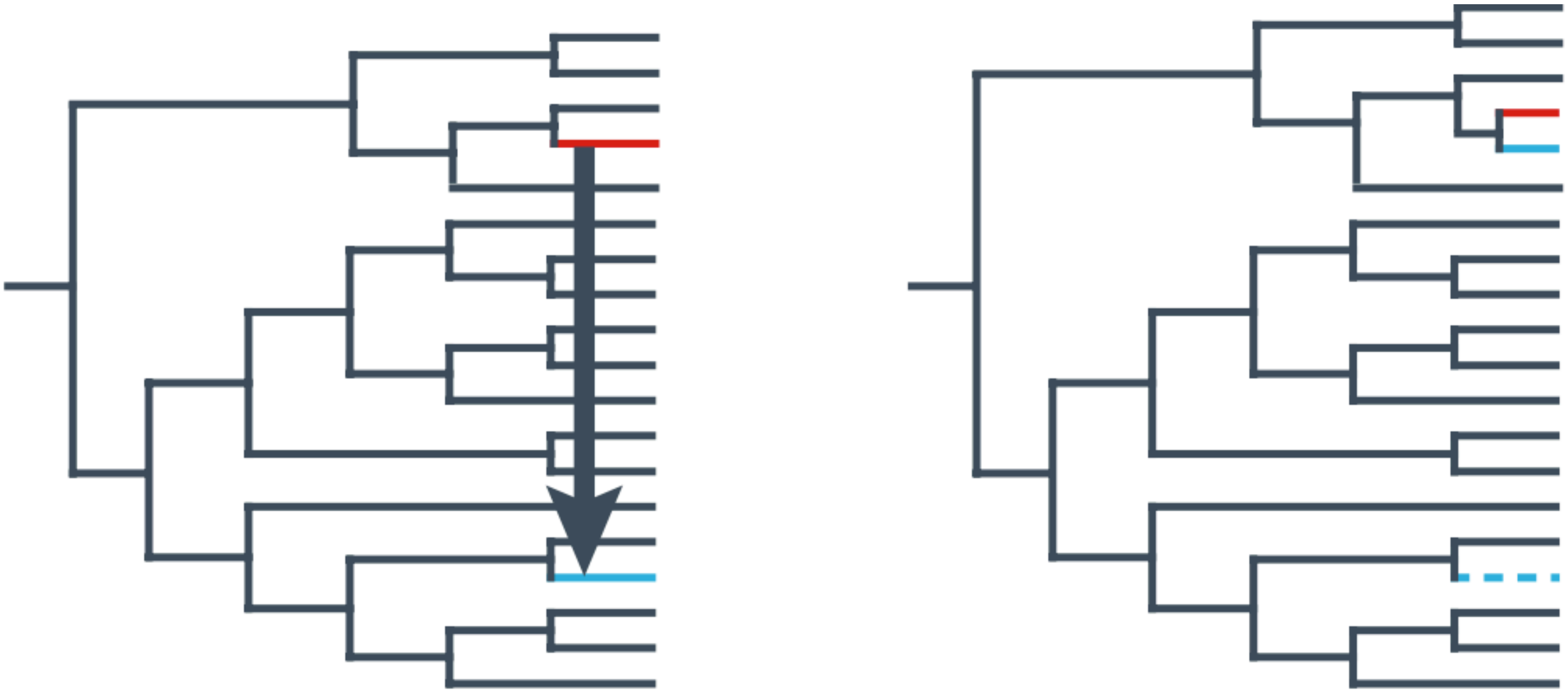
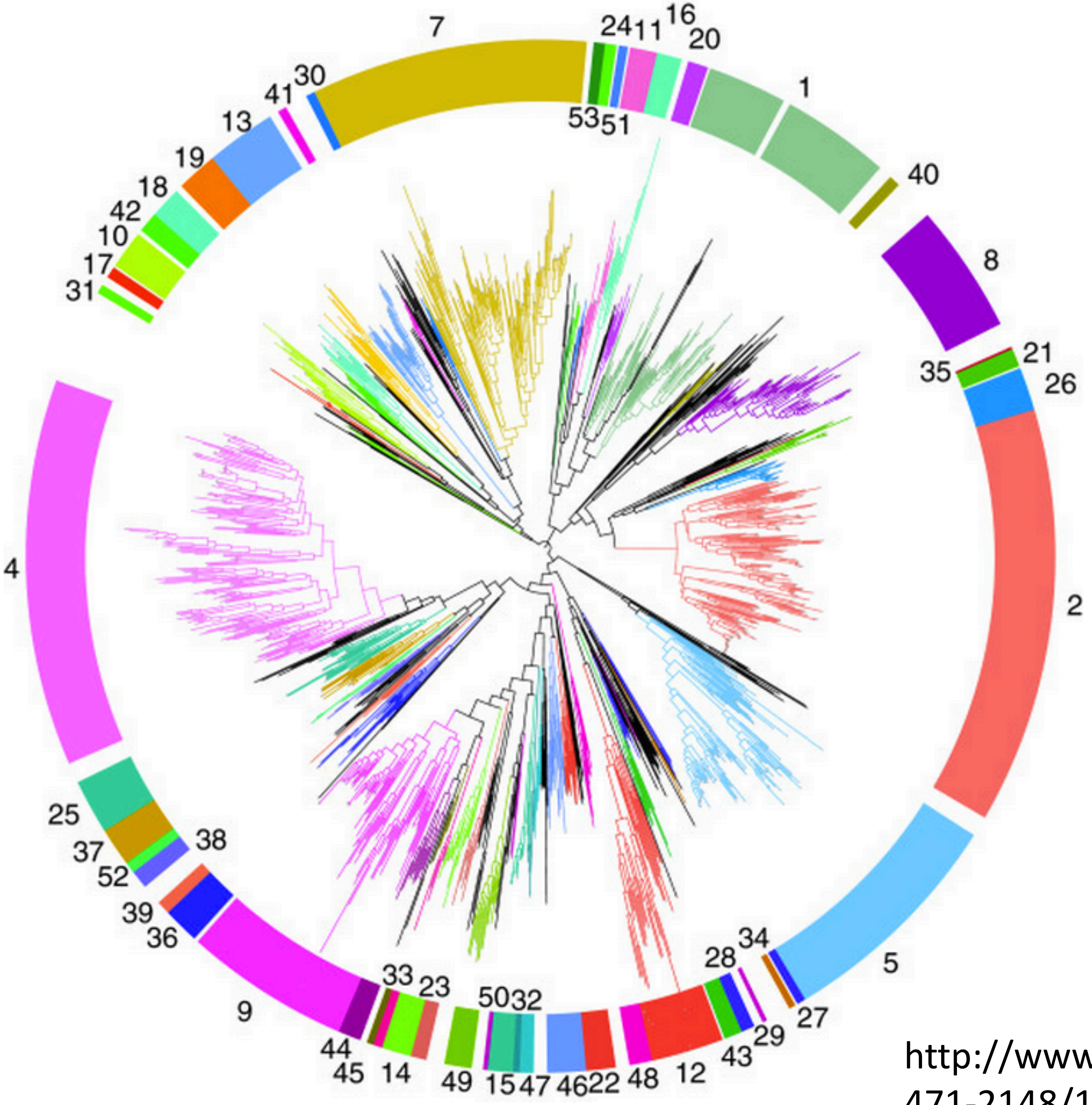


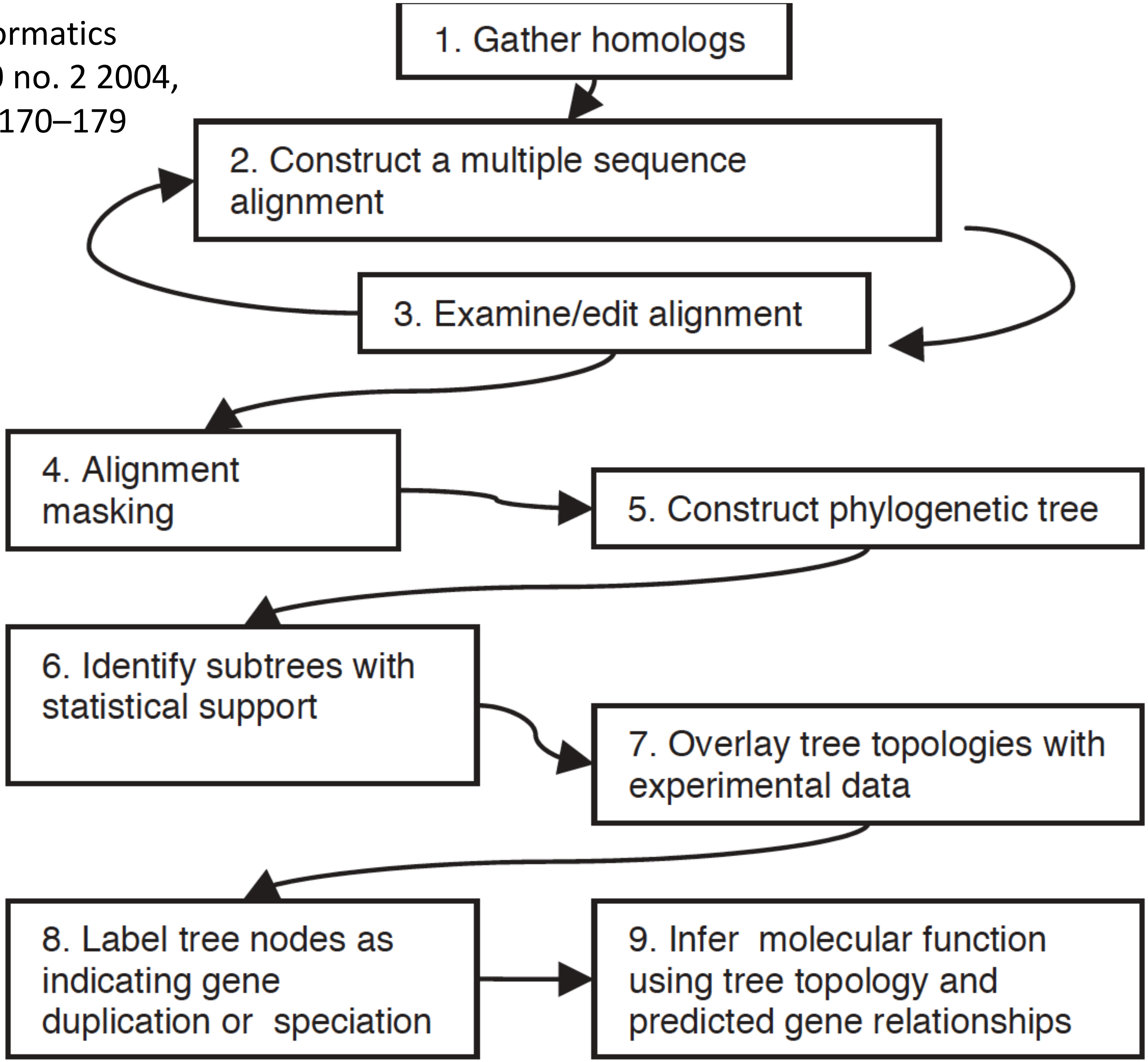
Figure 1.



http://www.biomedcentral.com/1471-2148/12/186

Phylogenetic tree of family GH5. In this circular phylogram, the branches corresponding to subfamilies 1–53

Bioinformatics
Vol. 20 no. 2 2004,
pages 170–179



1. Gather homologs
2. Construct a multiple sequence alignment
3. Examine/edit alignment
4. Alignment masking
5. Construct phylogenetic tree
6. Identify subtrees with statistical support
7. Overlay tree topologies with experimental data
8. Label tree nodes as indicating gene duplication or speciation
9. Infer molecular function using tree topology and predicted gene relationships

Step 1. Assembling a dataset

BLAST, FASTA, domain/family based (HMMER)

Step 2. Multiple sequence alignment

MAFFT, MUSCLE, Clustal Omega

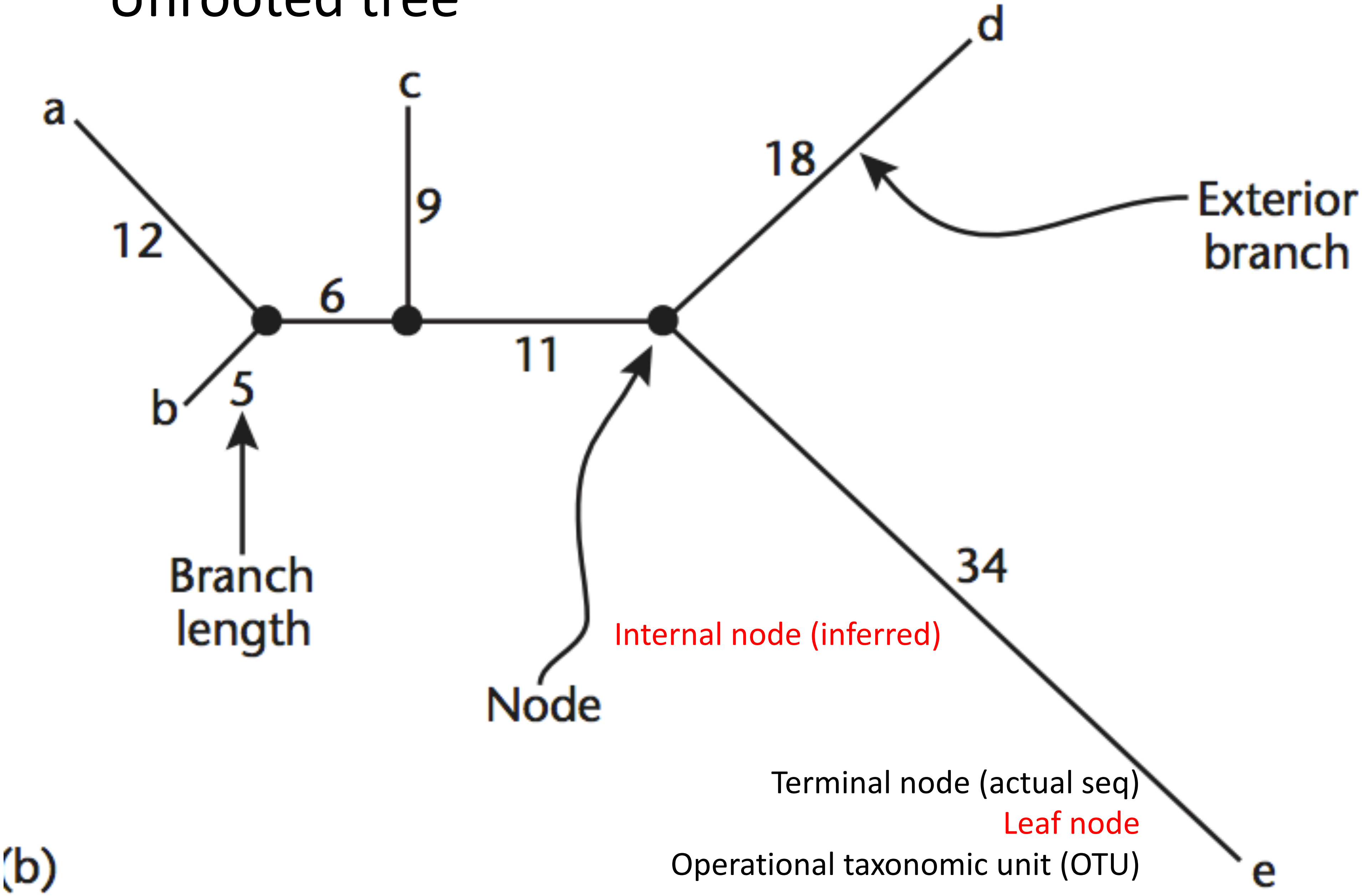
Step 3. Phylogeny reconstruction

MEGA5, PHYML, RAxML, GARLI, MrBayes, FastTree

Step 4. Tree visualization

TreeView, TreeDyn, MEGA5, iTOL

Unrooted tree



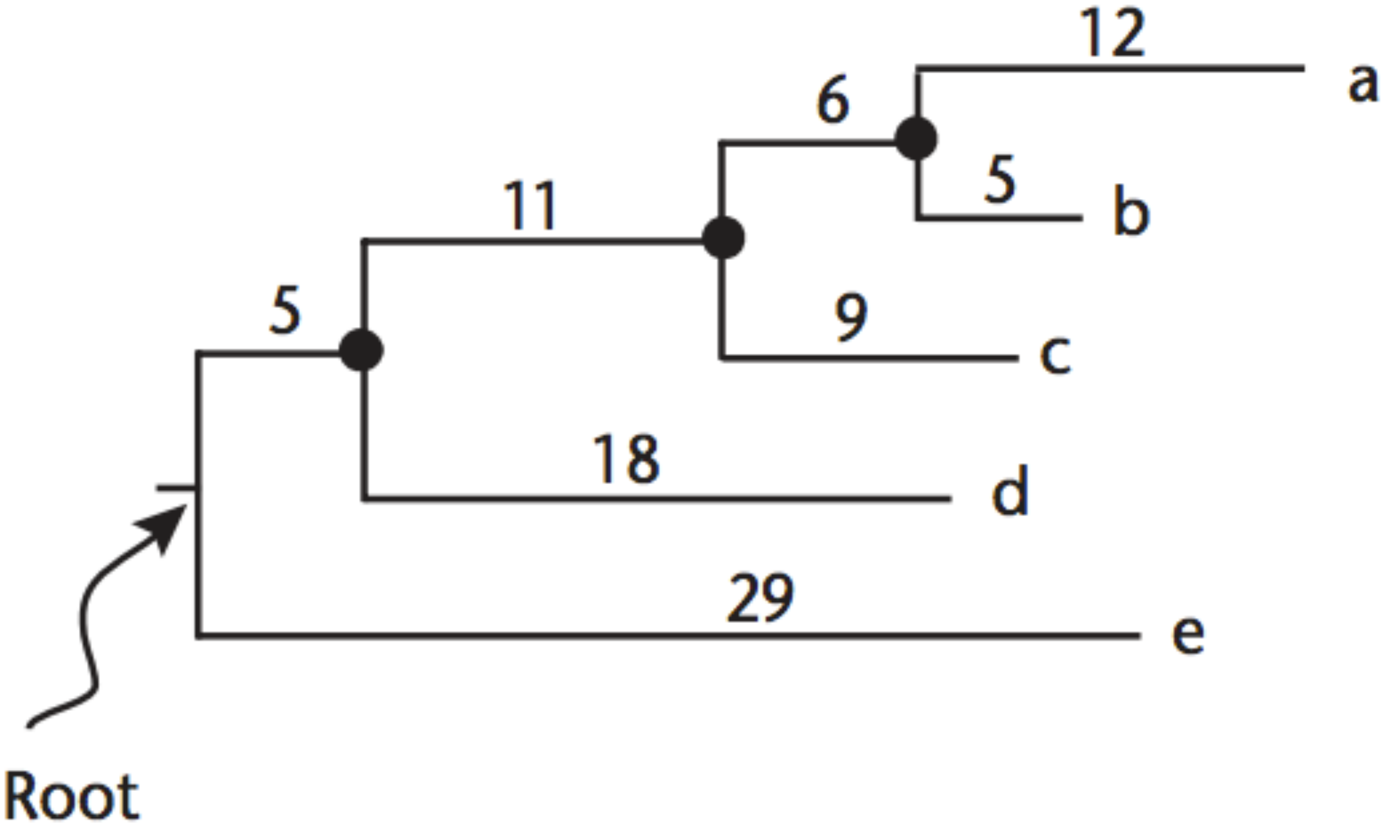
Internal node (inferred)

Terminal node (actual seq)

Leaf node

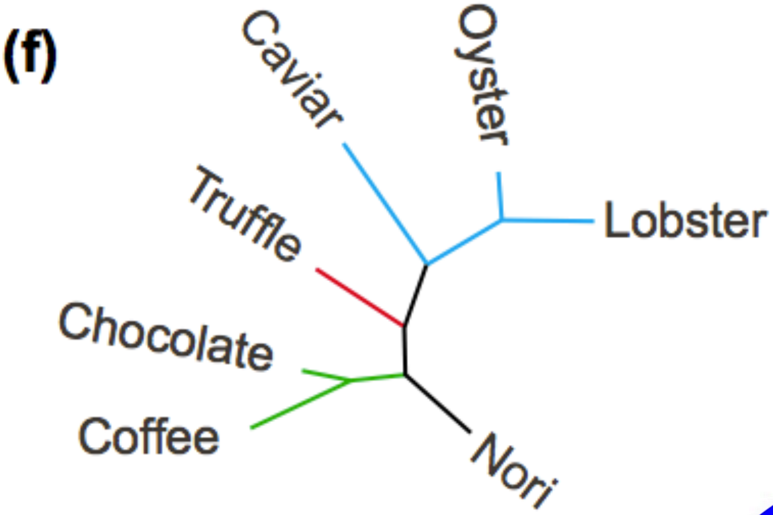
Operational taxonomic unit (OTU)

Rooted tree
Root is often selected based on prior knowledge

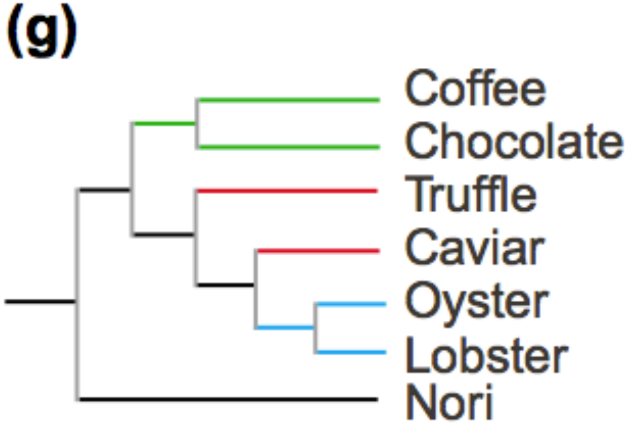


Branches are drawn with lengths proportional to the divergence (difference) between two nodes

Radial view

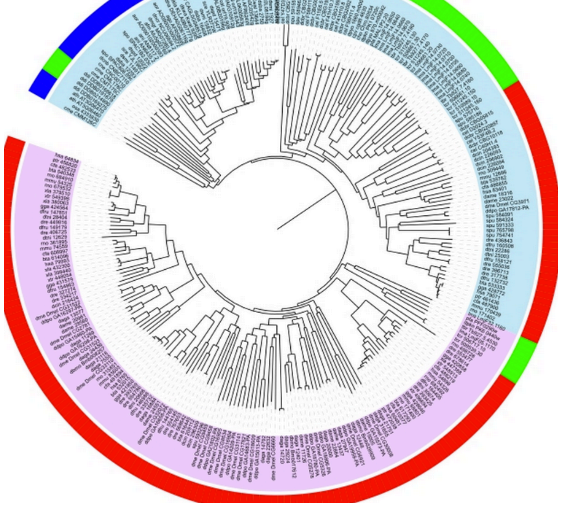


Rectangular view

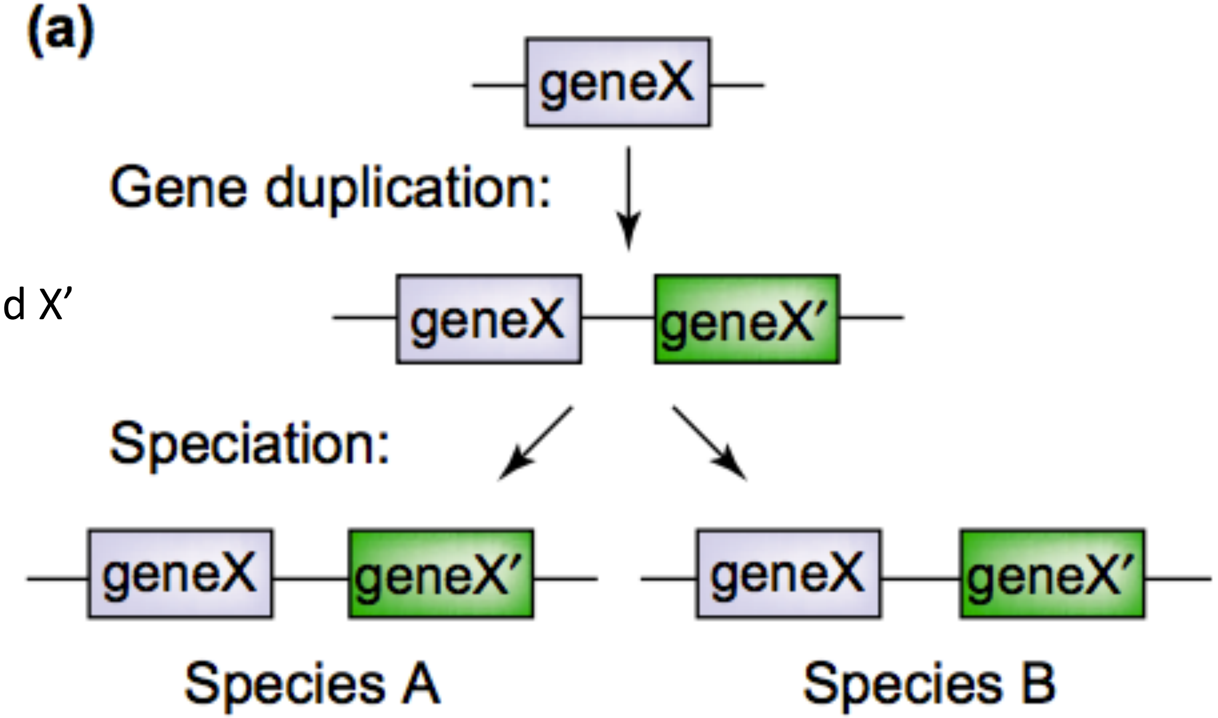


TRENDS in Genetics

Circular view



Paralog: X and X’



Ortholog:
X in A and X in B
X’ in A and X’ in B

What about X in A and X’ in B?
They are called out-paralog (not often used)

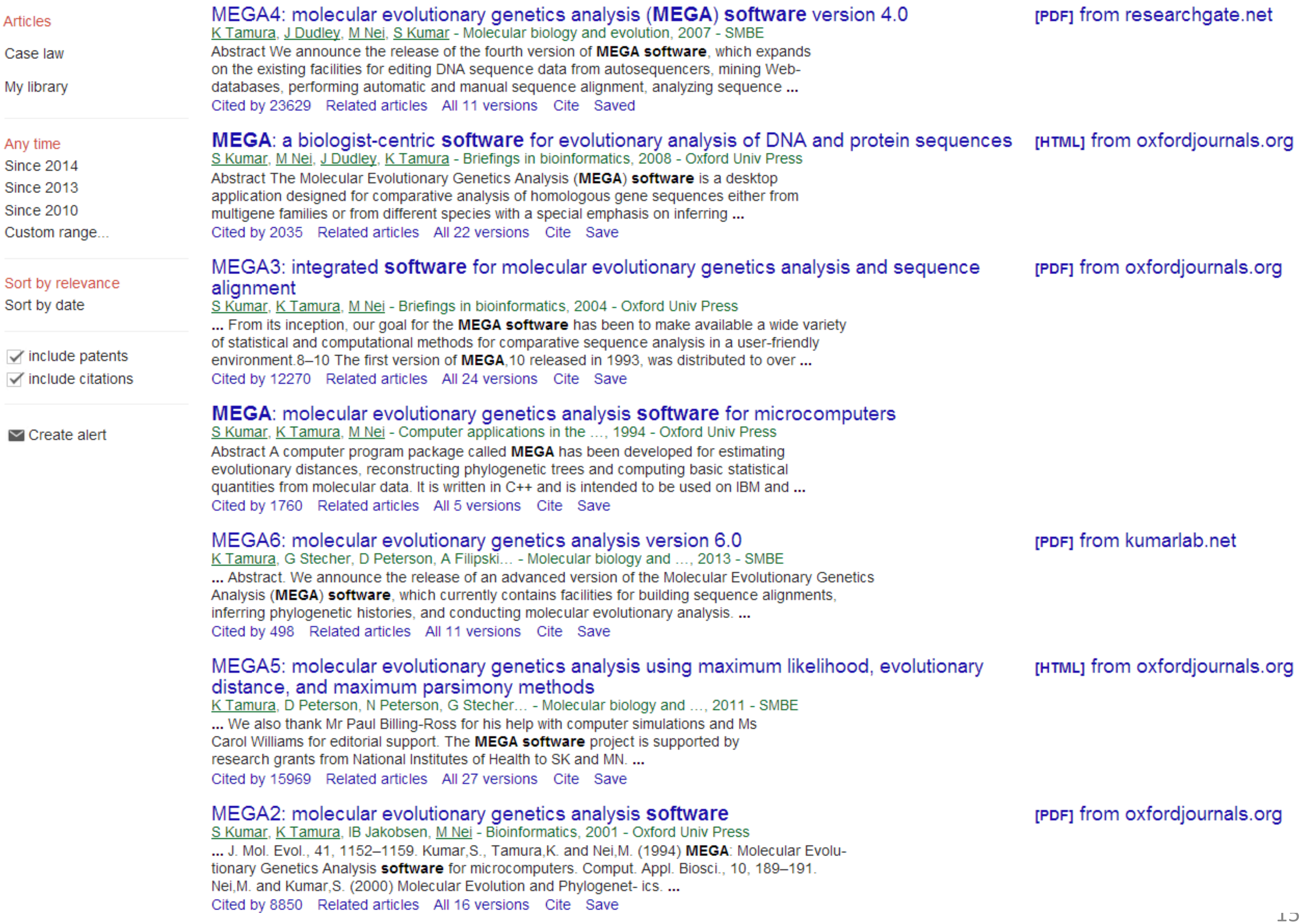
All the four genes together are called an orthologous group

MEGA: Molecular Evolutionary Genetics Analysis

MEGA is an integrated tool for conducting sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses. MEGA is used by biologists in a large number of laboratories for reconstructing the evolutionary histories of species and inferring the extent and nature of selective forces shaping the evolution of genes and species

Mega was developed as a software with GUI

The most cited phylogenetics analysis software package



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Sort by date

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☑ include citations

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MEGA4: molecular evolutionary genetics analysis (**MEGA**) **software** version 4.0 — **[PDF]** from researchgate.net

K Tamura, J Dudley, M Nei, S Kumar - Molecular biology and evolution, 2007 - SMBE

Abstract We announce the release of the fourth version of **MEGA software**, which expands on the existing facilities for editing DNA sequence data from autosequencers, mining Web-databases, performing automatic and manual sequence alignment, analyzing sequence **...**

Cited by 23629 Related articles All 11 versions Cite Saved

MEGA: a biologist-centric **software** for evolutionary analysis of DNA and protein sequences — **[HTML]** from oxfordjournals.org

S Kumar, M Nei, J Dudley, K Tamura - Briefings in bioinformatics, 2008 - Oxford Univ Press

Abstract The Molecular Evolutionary Genetics Analysis (**MEGA**) **software** is a desktop application designed for comparative analysis of homologous gene sequences either from multigene families or from different species with a special emphasis on inferring **...**

Cited by 2035 Related articles All 22 versions Cite Save

MEGA3: integrated **software** for molecular evolutionary genetics analysis and sequence alignment — **[PDF]** from oxfordjournals.org

S Kumar, K Tamura, M Nei - Briefings in bioinformatics, 2004 - Oxford Univ Press

... From its inception, our goal for the **MEGA software** has been to make available a wide variety of statistical and computational methods for comparative sequence analysis in a user-friendly environment.8–10 The first version of **MEGA**,10 released in 1993, was distributed to over **...**

Cited by 12270 Related articles All 24 versions Cite Save

MEGA: molecular evolutionary genetics analysis **software** for microcomputers

S Kumar, K Tamura, M Nei - Computer applications in the ..., 1994 - Oxford Univ Press

Abstract A computer program package called **MEGA** has been developed for estimating evolutionary distances, reconstructing phylogenetic trees and computing basic statistical quantities from molecular data. It is written in C++ and is intended to be used on IBM and **...**

Cited by 1760 Related articles All 5 versions Cite Save

MEGA6: molecular evolutionary genetics analysis version 6.0 — **[PDF]** from kumarlab.net

K Tamura, G Stecher, D Peterson, A Filipski... - Molecular biology and ..., 2013 - SMBE

... Abstract. We announce the release of an advanced version of the Molecular Evolutionary Genetics Analysis (**MEGA**) **software**, which currently contains facilities for building sequence alignments, inferring phylogenetic histories, and conducting molecular evolutionary analysis. **...**

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MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods — **[HTML]** from oxfordjournals.org

K Tamura, D Peterson, N Peterson, G Stecher... - Molecular biology and ..., 2011 - SMBE

... We also thank Mr Paul Billing-Ross for his help with computer simulations and Ms Carol Williams for editorial support. The **MEGA software** project is supported by research grants from National Institutes of Health to SK and MN. **...**

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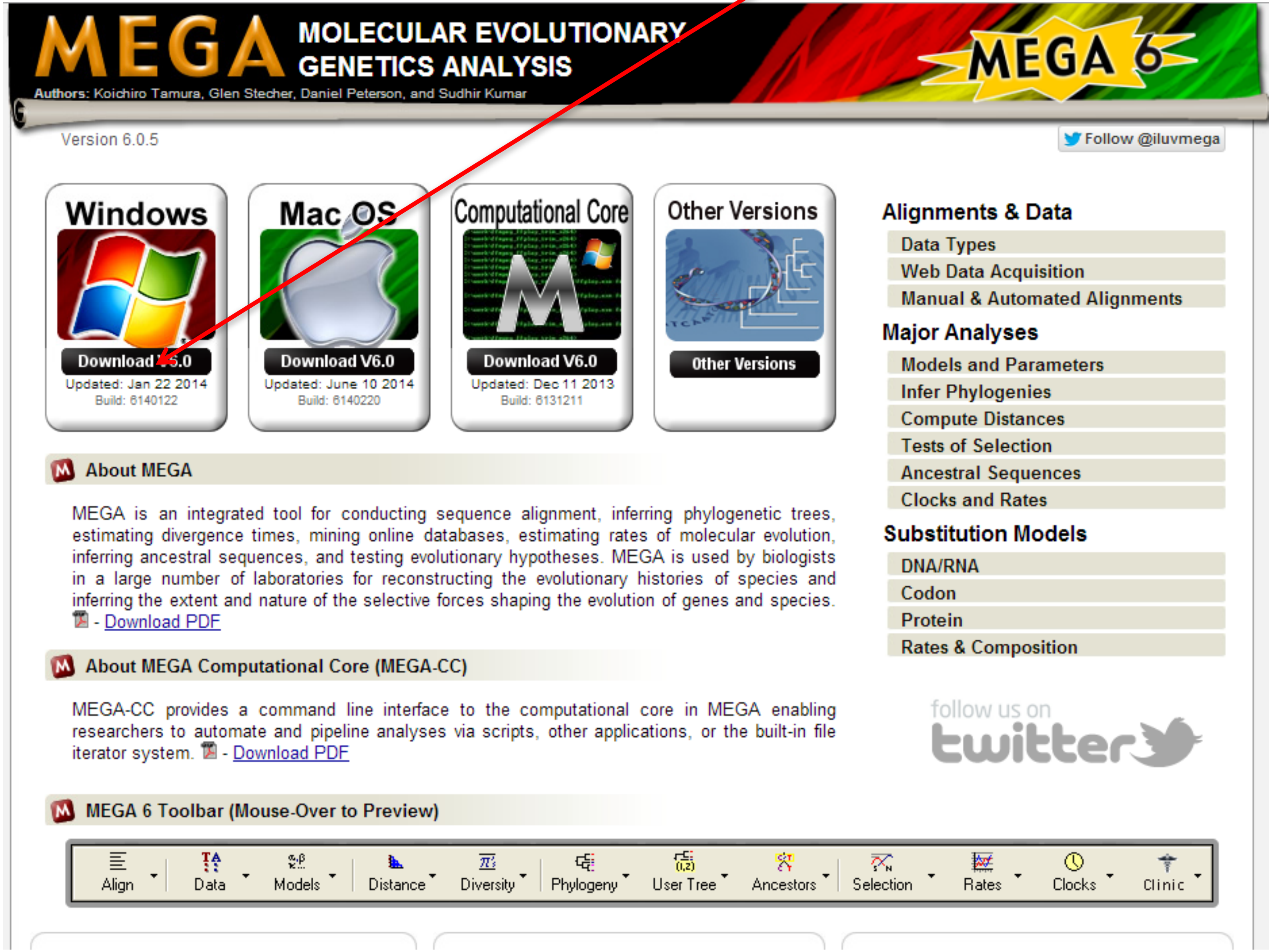
MEGA2: molecular evolutionary genetics analysis **software** — **[PDF]** from oxfordjournals.org

S Kumar, K Tamura, IB Jakobsen, M Nei - Bioinformatics, 2001 - Oxford Univ Press

... J. Mol. Evol., 41, 1152–1159. Kumar,S., Tamura,K. and Nei,M. (1994) **MEGA**: Molecular Evolutionary Genetics Analysis **software** for microcomputers. Comput. Appl. Biosci., 10, 189–191. Nei,M. and Kumar,S. (2000) Molecular Evolution and Phylogenet- ics. **...**

Cited by 8850 Related articles All 16 versions Cite Save http://www.megasoftware.net/

Free download for different OSs, e.g. WINDOWS



Authors: Koichiro Tamura, Glen Stecher, Daniel Peterson, and Sudhir Kumar

About MEGA

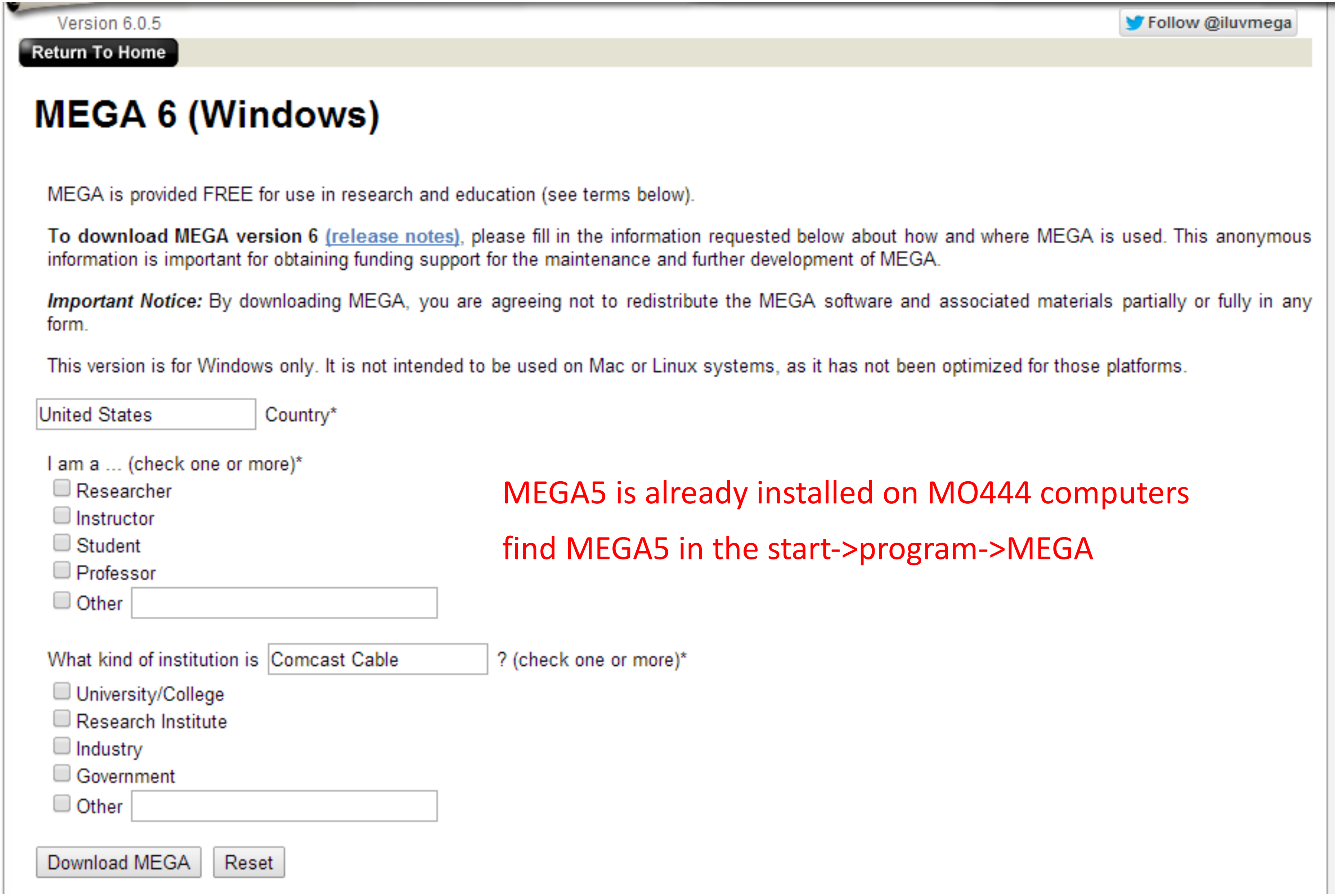
MEGA is an integrated tool for conducting sequence alignment, inferring phylogenetic trees, estimating divergence times, mining online databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses. MEGA is used by biologists in a large number of laboratories for reconstructing the evolutionary histories of species and inferring the extent and nature of the selective forces shaping the evolution of genes and species. - Download PDF

About MEGA Computational Core (MEGA-CC)

MEGA-CC provides a command line interface to the computational core in MEGA enabling researchers to automate and pipeline analyses via scripts, other applications, or the built-in file iterator system. - Download PDF

MEGA 6 Toolbar (Mouse-Over to Preview)

it's free, but you need to fill out an on-line form to download



MEGA 6 (Windows)

MEGA is provided FREE for use in research and education (see terms below).

To download MEGA version 6 (release notes), please fill in the information requested below about how and where MEGA is used. This anonymous information is important for obtaining funding support for the maintenance and further development of MEGA.

Important Notice: By downloading MEGA, you are agreeing not to redistribute the MEGA software and associated materials partially or fully in any form.

This version is for Windows only. It is not intended to be used on Mac or Linux systems, as it has not been optimized for those platforms.

United States Country*

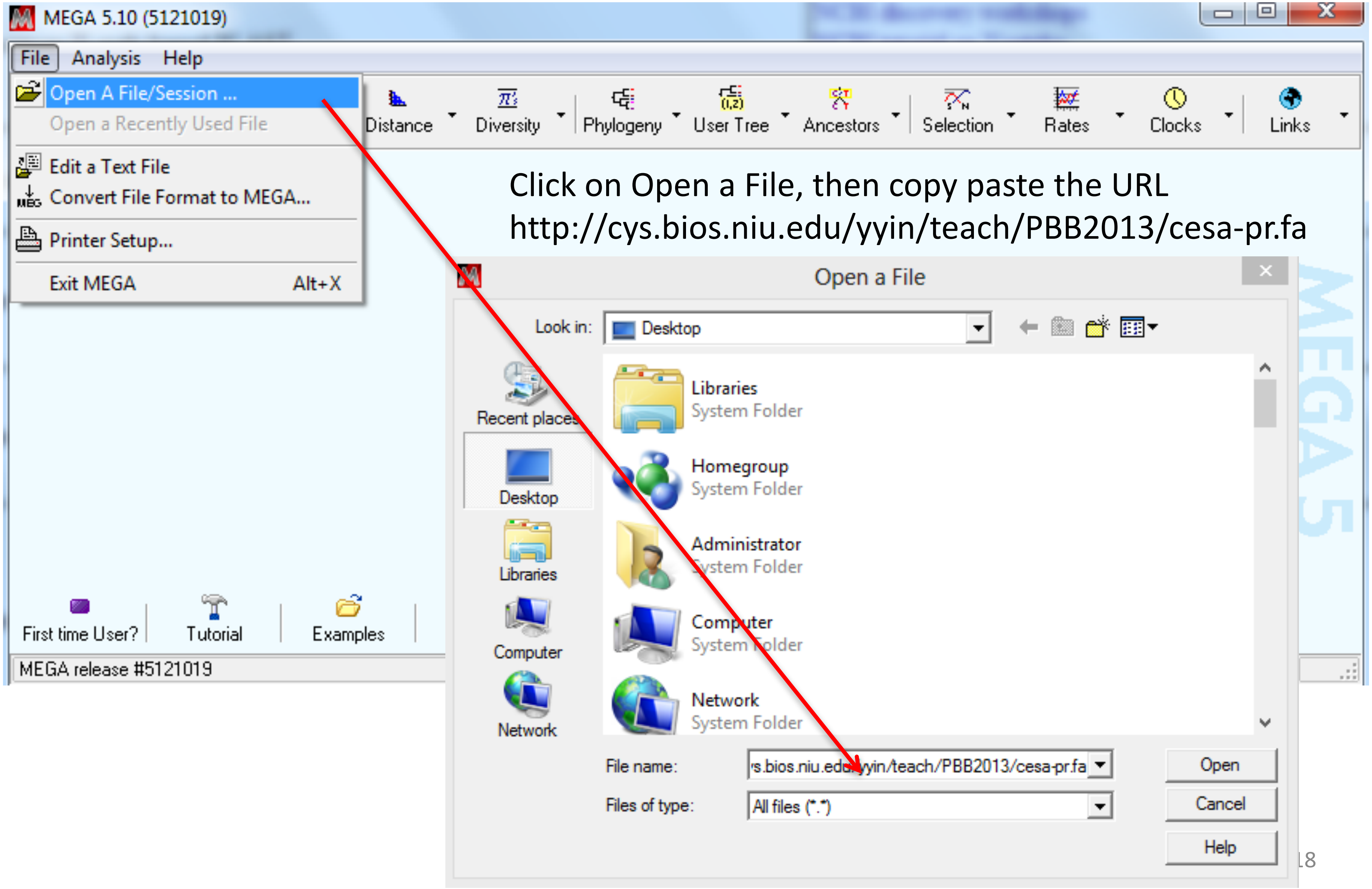
I am a ... (check one or more)*
☐ Researcher
☐ Instructor
☐ Student
☐ Professor
☐ Other

What kind of institution is Comcast Cable ? (check one or more)*
☐ University/College
☐ Research Institute
☐ Industry
☐ Government
☐ Other

MEGA5 is already installed on MO444 computers

find MEGA5 in the start->program->MEGA

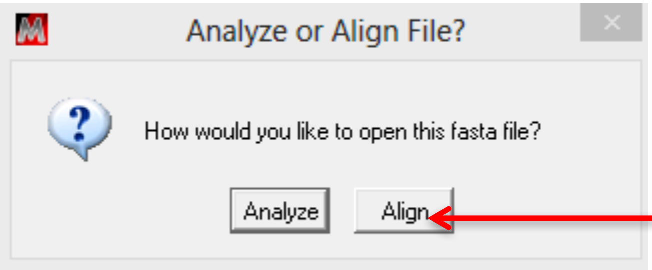
We're gonna use MEGA to do the alignment first, then build the phylogeny



Click on Open a File, then copy paste the URL http://cys.bios.niu.edu/yyin/teach/PBB2013/cesa-pr.fa

File name: s.bios.niu.edu/yyin/teach/PBB2013/cesa-pr.fa

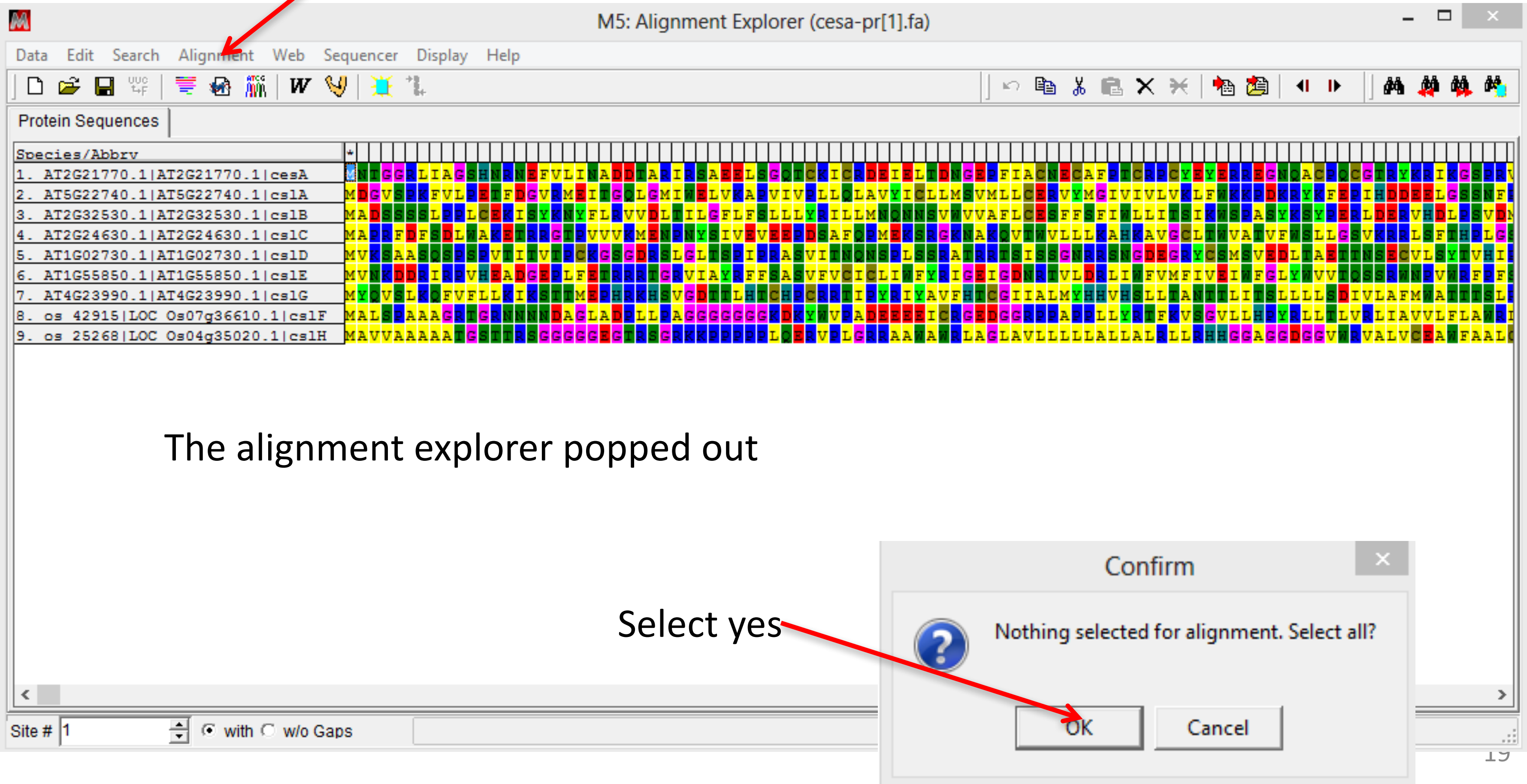
Files of type: All files (*.*)



How would you like to open this fasta file?

Align the seq first

Click on alignment then choose align by muscle



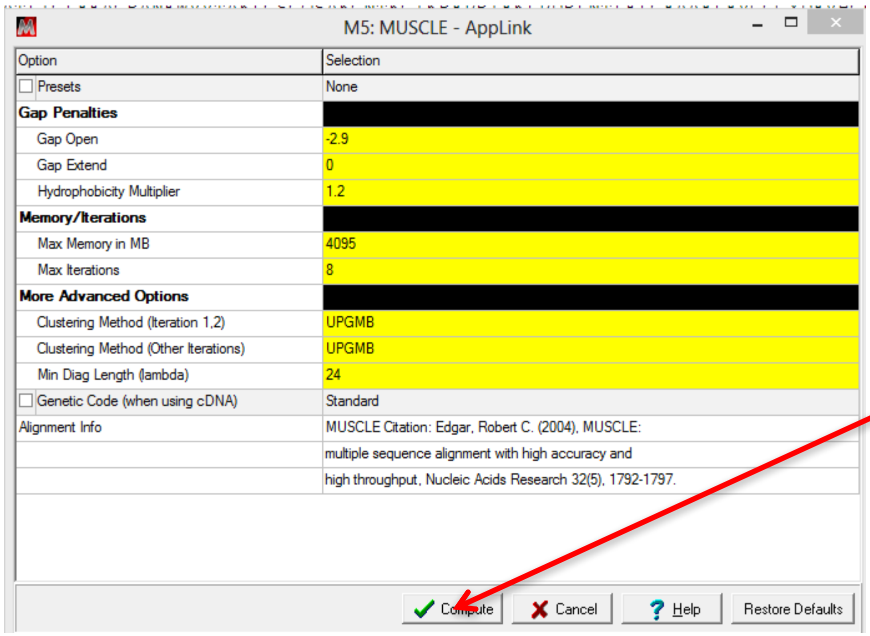
1. AT2G21770.1|AT2G21770.1|cesA
2. AT5G22740.1|AT5G22740.1|cslA
3. AT2G32530.1|AT2G32530.1|cslB
4. AT2G24630.1|AT2G24630.1|cslC
5. AT1G02730.1|AT1G02730.1|cslD
6. AT1G55850.1|AT1G55850.1|cslE
7. AT4G23990.1|AT4G23990.1|cslG
8. os 42915|LOC Os07g36610.1|cslF
9. os 25268|LOC Os04g35020.1|cslH

Site # 1 ● with ○ w/o Gaps

Nothing selected for alignment. Select all?

The alignment explorer popped out

Select yes

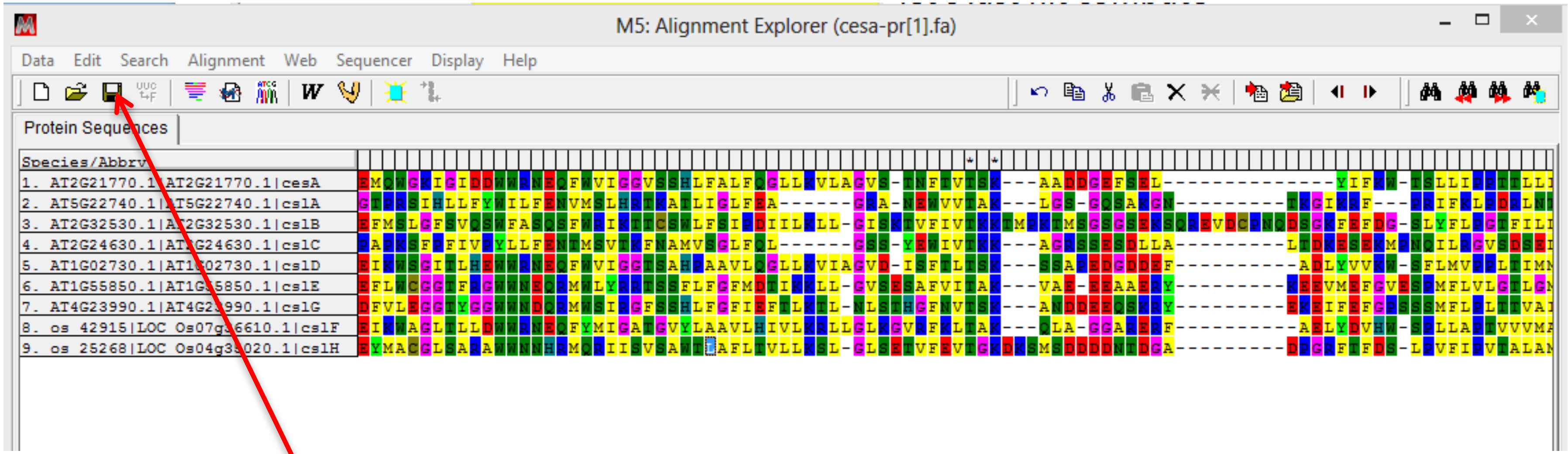


M5: MUSCLE - AppLink

Option	Selection
☐ Presets	None
Gap Penalties	
Gap Open	-2.9
Gap Extend	0
Hydrophobicity Multiplier	1.2
Memory/Iterations	
Max Memory in MB	4095
Max Iterations	8
More Advanced Options	
Clustering Method (Iteration 1,2)	UPGMB
Clustering Method (Other Iterations)	UPGMB
Min Diag Length (lambda)	24
☐ Genetic Code (when using cDNA)	Standard
Alignment Info	MUSCLE Citation: Edgar, Robert C. (2004), MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Research 32(5), 1792-1797.

Popped out window to allow option change

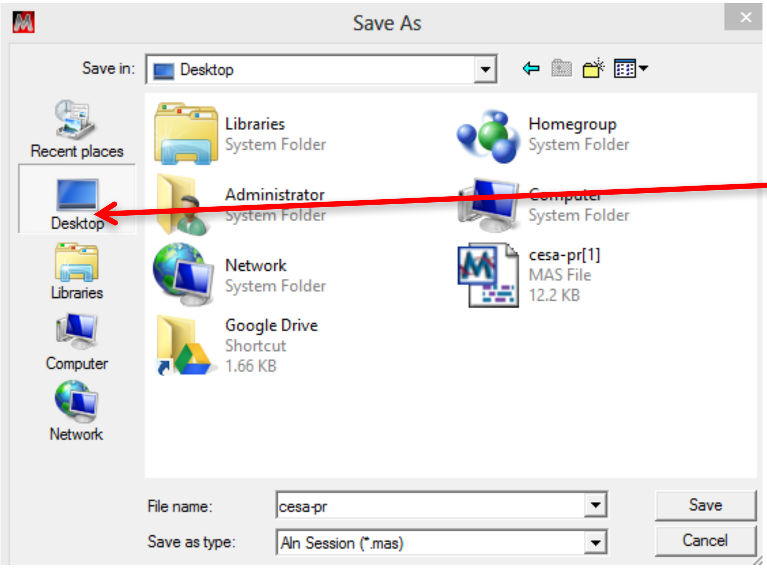
let’s just hit compute



M5: Alignment Explorer (cesa-pr[1].fa)

Species/Abbrv
1. AT2G21770.1|AT2G21770.1|cesA
2. AT5G22740.1|AT5G22740.1|cslA
3. AT2G32530.1|AT2G32530.1|cslB
4. AT2G24630.1|AT2G24630.1|cslC
5. AT1G02730.1|AT1G02730.1|cslD
6. AT1G55850.1|AT1G55850.1|cslE
7. AT4G23990.1|AT4G23990.1|cslG
8. os 42915|LOC Os07g36610.1|cslF
9. os 25268|LOC Os04g35020.1|cslH

Now the alignment explorer shows the aligned seqs
Next hit the save icon to save the alignment as a MEGA format



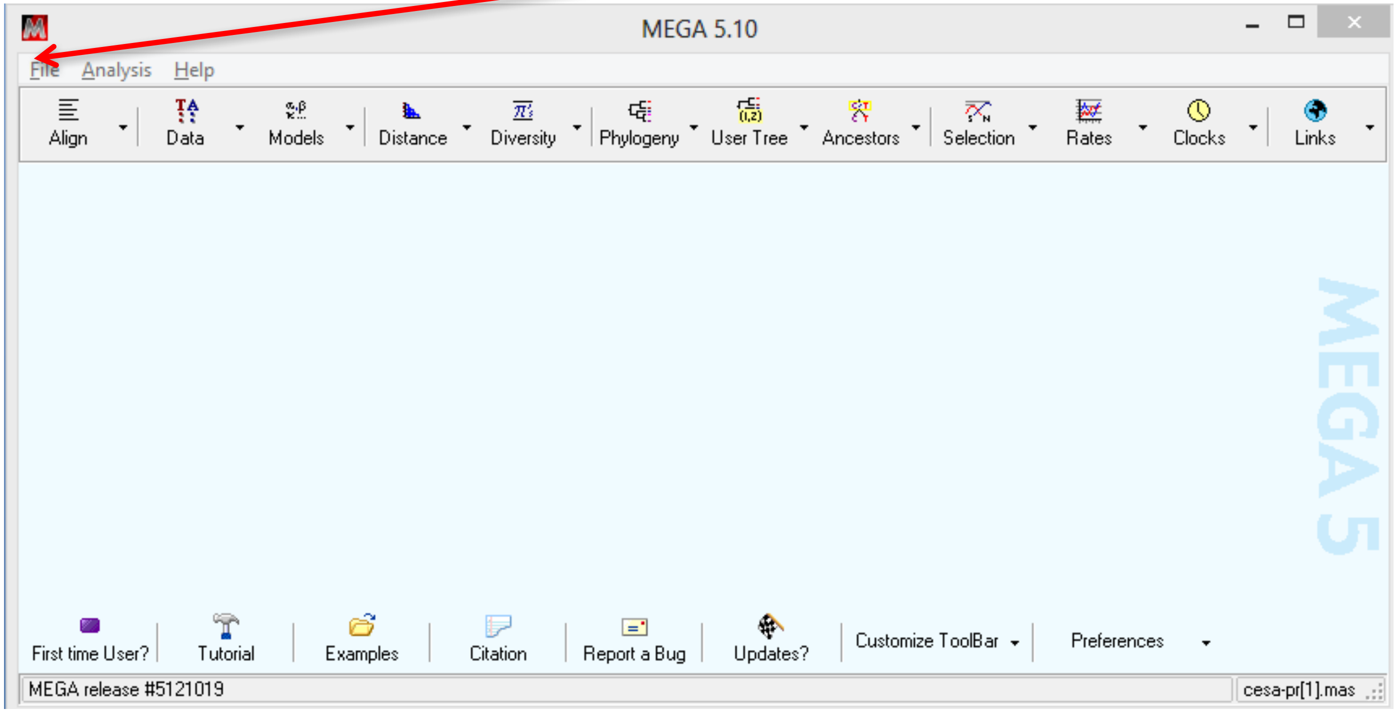
Save in: Desktop

File name: cesa-pr

Save as type: Aln Session (*.mas)

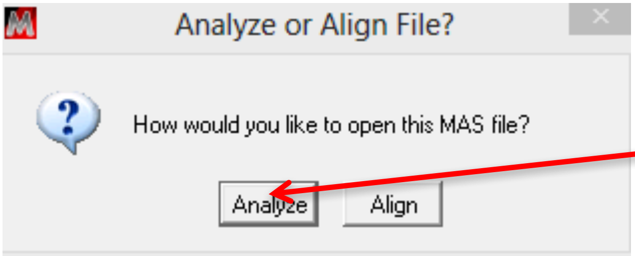
Now I saved it in the desktop folder

Now go back to the main window, click on File to open the saved mas file



MEGA release #5121019

cesa-pr[1].mas

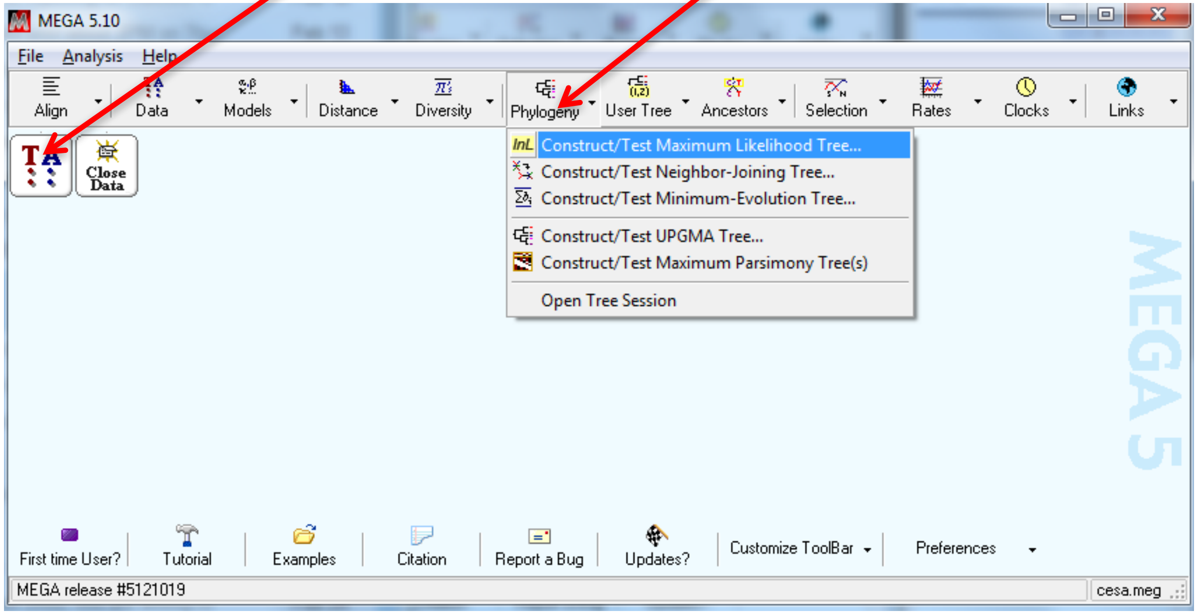


How would you like to open this MAS file?

This time choose analyze as it’s an aligned file

This window changed, meaning the data is loaded; we can build the tree now

You may choose from a list of different building algorithms basically, maximum likelihood is the most accurate but also the slowest neighbor-joining and maximum parsimony are also very popular and faster if you have over 50 sequences or longer sequences



Phylogenetic trees are calculated by applying mathematical models to infer evolutionary relationships between molecules or organisms (here sequences), based on a set of characters that describe their differences.

Four main categories of phylogenetic reconstruction methods:

1. Maximum parsimony approaches create trees using the minimum number of ancestors needed to explain the observed characters

2. Distance matrix methods, such as neighbor joining, allow more sophisticated evolutionary models than parsimony

3. Maximum likelihood methods search a set of tree and evolutionary models to find the ones most likely to generate the observed characters

4. Bayesian approaches offer more flexibility, as they allow optimization of all aspects of a tree (model, topology, branch length)

Syst. Biol. 55(2):314–328, 2006

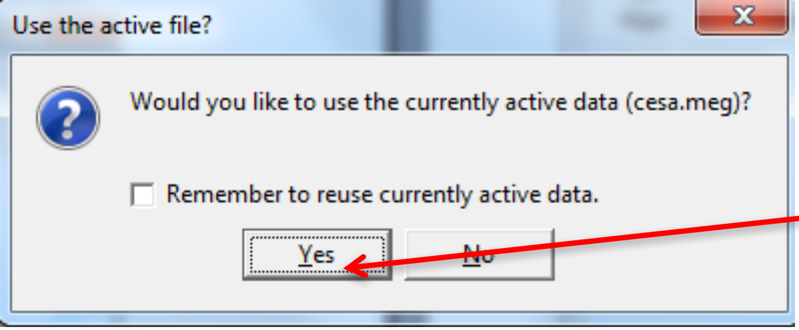
Maximum likelihood and Bayesian, in general, outperformed neighbor joining and maximum parsimony in terms of tree reconstruction accuracy.

In general, our results indicate that as alignment error increases, topological accuracy decreases.

Results also indicated that as the length of the branch and of the neighboring branches increase, alignment accuracy decreases, and the length of the neighboring branches is the major factor in topological accuracy.

Mol Biol Evol (2005) 22 (3): 792-802.

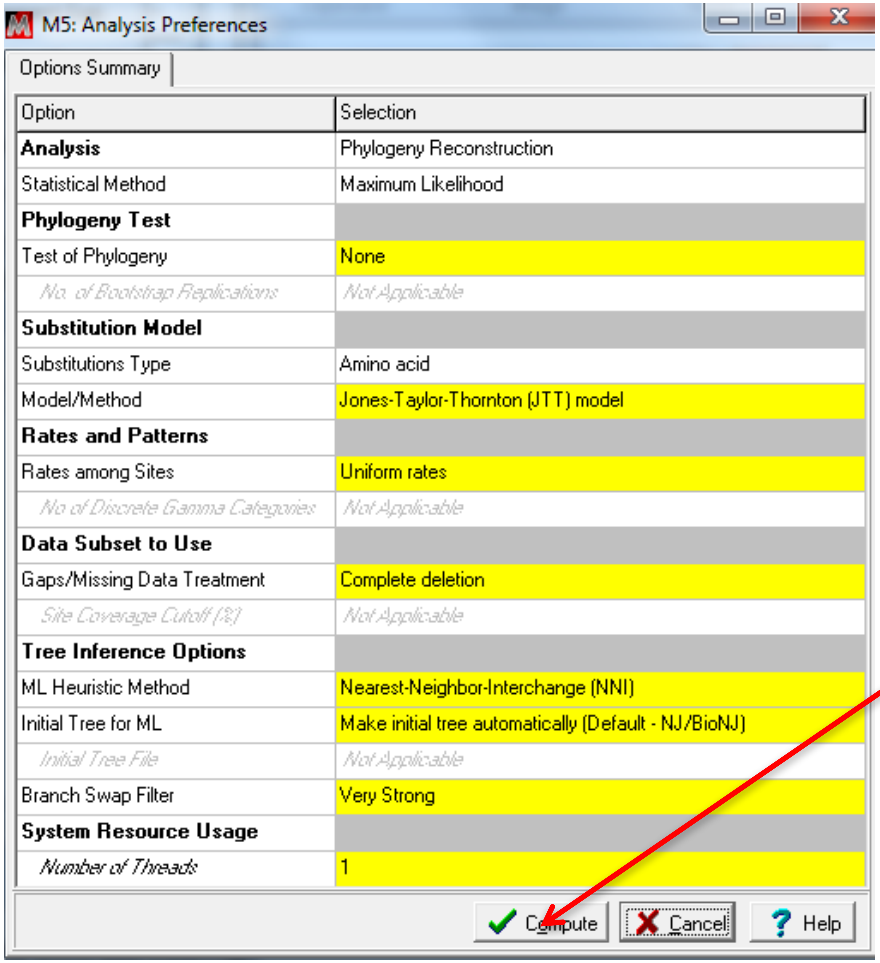
Over the variety of conditions tested, Bayesian trees estimated from DNA sequences that had been aligned according to the alignment of the corresponding protein sequences were the most accurate, followed by Maximum Likelihood trees estimated from DNA sequences and Parsimony trees estimated from protein sequences



Would you like to use the currently active data (cesa.meg)?

☐ Remember to reuse currently active data.

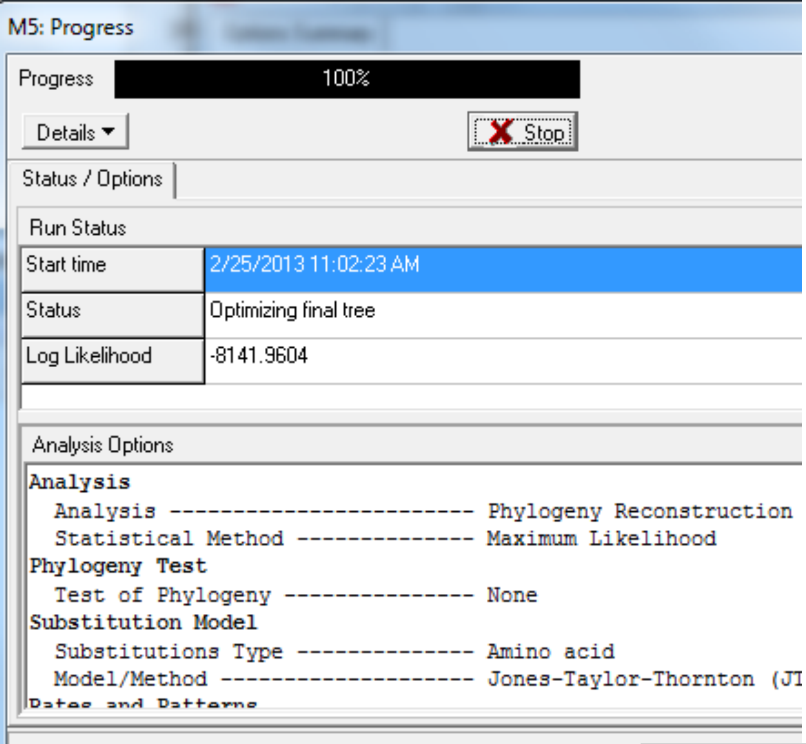
Choose yes



Option	Selection
Analysis	Phylogeny Reconstruction
Statistical Method	Maximum Likelihood
Phylogeny Test	
Test of Phylogeny	None
No. of Bootstrap Replications	*Not Applicable*
Substitution Model	
Substitutions Type	Amino acid
Model/Method	Jones-Taylor-Thornton (JTT) 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*
Tree Inference Options	
ML Heuristic Method	Nearest-Neighbor-Interchange (NNI)
Initial Tree for ML	Make initial tree automatically (Default - NJ/BioNJ)
Initial Tree File	*Not Applicable*
Branch Swap Filter	Very Strong
System Resource Usage	
Number of Threads	1

You may choose parameters for tree building

Let's just hit compute



Progress 100%

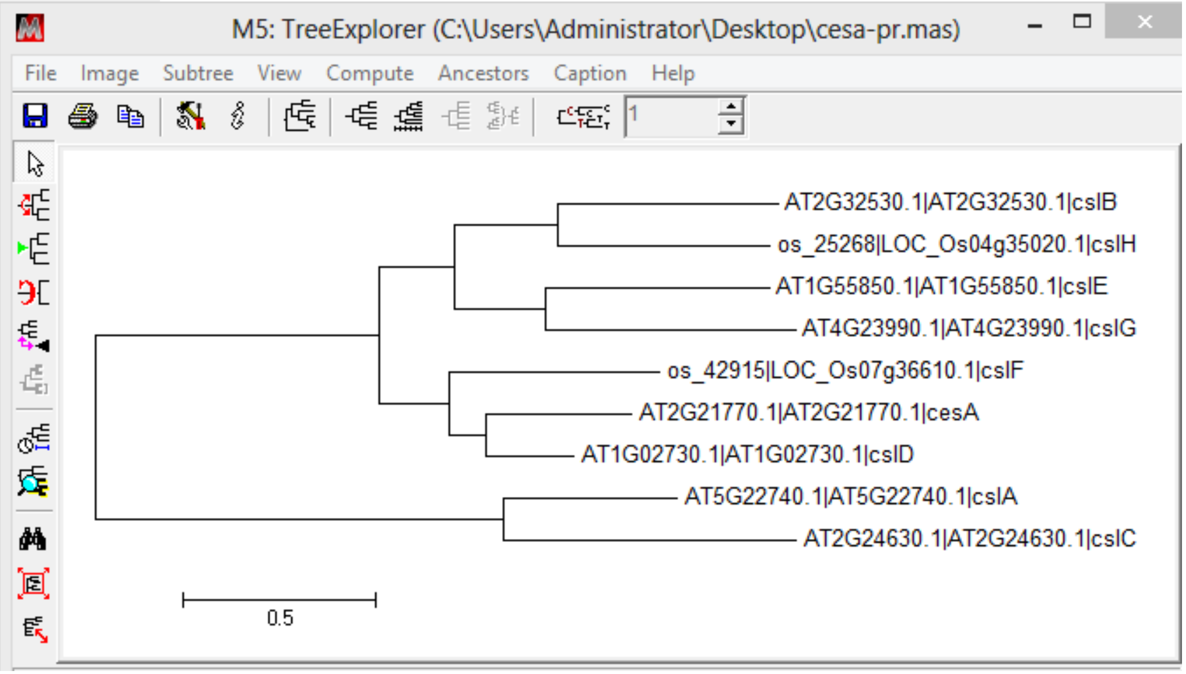
Run Status

Start time	2/25/2013 11:02:23 AM
Status	Optimizing final tree
Log Likelihood	-8141.9604

Analysis Options

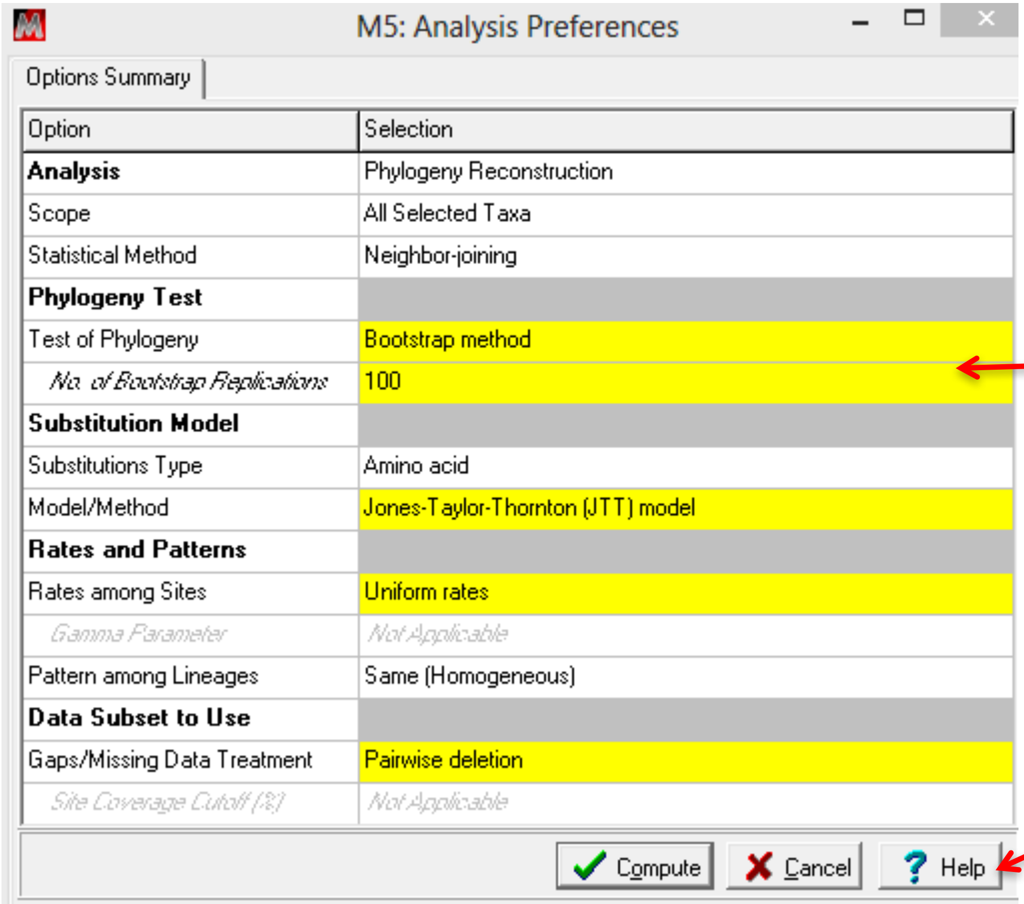
```
Analysis
  Analysis ------------------------ Phylogeny Reconstruction
  Statistical Method -------------- Maximum Likelihood
Phylogeny Test
  Test of Phylogeny --------------- None
Substitution Model
  Substitutions Type -------------- Amino acid
  Model/Method -------------------- Jones-Taylor-Thornton (JT
Rates and Patterns
```

the tree graph is shown after it's done



if we want to have statistical values on the clustering

this time we want to choose neighbor-joining algorithm because it is much faster than maximum likelihood. Here we also want to choose bootstrap method to test the phylogeny then we will have statistical values for each node.



Option	Selection
Analysis	Phylogeny Reconstruction
Scope	All Selected Taxa
Statistical Method	Neighbor-joining
Phylogeny Test	
Test of Phylogeny	Bootstrap method
No. of Bootstrap Replications	100
Substitution Model	
Substitutions Type	Amino acid
Model/Method	Jones-Taylor-Thornton (JTT) model
Rates and Patterns	
Rates among Sites	Uniform rates
Gamma Parameter	*Not Applicable*
Pattern among Lineages	Same (Homogeneous)
Data Subset to Use	
Gaps/Missing Data Treatment	Pairwise deletion
Site Coverage Cutoff (%)	*Not Applicable*

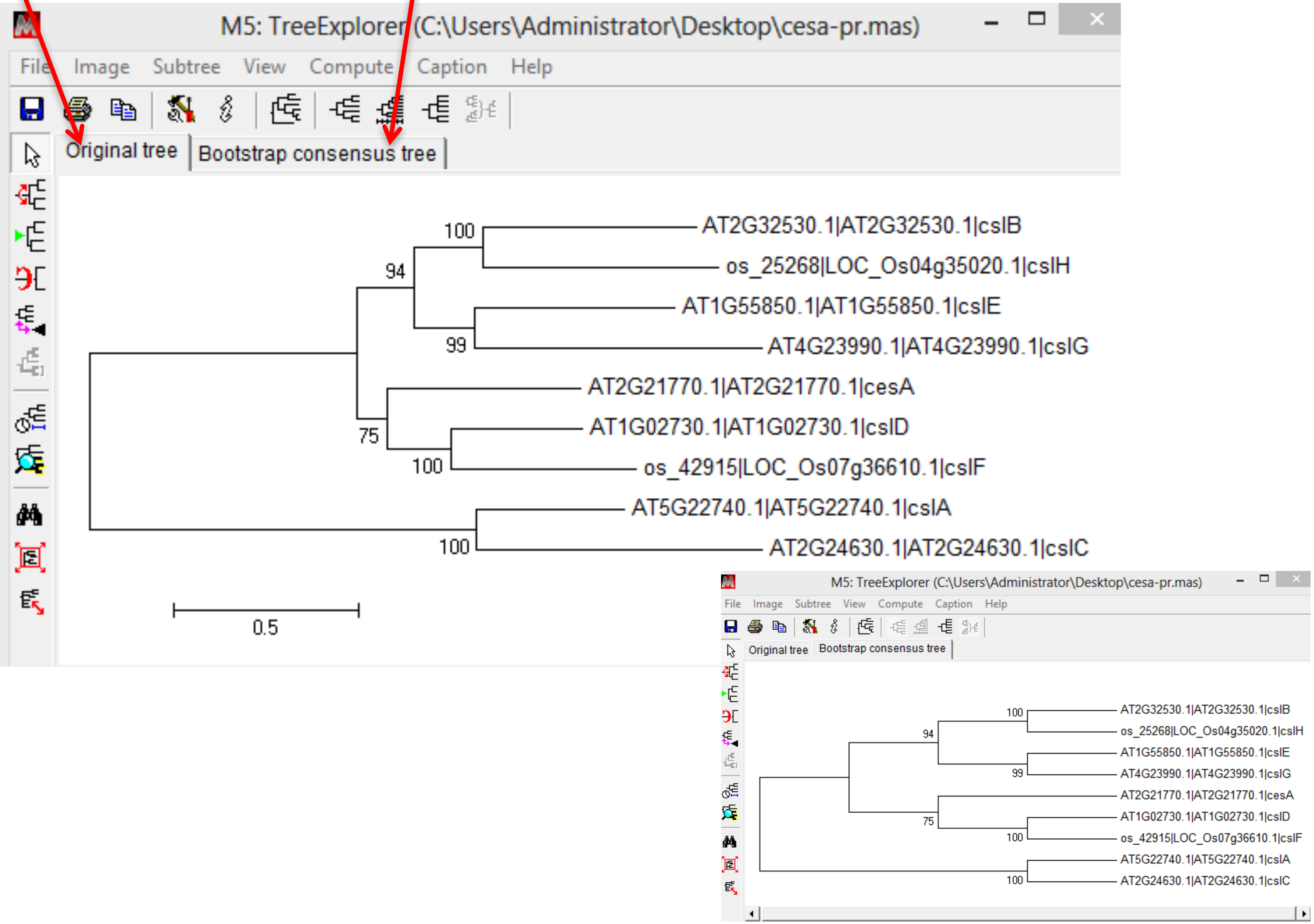
Now change here

Yellow are where you can change

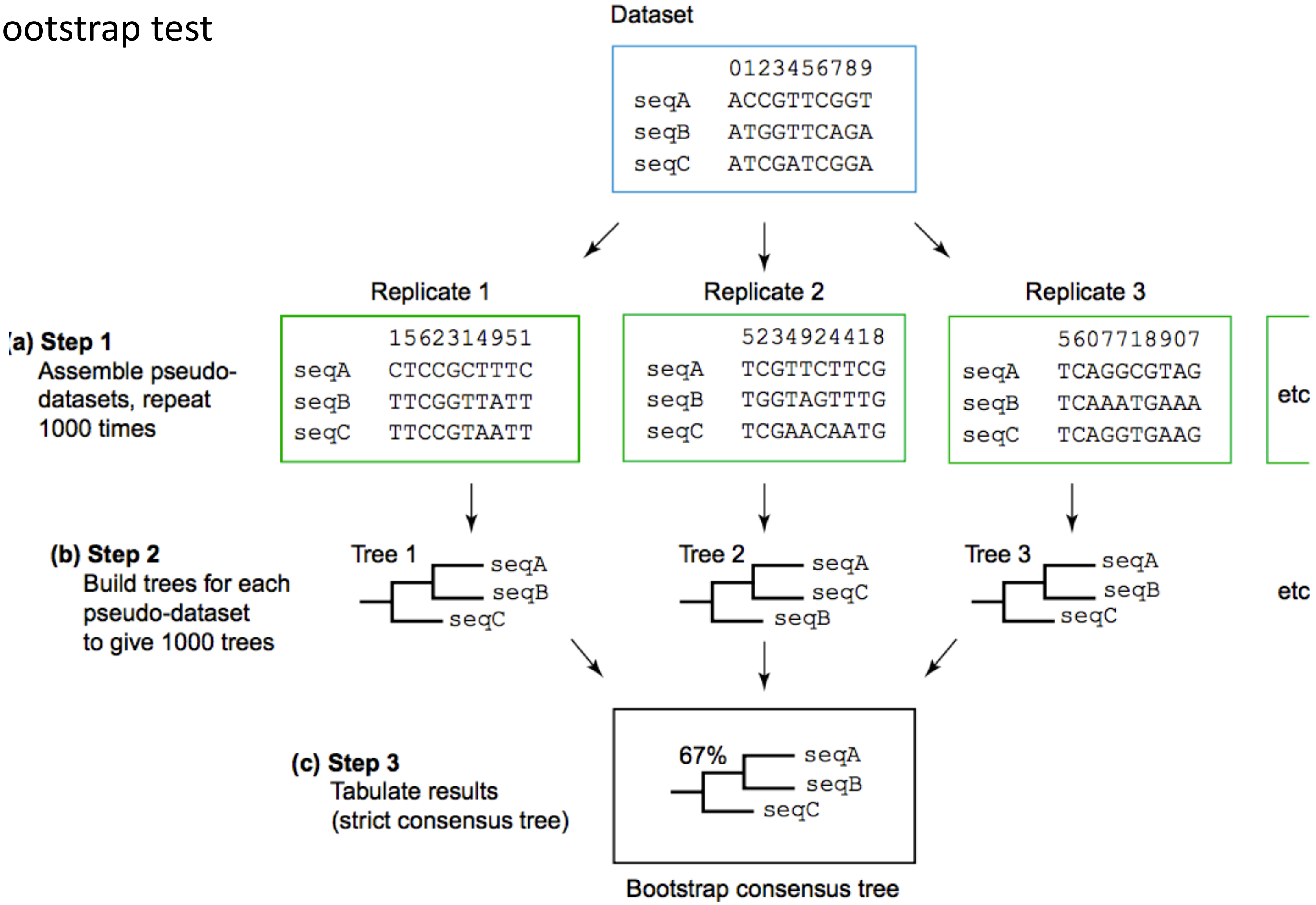
To learn what do these options mean
Click on help

This is the original tree with bootstrap support values at each internal node

Consensus tree from bootstrap test



Bootstrap test



Dataset

```
        0123456789
seqA    ACCGTTCGGT
seqB    ATGGTTCAGA
seqC    ATCGATCGGA
```

Replicate 1

```
        1562314951
seqA    CTCCGCTTTC
seqB    TTCGGTTATT
seqC    TTCCGTAATT
```

Replicate 2

```
        5234924418
seqA    TCGTTCTTCG
seqB    TGGTAGTTTG
seqC    TCGAACAATG
```

Replicate 3

```
        5607718907
seqA    TCAGGCGTAG
seqB    TCAAATGAAA
seqC    TCAGGTGAAG
```

etc

(a) Step 1
Assemble pseudo-datasets, repeat 1000 times

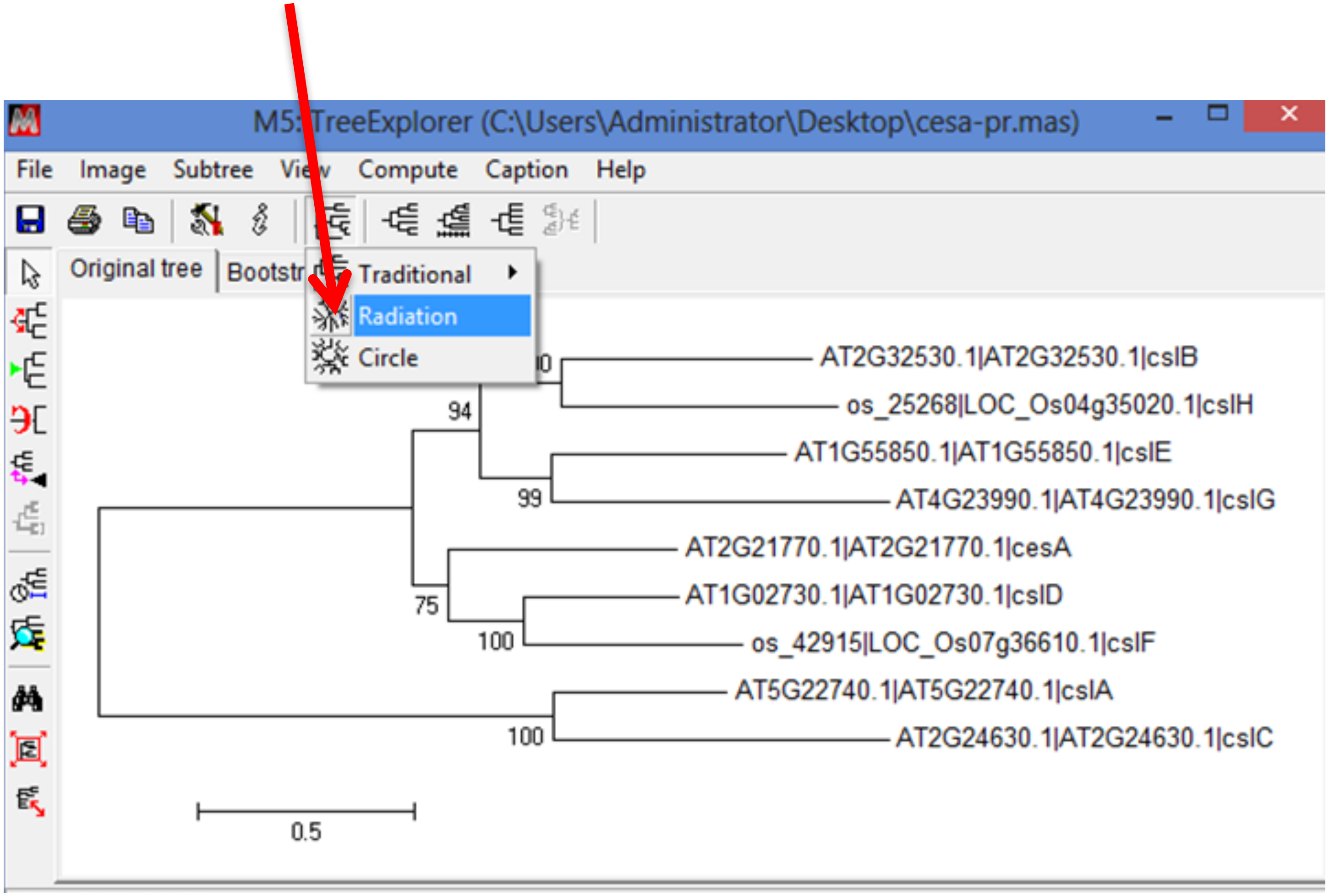
(b) Step 2
Build trees for each pseudo-dataset to give 1000 trees

(c) Step 3
Tabulate results (strict consensus tree)

Bootstrap consensus tree

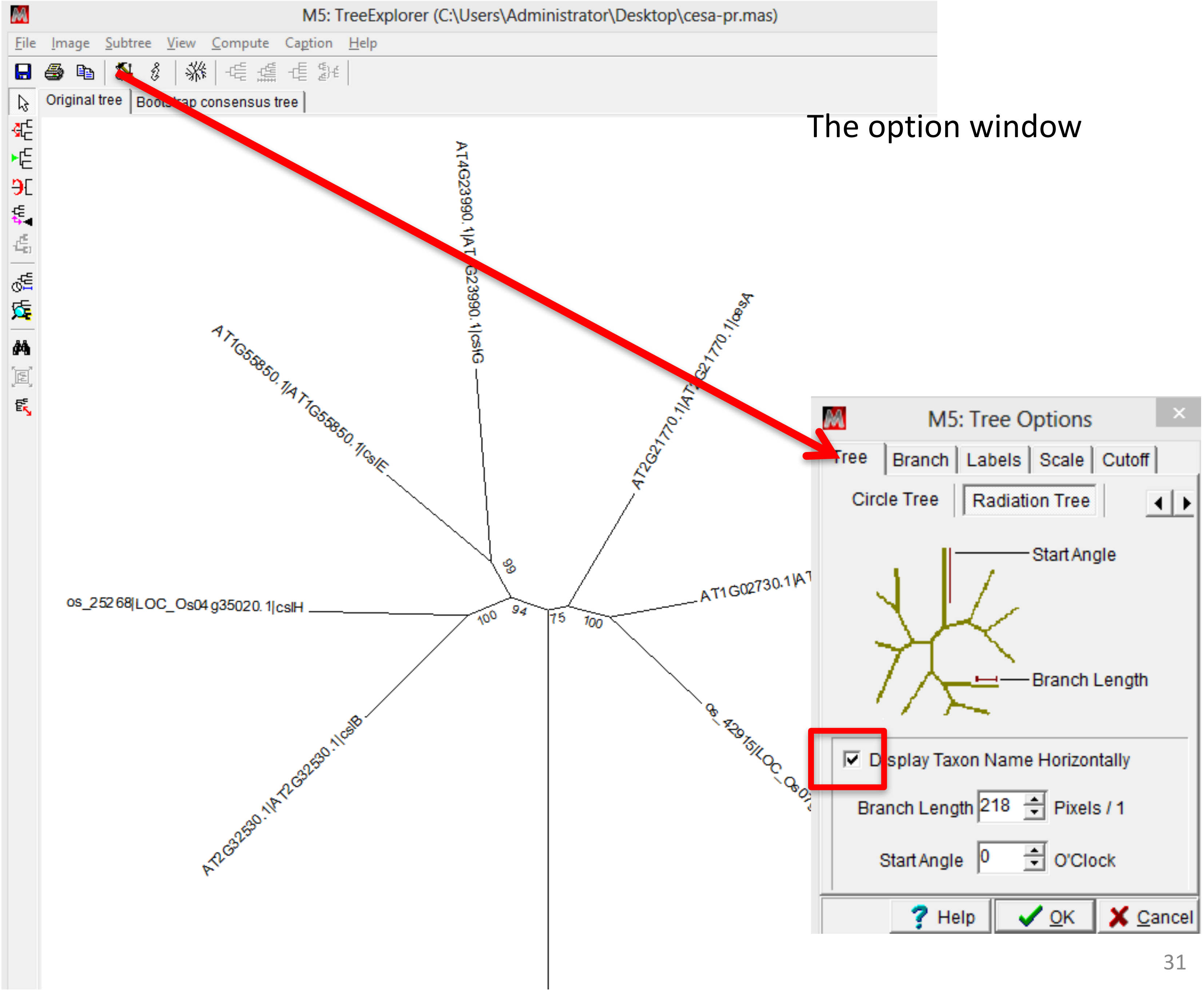
TRENDS in Genetics

Different presentation views of phylograms



M5: TreeExplorer (C:\Users\Administrator\Desktop\cesa-pr.mas)

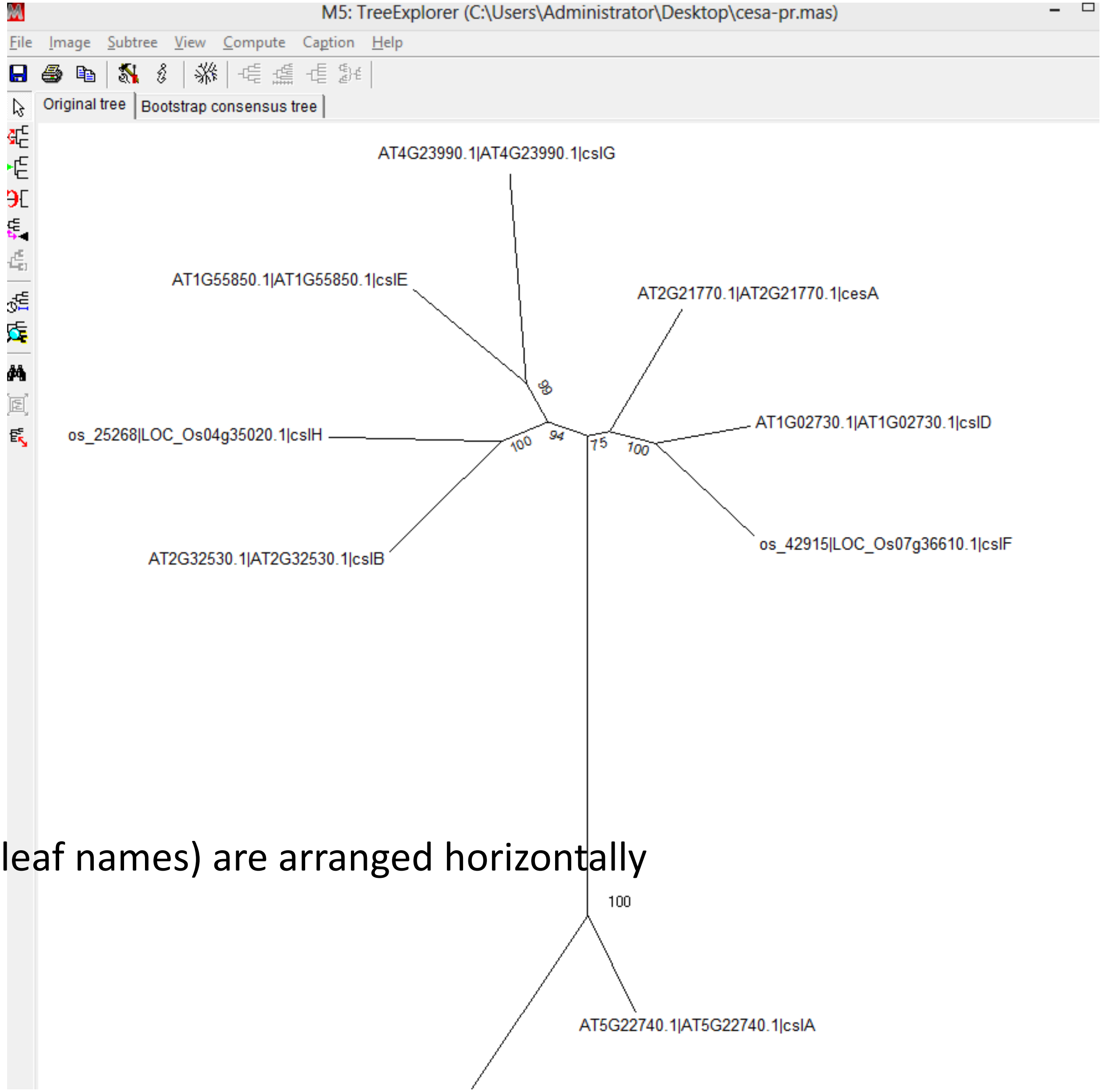
The option window



☑ Display Taxon Name Horizontally

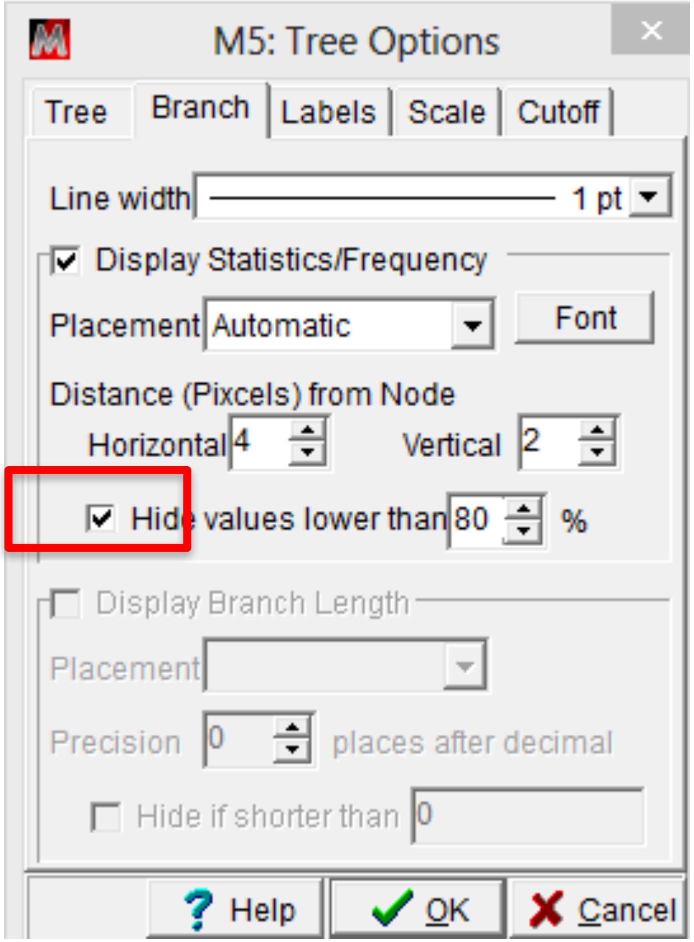
Branch Length 218 Pixels / 1

Start Angle 0 O'Clock



Now the IDs (leaf names) are arranged horizontally

To only show good bootstrap values higher than certain values



Line width 1 pt

☑ Display Statistics/Frequency

Placement Automatic

Distance (Pixcels) from Node

Horizontal 4

Vertical 2

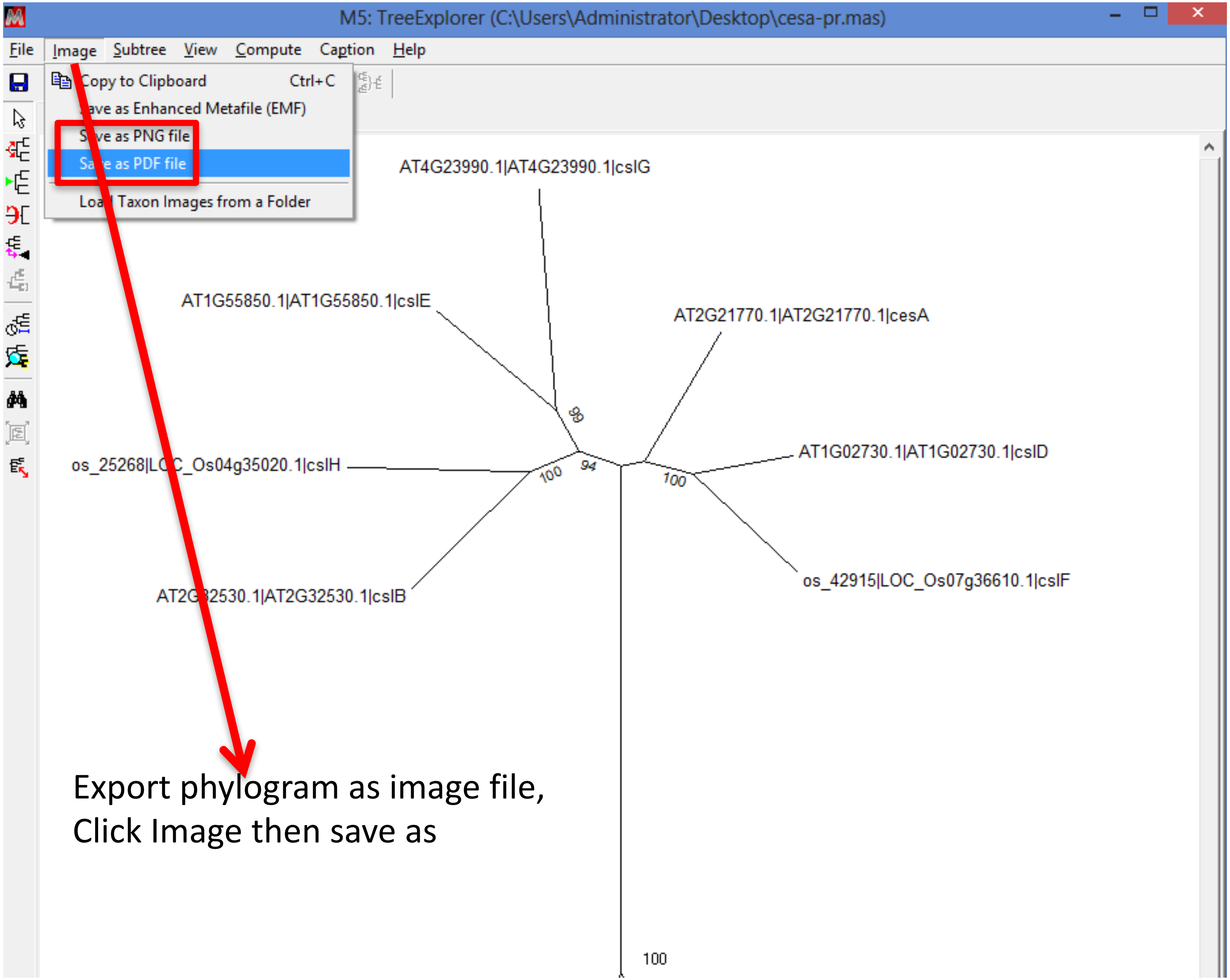
☑ Hide values lower than 80 %

☐ Display Branch Length

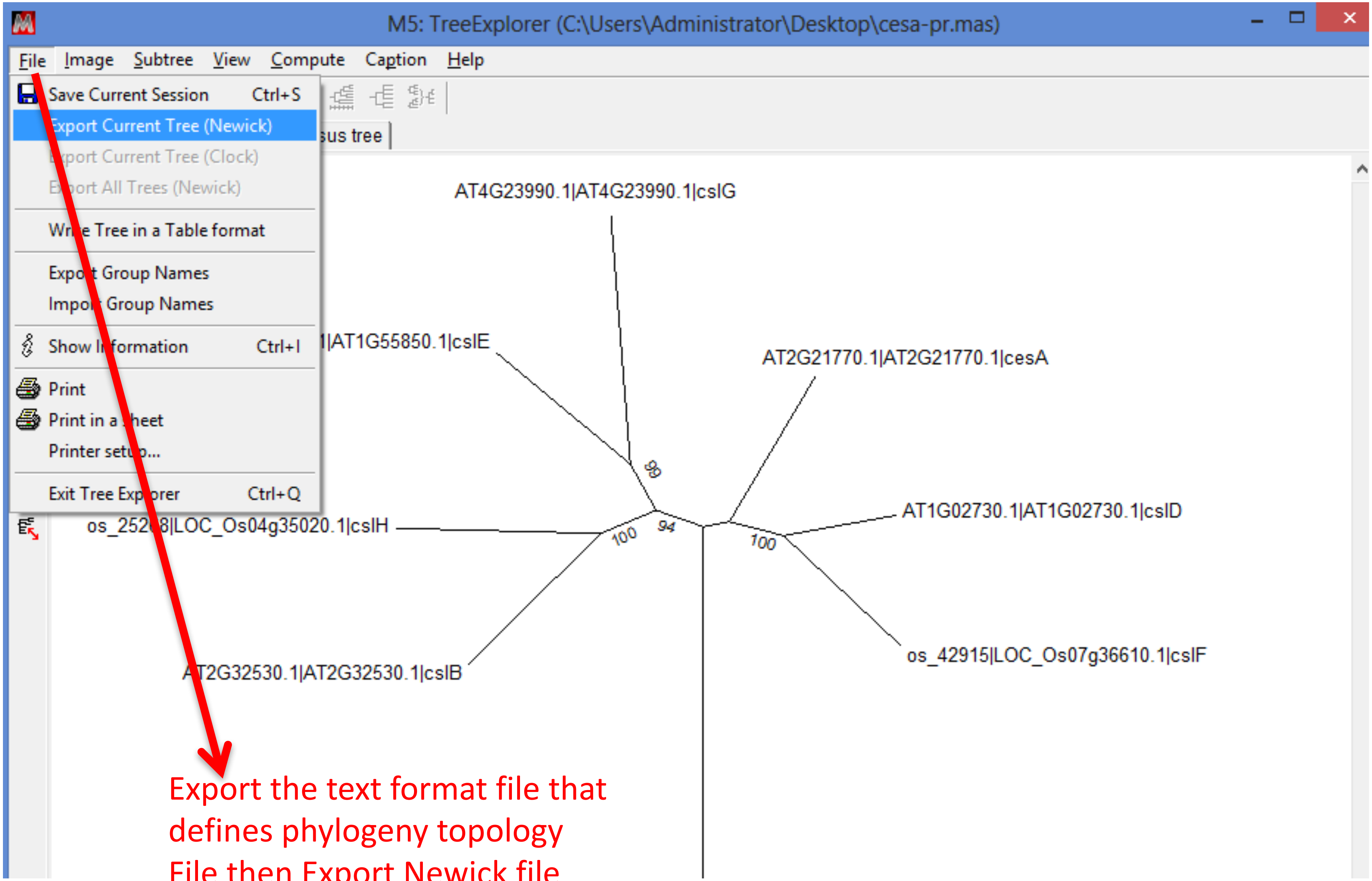
Placement

Precision 0 places after decimal

☐ Hide if shorter than 0



Export phylogram as image file,
Click Image then save as



Export the text format file that defines phylogeny topology
File then Export Newick file

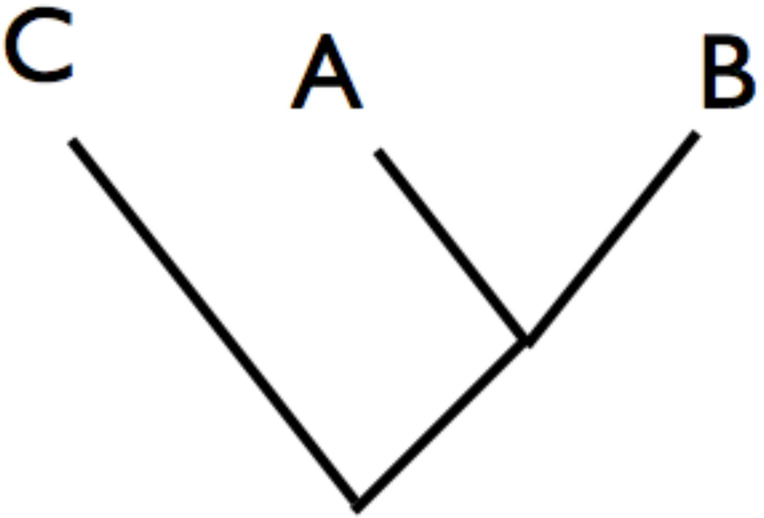
Open the saved newick format file in notepad

```
((((AT2G32530.1|AT2G32530.1|cslB:0.57646262,'os_25268|LOC_Os04g35020.1|cslH':0.
63658065)1.0000:0.18712502,(AT1G55850.1|AT1G55850.1|cslE:0.54168375,AT4G23990
.1|AT4G23990.1|cslG:0.77646829)0.9900:0.16421052)0.9400:0.15649299,(AT2G21770.1
|AT2G21770.1|cesA:0.52631255,(AT1G02730.1|AT1G02730.1|cslD:0.35504124,'os_429
15|LOC_Os07g36610.1|cslF':0.50349483)1.0000:0.17352695)0.7500:0.08201111)1.0000
:0.72454177,(AT5G22740.1|AT5G22740.1|cslA:0.39871493,AT2G24630.1|AT2G24630.1|
cslC:0.77203016)1.0000:1.04968340);
```

Not for human read!!!

Newick format uses parenthesis to group two nodes at a time to describe the groupings

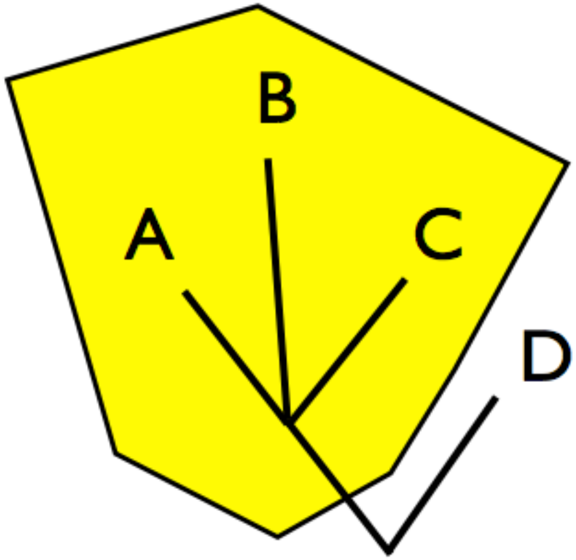
A most simplified example



`((A,B),C);`

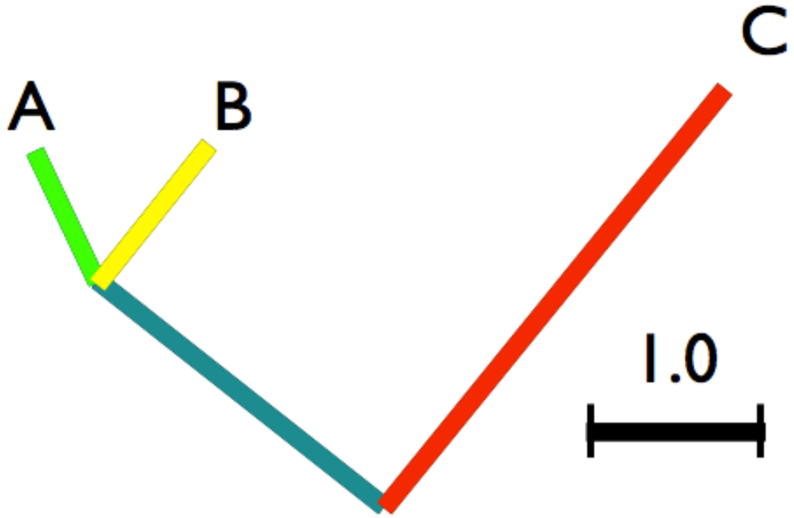
http://www.embl.de/~seqanal/courses/molEvolSofiaMar2012/newickPhylipTreeFormat.pdf polytomy/multifurcation

`((A,B,C),D);`



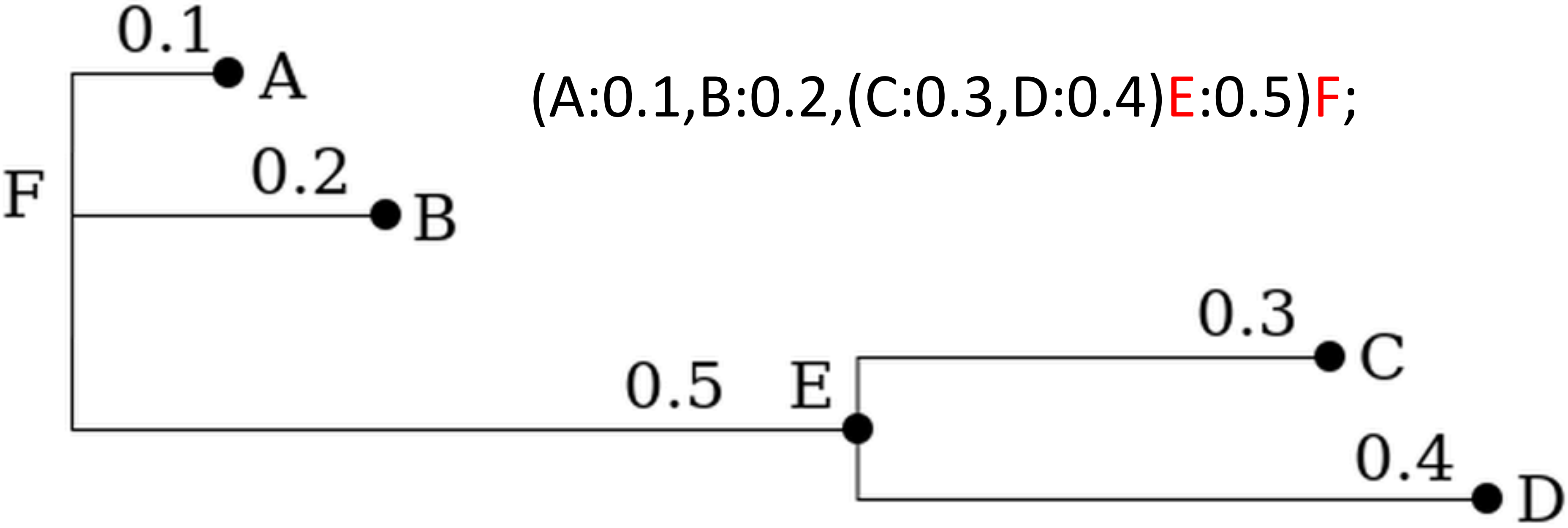
Add the branch length

((A:1,B:1):2,C:3)

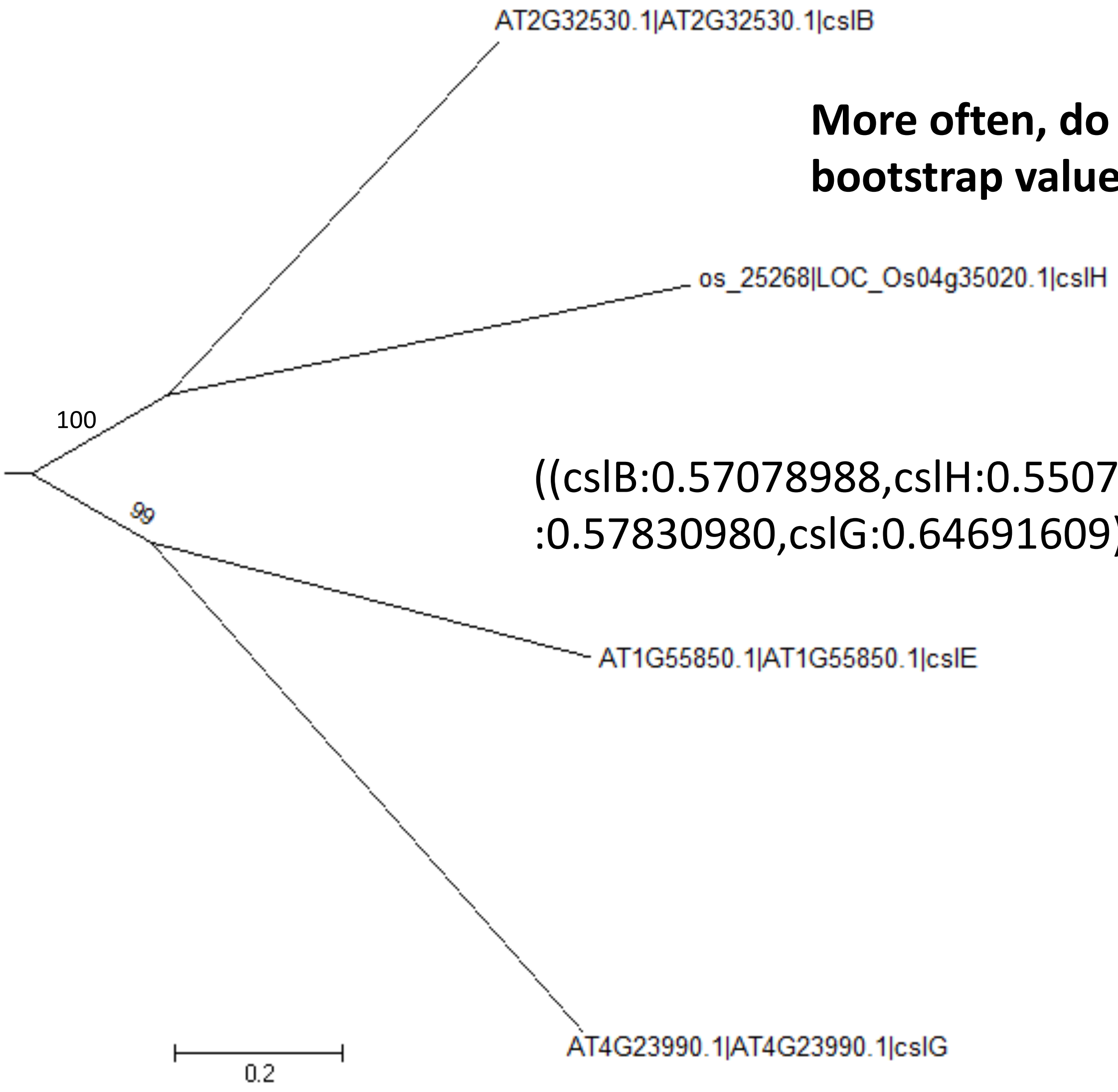


Add the internal node name

(A:0.1,B:0.2,(C:0.3,D:0.4)E:0.5)F;

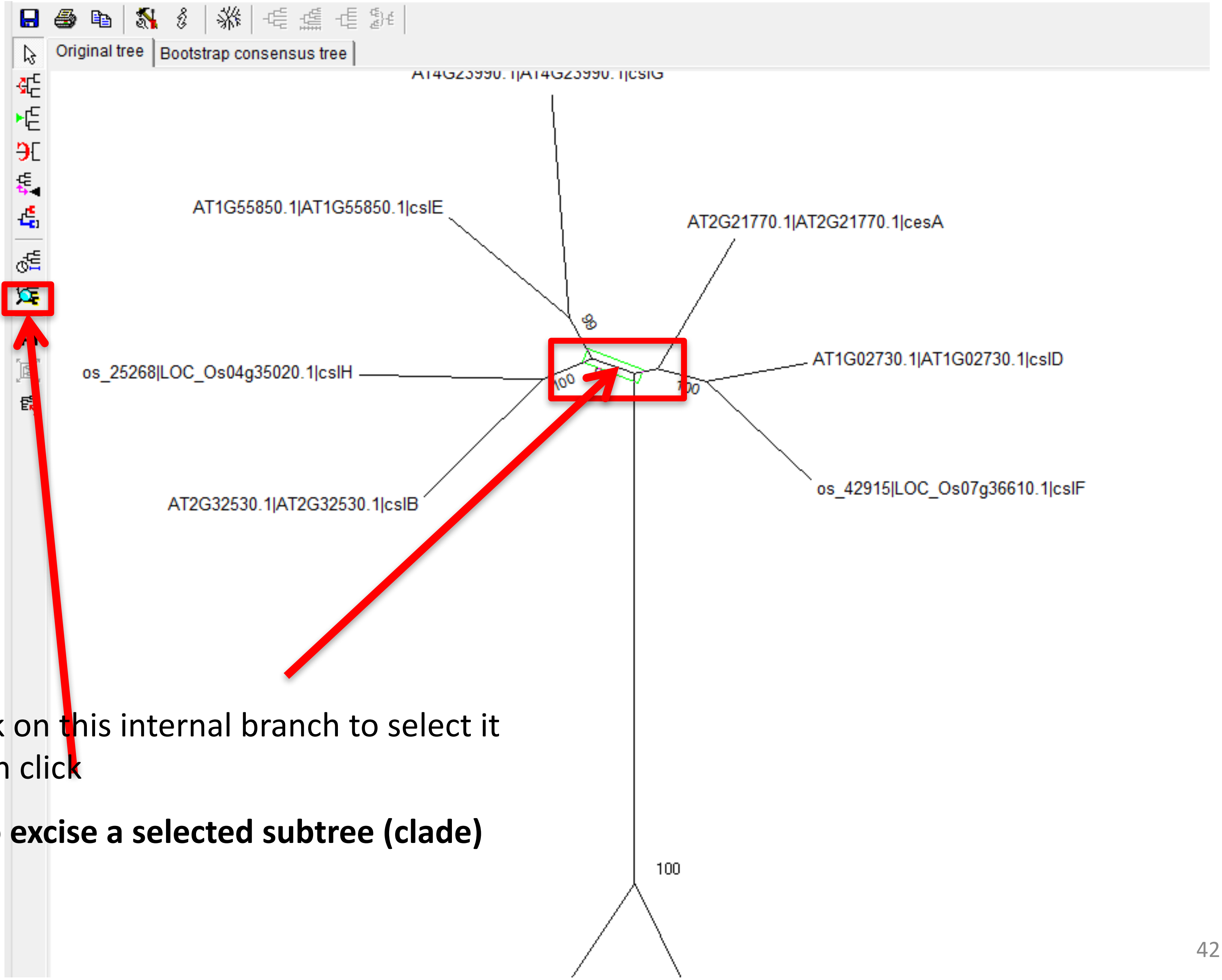


E and F and inferred nodes, not from the input



More often, do not add internal nodes but add bootstrap values

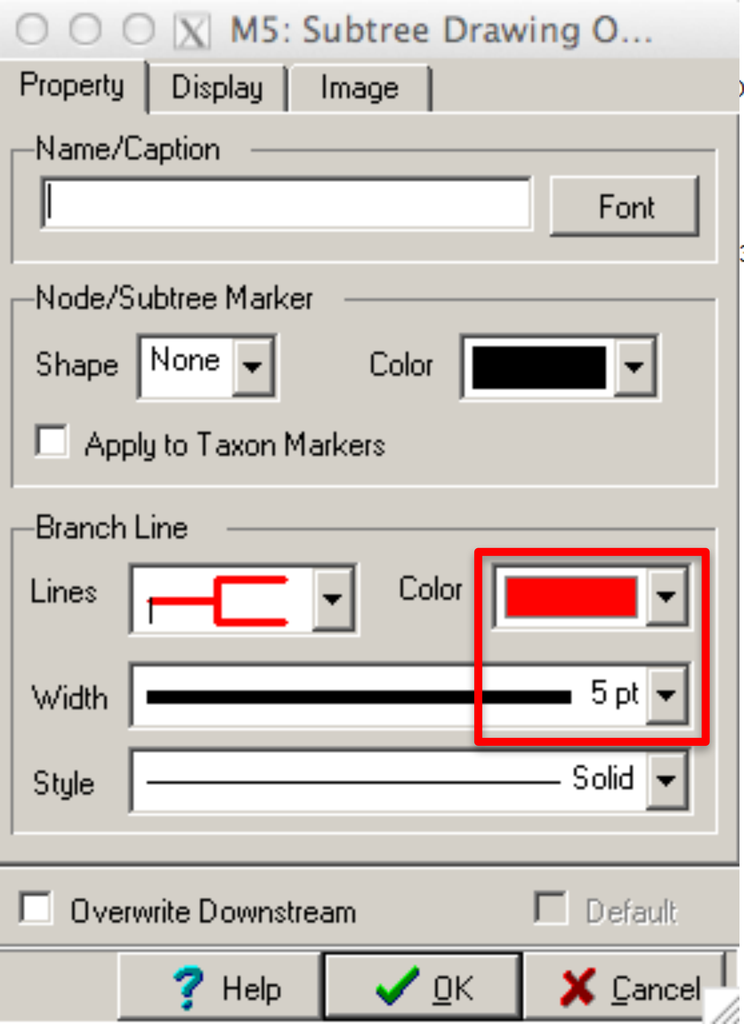
```
((cslB:0.57078988,cslH:0.55075714)1.000:0.26338963,(cslE:0.57830980,cslG:0.64691609)0.9900:0.23352951);
```



Click on this internal branch to select it
Then click

To excise a selected subtree (clade)

To color branches


M5: Subtree Drawing O...

Property | Display | Image

Name/Caption

Font

Node/Subtree Marker

Shape None

Color

☐ Apply to Taxon Markers

Branch Line

Lines

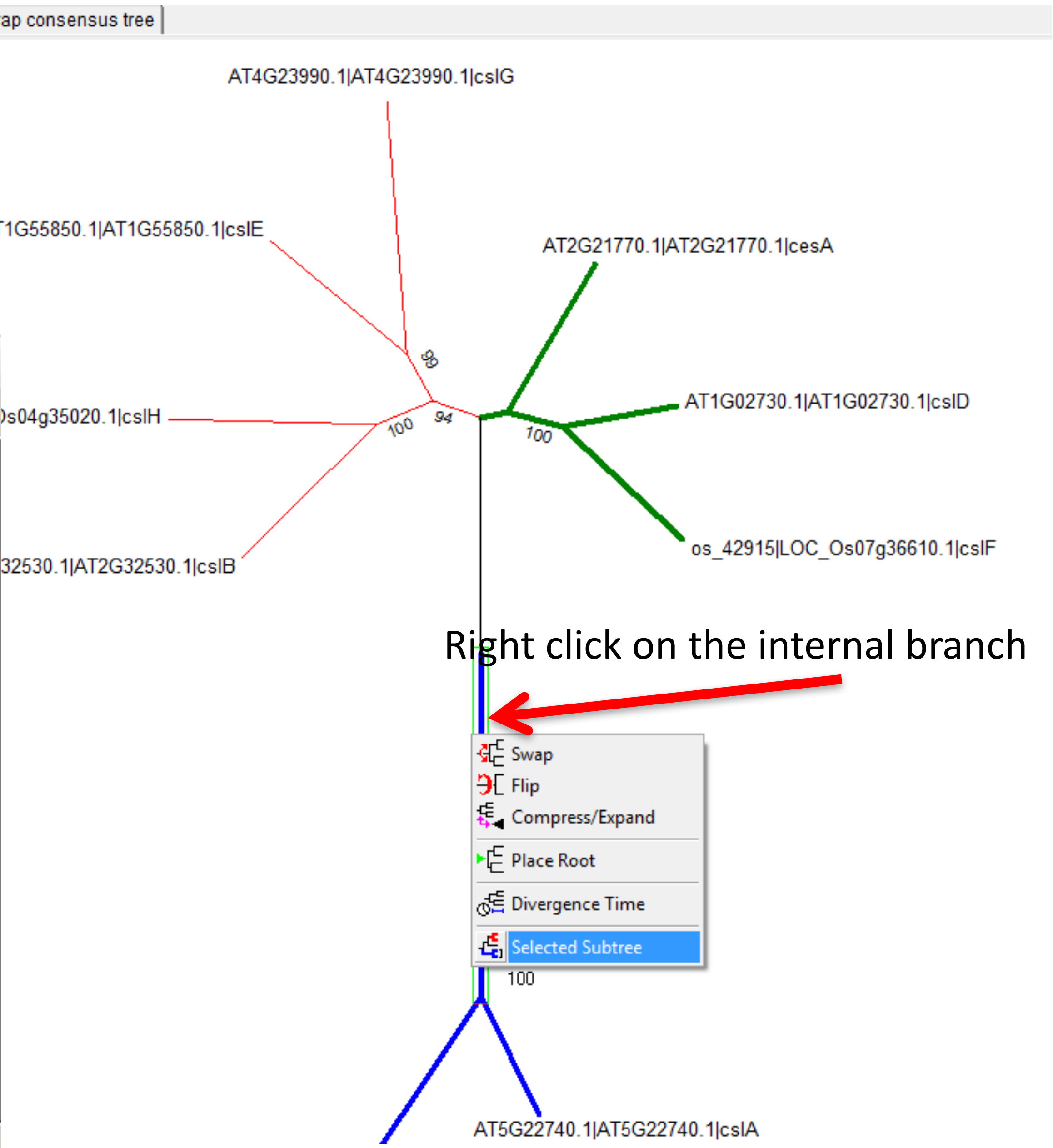
Color 🟥

Width 5 pt

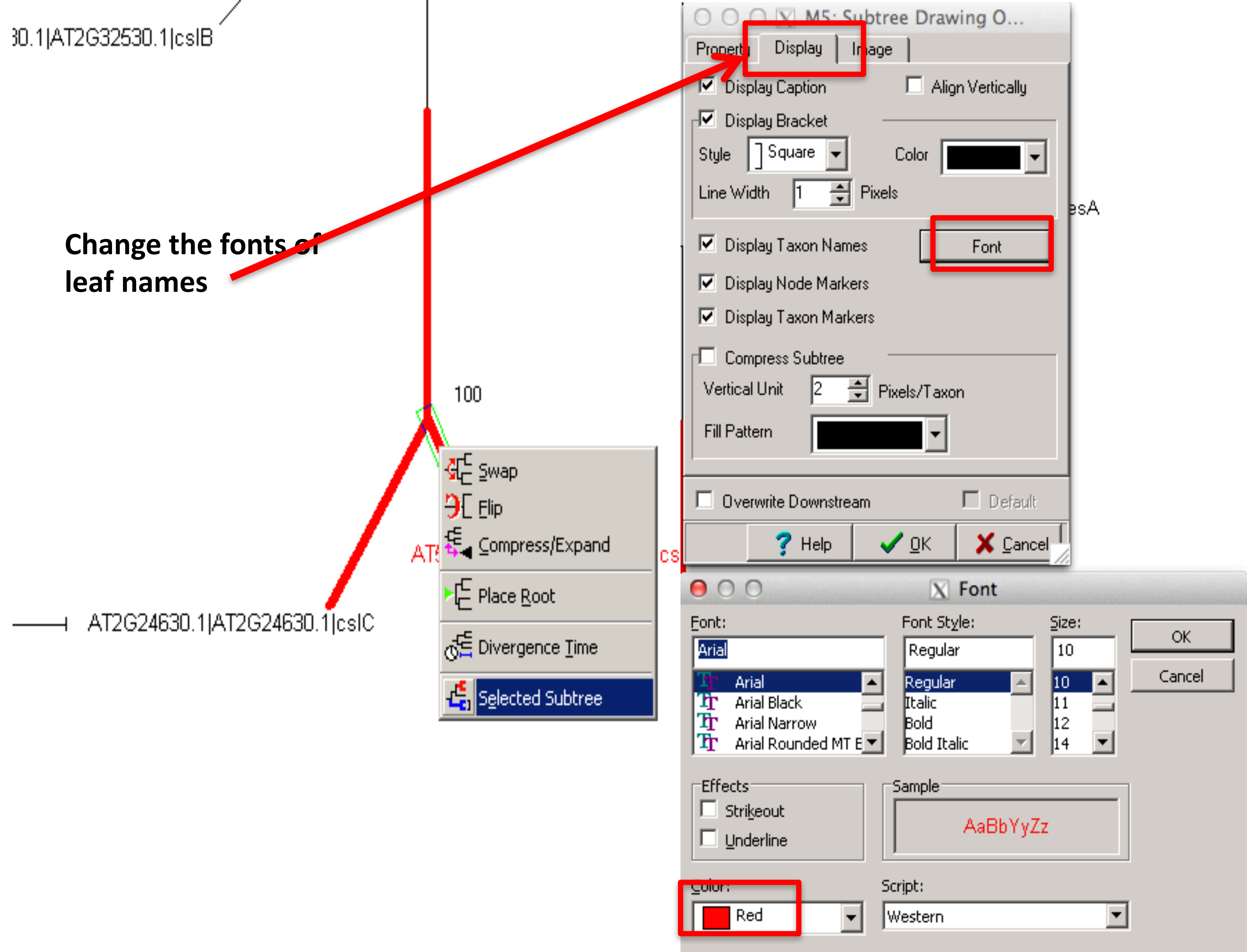
Style Solid

☐ Overwrite Downstream

☐ Default



Right click on the internal branch



Change the fonts of leaf names

M5: Subtree Drawing O...

Property | Display | Image

☑ Display Caption ☐ Align Vertically

☑ Display Bracket

Style: Square Color: ■

Line Width: 1 Pixels

☑ Display Taxon Names [Font]

☑ Display Node Markers

☑ Display Taxon Markers

☐ Compress Subtree

Vertical Unit: 2 Pixels/Taxon

Fill Pattern: ■

☐ Overwrite Downstream ☐ Default

Font

Font: Arial

Font Style: Regular

Size: 10

Effects

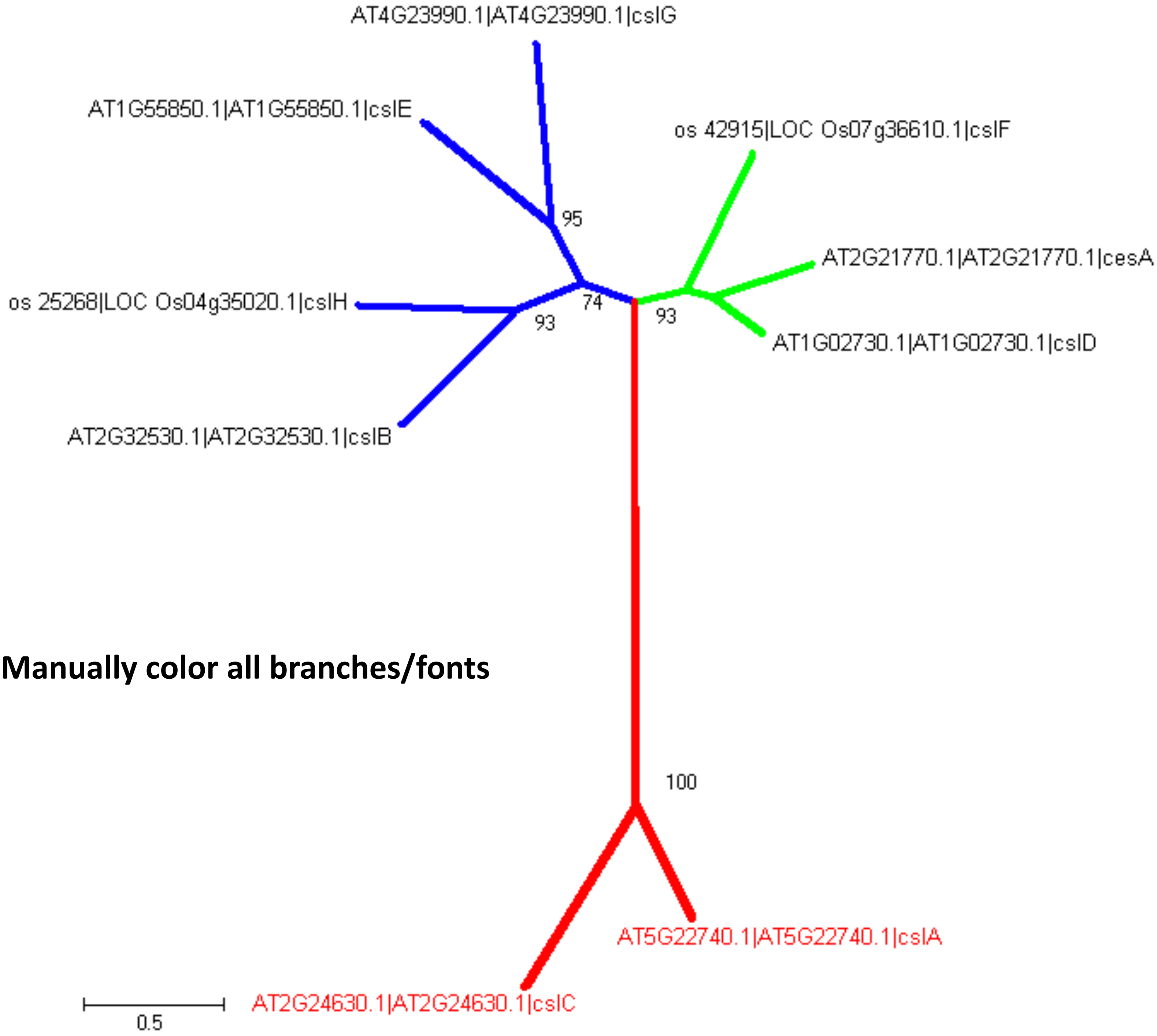
☐ Strikeout

☐ Underline

Sample: AaBbYyZz

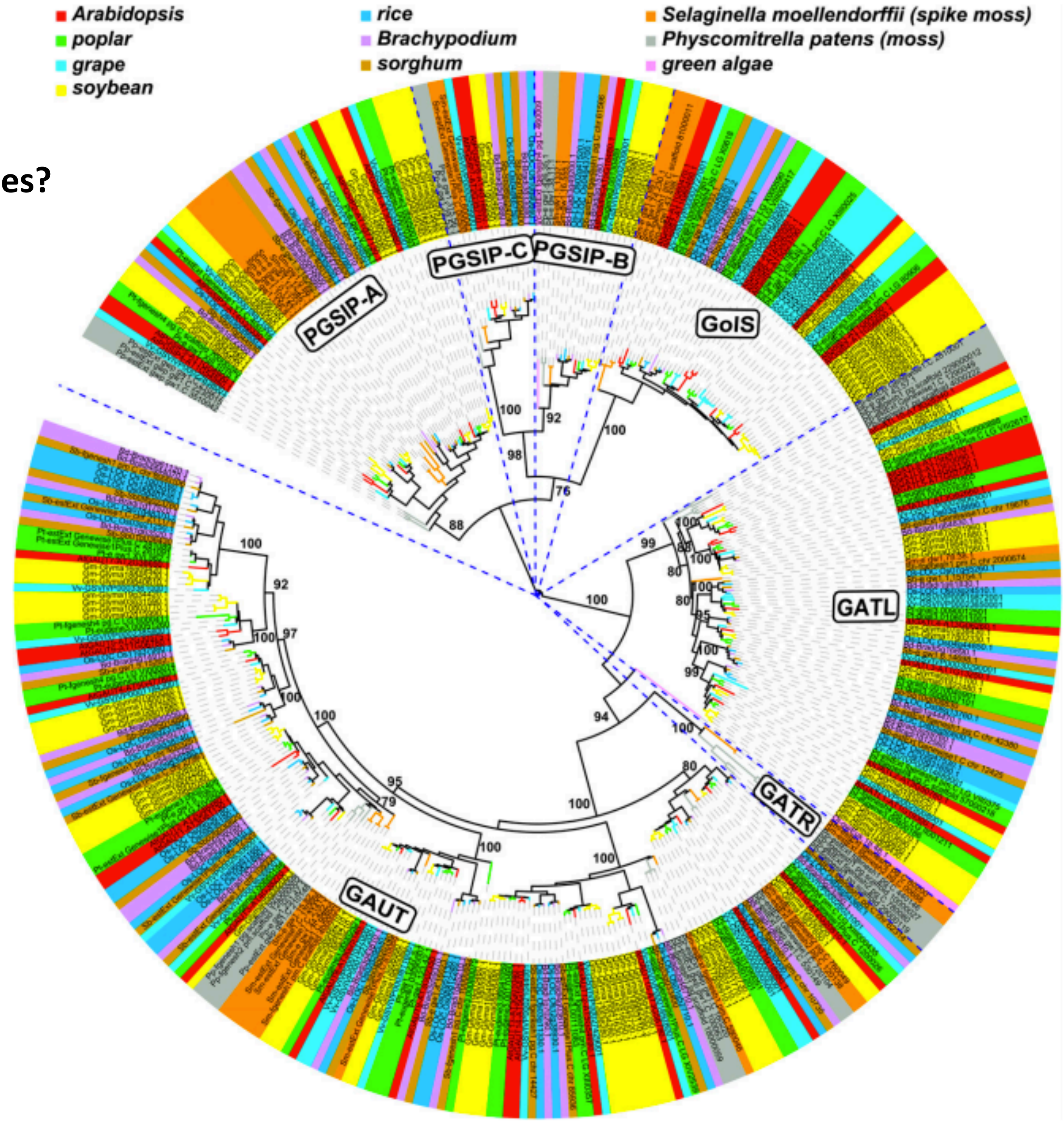
Color: 🟥 Red

Script: Western

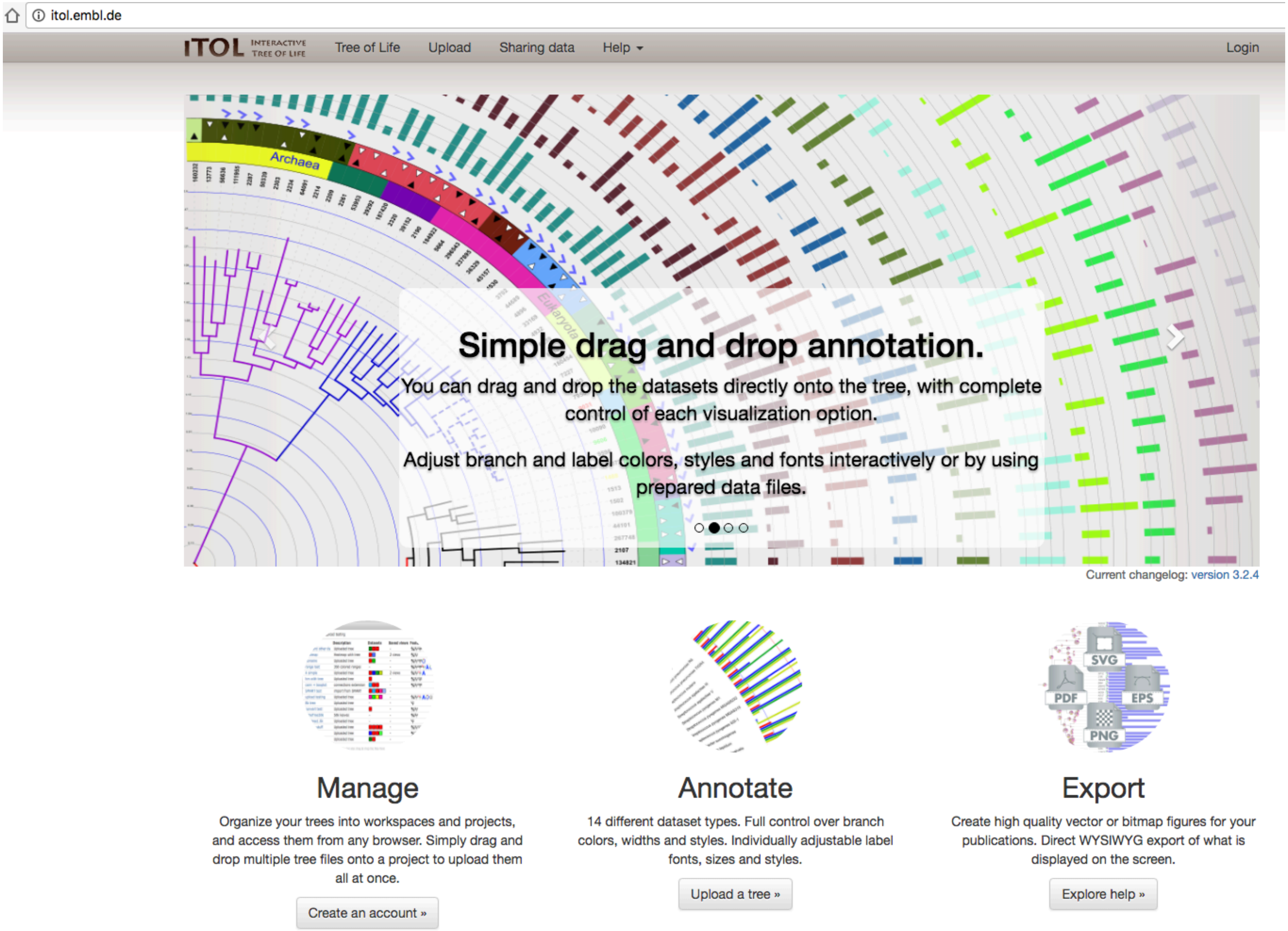


Manually color all branches/fonts

What if we have hundreds of genes?



http://itol.embl.de/



Simple drag and drop annotation.

You can drag and drop the datasets directly onto the tree, with complete control of each visualization option.

Adjust branch and label colors, styles and fonts interactively or by using prepared data files.

Current changelog: version 3.2.4

Manage

Organize your trees into workspaces and projects, and access them from any browser. Simply drag and drop multiple tree files onto a project to upload them all at once.

Annotate

14 different dataset types. Full control over branch colors, widths and styles. Individually adjustable label fonts, sizes and styles.

Export

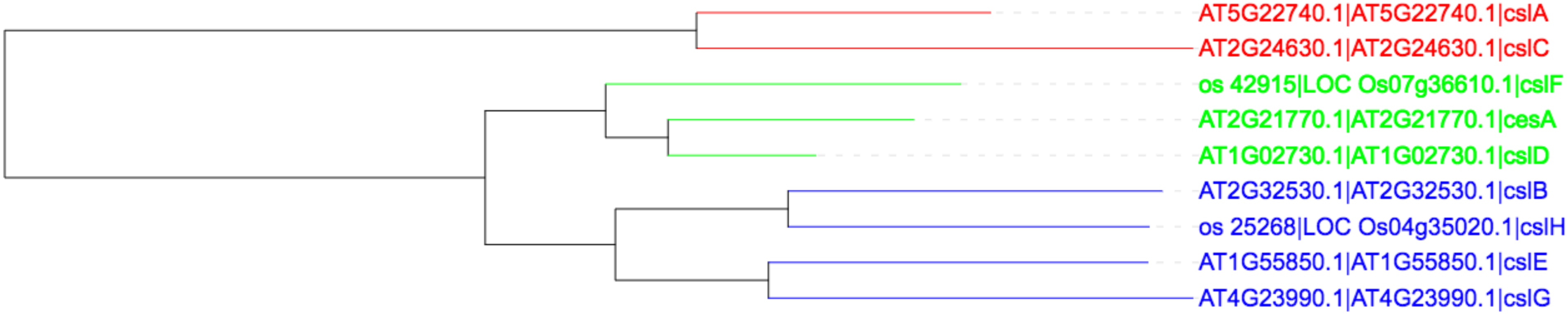
Create high quality vector or bitmap figures for your publications. Direct WYSIWYG export of what is displayed on the screen.

Automatically define branch and font colors by uploading **color definition files**

You can define your own colors for each branch/leaf separately.
Use standard hexadecimal color notation (for example, #ff0000 for red)

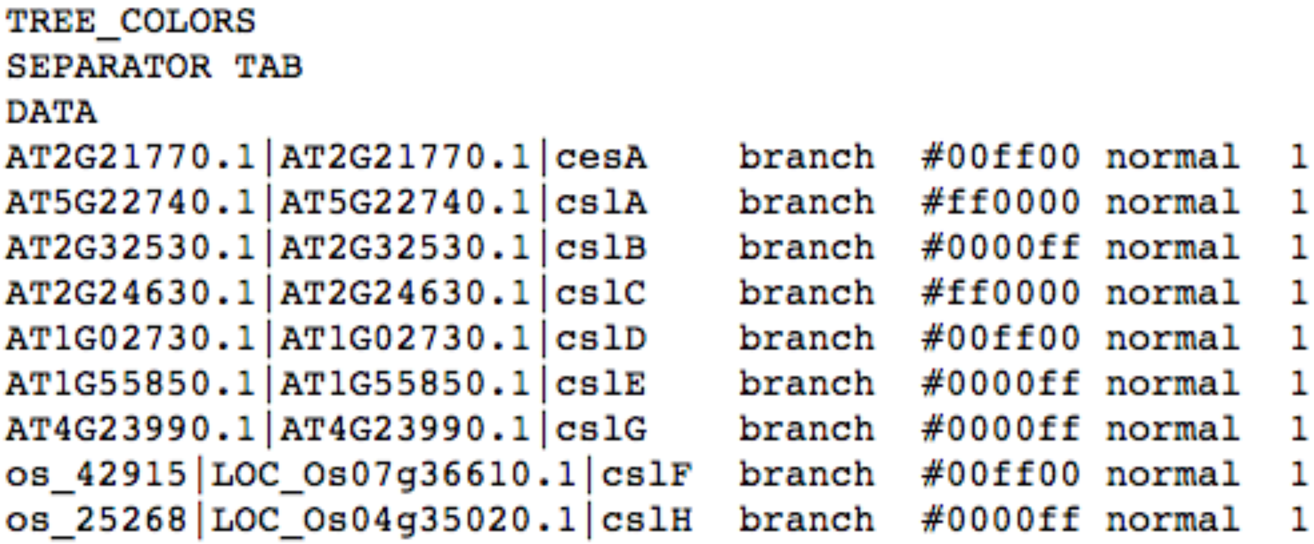
http://www.w3schools.com/html/html_colors.asp

```
((((AT2G32530.1|AT2G32530.1|cslB:0.57078988,os_25268|LOC_Os04g35020.1|cslH:0.55075714)0.9300:0.26338963,(AT1G55850.1|AT1G55850.1|cslE:0.57830980,AT4G23990.1|AT4G23990.1|cslG:0.64691609)0.9500:0.23352951)0.7400:0.19857786,(os_42915|LOC_Os07g36610.1|cslF:0.54191868,(AT2G21770.1|AT2G21770.1|cesA:0.37516472,AT1G02730.1|AT1G02730.1|cslD:0.22502015)0.6600:0.09521396)0.9300:0.18369951)1.0000:0.73286595,(AT5G22740.1|AT5G22740.1|cslA:0.44848889,AT2G24630.1|AT2G24630.1|cslC:0.75671710)1.0000:1.05517231);
```



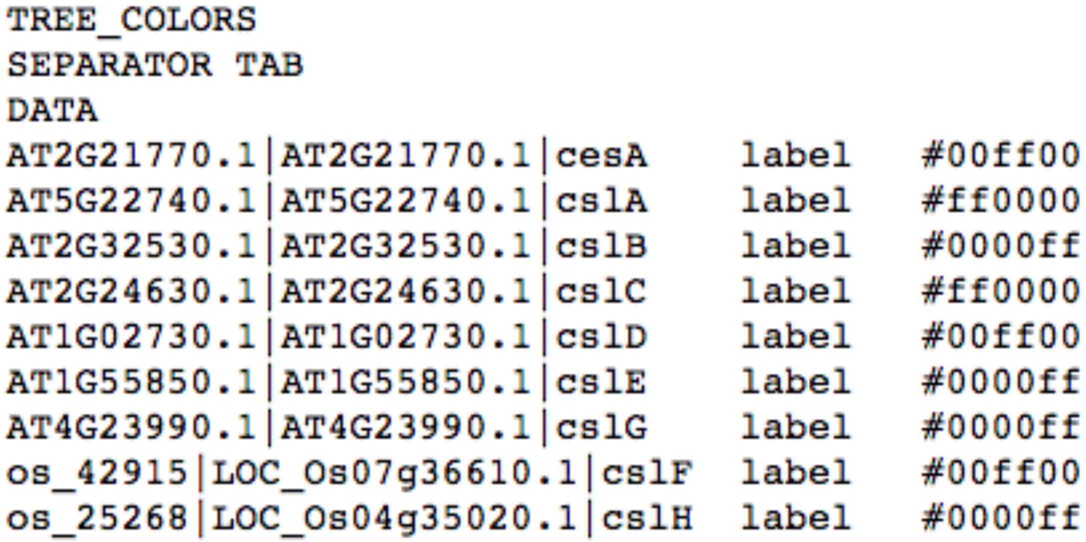
http://itol.embl.de/help/help.shtml

http://cys.bios.niu.edu/yyin/teach/PBB/cesa-pr.fa.br.txt

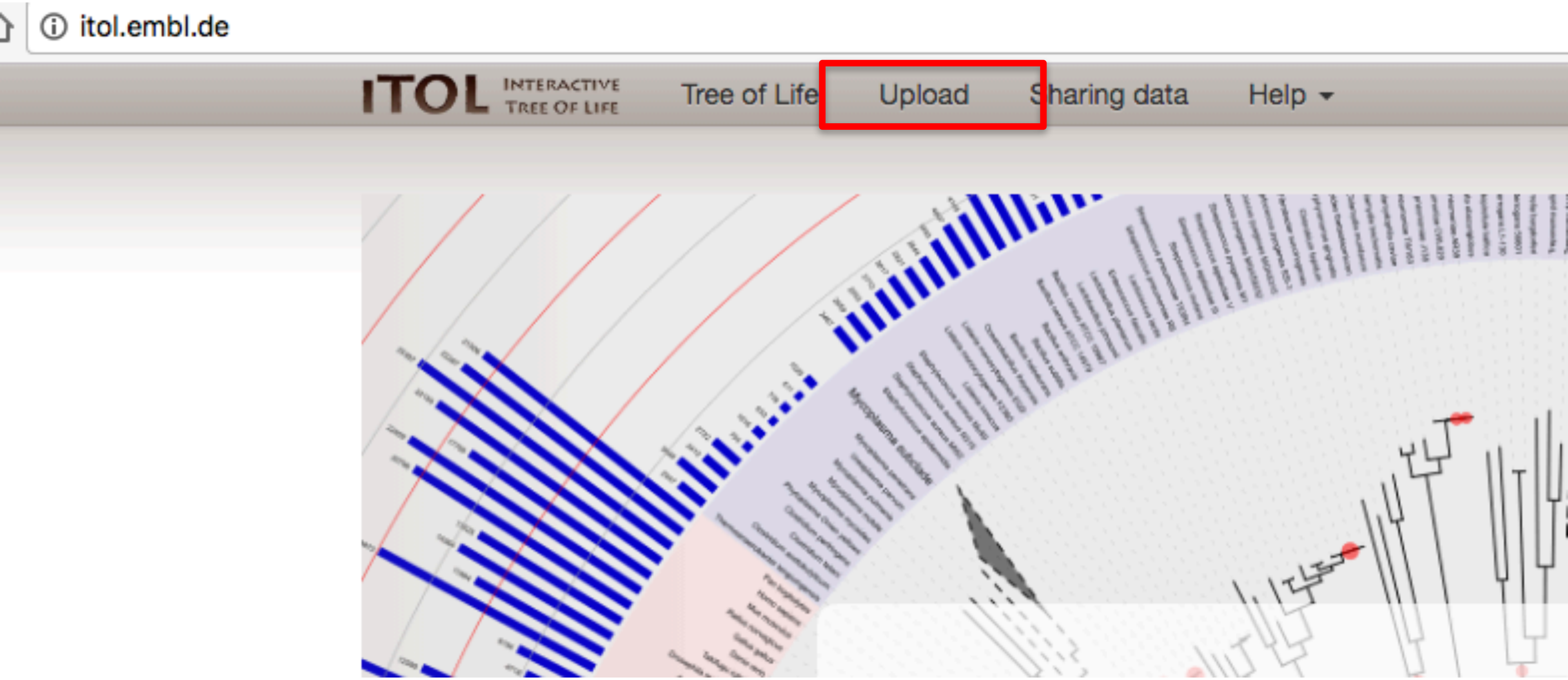


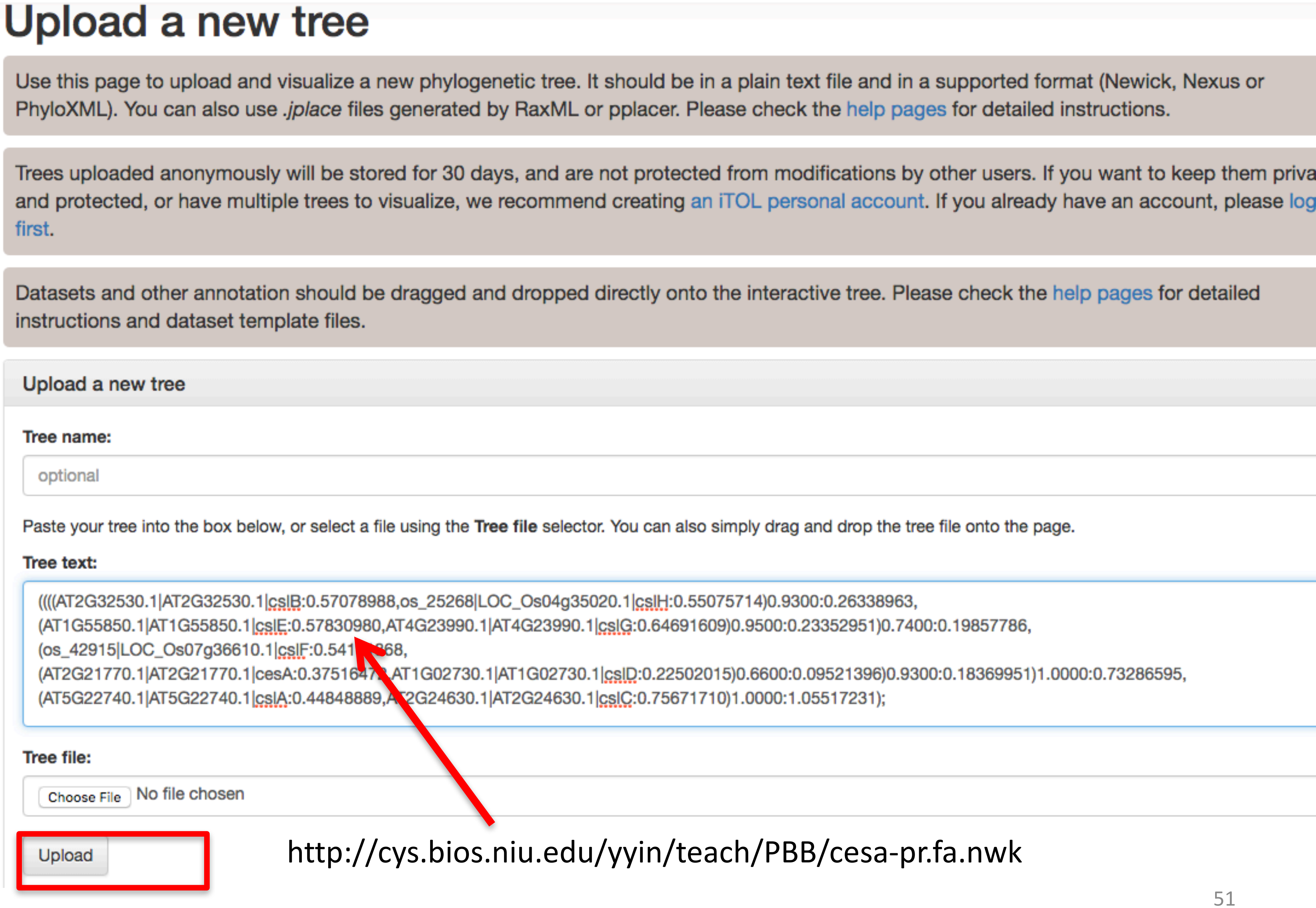
```
TREE_COLORS
SEPARATOR TAB
DATA
AT2G21770.1|AT2G21770.1|cesA	branch	#00ff00	normal	1
AT5G22740.1|AT5G22740.1|cslA	branch	#ff0000	normal	1
AT2G32530.1|AT2G32530.1|cslB	branch	#0000ff	normal	1
AT2G24630.1|AT2G24630.1|cslC	branch	#ff0000	normal	1
AT1G02730.1|AT1G02730.1|cslD	branch	#00ff00	normal	1
AT1G55850.1|AT1G55850.1|cslE	branch	#0000ff	normal	1
AT4G23990.1|AT4G23990.1|cslG	branch	#0000ff	normal	1
os_42915|LOC_Os07g36610.1|cslF	branch	#00ff00	normal	1
os_25268|LOC_Os04g35020.1|cslH	branch	#0000ff	normal	1
```

http://cys.bios.niu.edu/yyin/teach/PBB/cesa-pr.fa.col.txt



```
TREE_COLORS
SEPARATOR TAB
DATA
AT2G21770.1|AT2G21770.1|cesA	label	#00ff00
AT5G22740.1|AT5G22740.1|cslA	label	#ff0000
AT2G32530.1|AT2G32530.1|cslB	label	#0000ff
AT2G24630.1|AT2G24630.1|cslC	label	#ff0000
AT1G02730.1|AT1G02730.1|cslD	label	#00ff00
AT1G55850.1|AT1G55850.1|cslE	label	#0000ff
AT4G23990.1|AT4G23990.1|cslG	label	#0000ff
os_42915|LOC_Os07g36610.1|cslF	label	#00ff00
os_25268|LOC_Os04g35020.1|cslH	label	#0000ff
```


itol.embl.de
ITOL
INTERACTIVE
TREE OF LIFE
Tree of Life
Upload
Sharing data
Help
Mycoplasma mobile clade



Upload a new tree

Use this page to upload and visualize a new phylogenetic tree. It should be in a plain text file and in a supported format (Newick, Nexus or PhyloXML). You can also use *.jplace* files generated by RaxML or pplacer. Please check the help pages for detailed instructions.

Trees uploaded anonymously will be stored for 30 days, and are not protected from modifications by other users. If you want to keep them priva and protected, or have multiple trees to visualize, we recommend creating an iTOL personal account. If you already have an account, please log first.

Datasets and other annotation should be dragged and dropped directly onto the interactive tree. Please check the help pages for detailed instructions and dataset template files.

Upload a new tree

Tree name:

optional

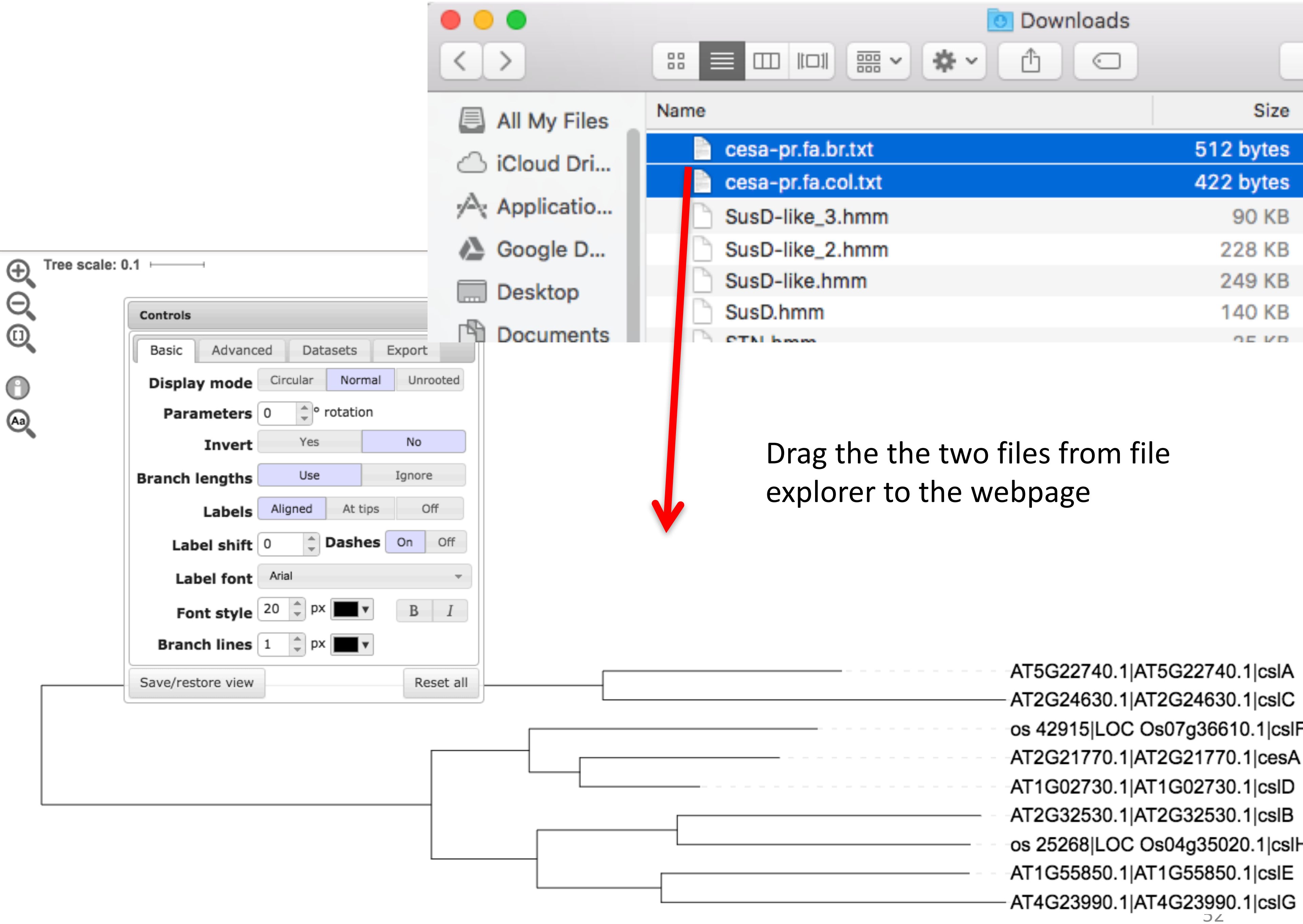
Paste your tree into the box below, or select a file using the **Tree file** selector. You can also simply drag and drop the tree file onto the page.

Tree text:

```
((((AT2G32530.1|AT2G32530.1|cslB:0.57078988,os_25268|LOC_Os04g35020.1|cslH:0.55075714)0.9300:0.26338963,
(AT1G55850.1|AT1G55850.1|cslE:0.57830980,AT4G23990.1|AT4G23990.1|cslG:0.64691609)0.9500:0.23352951)0.7400:0.19857786,
(os_42915|LOC_Os07g36610.1|cslF:0.541[illegible]868,
(AT2G21770.1|AT2G21770.1|cesA:0.3751647[illegible]AT1G02730.1|AT1G02730.1|cslD:0.22502015)0.6600:0.09521396)0.9300:0.18369951)1.0000:0.73286595,
(AT5G22740.1|AT5G22740.1|cslA:0.44848889,A[illegible]2G24630.1|AT2G24630.1|cslC:0.75671710)1.0000:1.05517231);
```

Tree file:

http://cys.bios.niu.edu/yyin/teach/PBB/cesa-pr.fa.nwk



Downloads

Name	Size
cesa-pr.fa.br.txt	512 bytes
cesa-pr.fa.col.txt	422 bytes
SusD-like_3.hmm	90 KB
SusD-like_2.hmm	228 KB
SusD-like.hmm	249 KB
SusD.hmm	140 KB

Tree scale: 0.1

Controls

Basic | Advanced | Datasets | Export

Display mode: Normal

Parameters: 0 ° rotation

Invert: No

Branch lengths: Use

Labels: Aligned

Label shift: 0

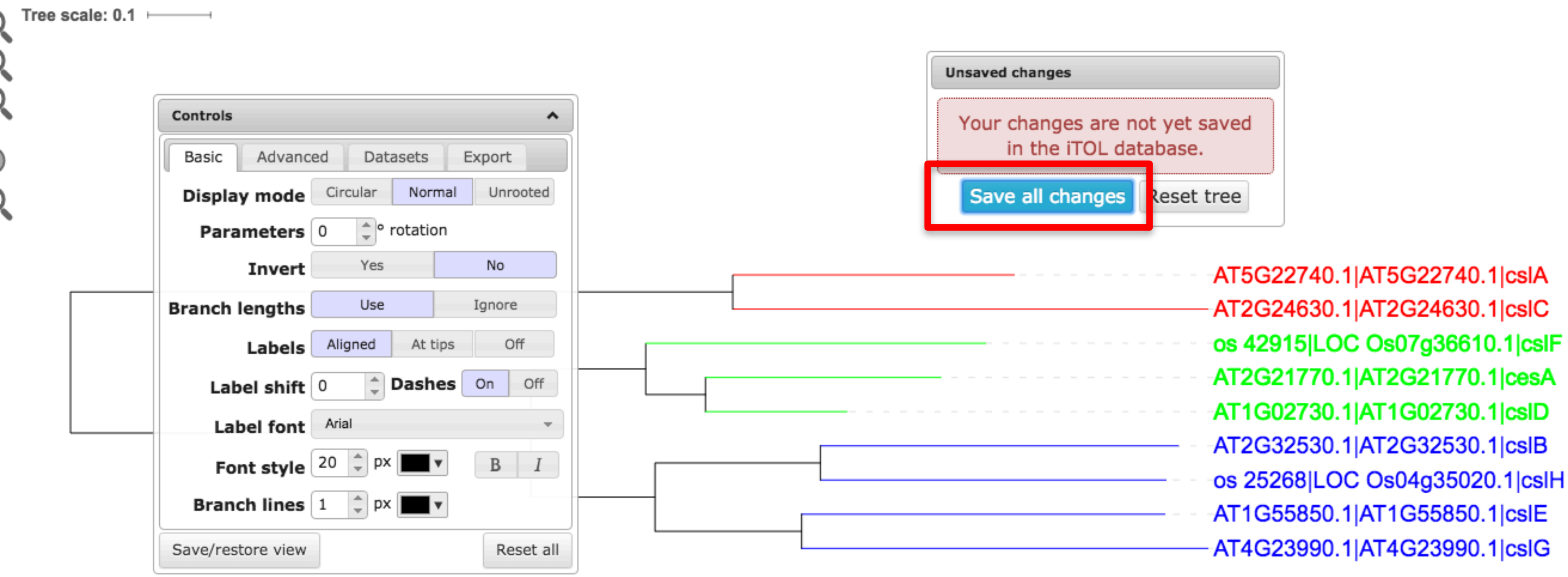
Dashes: On

Label font: Arial

Font style: 20 px

Branch lines: 1 px

Drag the the two files from file explorer to the webpage



Display mode Circular [✓ Normal] Unrooted

Parameters 0 ° rotation

Invert Yes [✓ No]

Branch lengths [✓ Use] Ignore

Labels [✓ Aligned] At tips Off

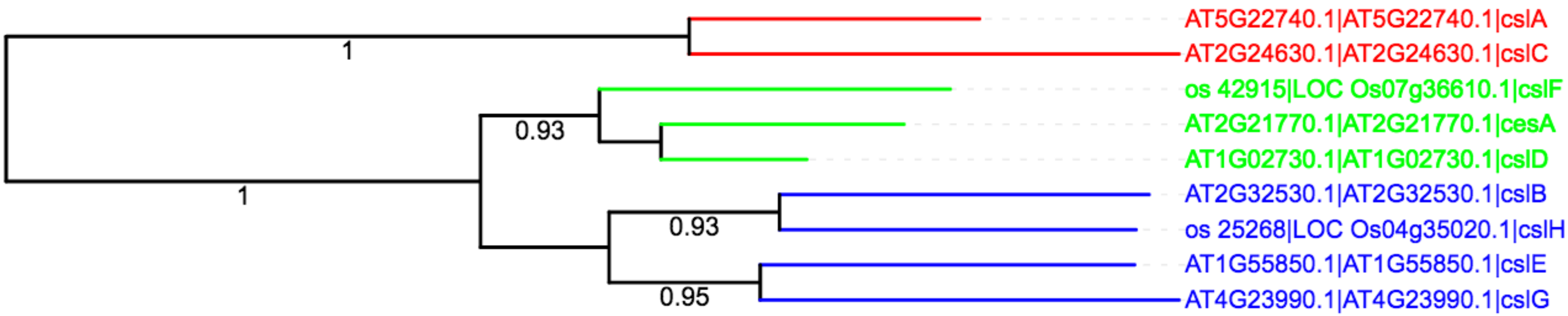
Label shift 0 **Dashes** [✓ On] Off

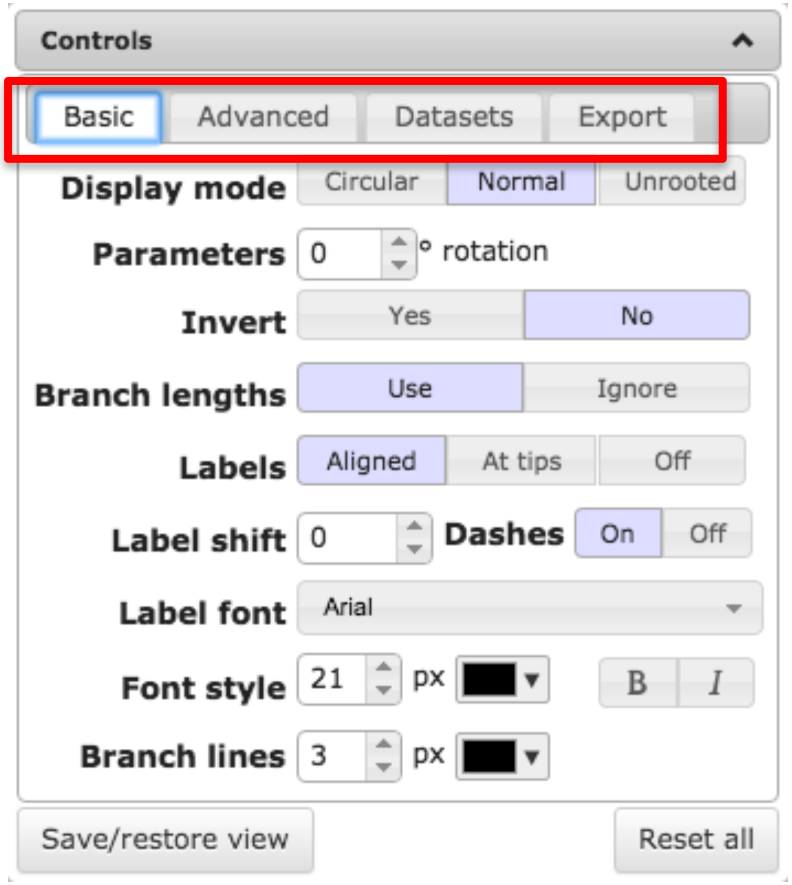
Label font Arial

Font style 20 px B *I*

Branch lines 1 px

Your changes are not yet saved in the iTOL database.





Display mode Circular [✓ Normal] Unrooted

Parameters 0 ° rotation

Invert Yes [✓ No]

Branch lengths [✓ Use] Ignore

Labels [✓ Aligned] At tips Off

Label shift 0 **Dashes** [✓ On] Off

Label font Arial

Font style 21 px ■ B *I*

Branch lines 3 px ■