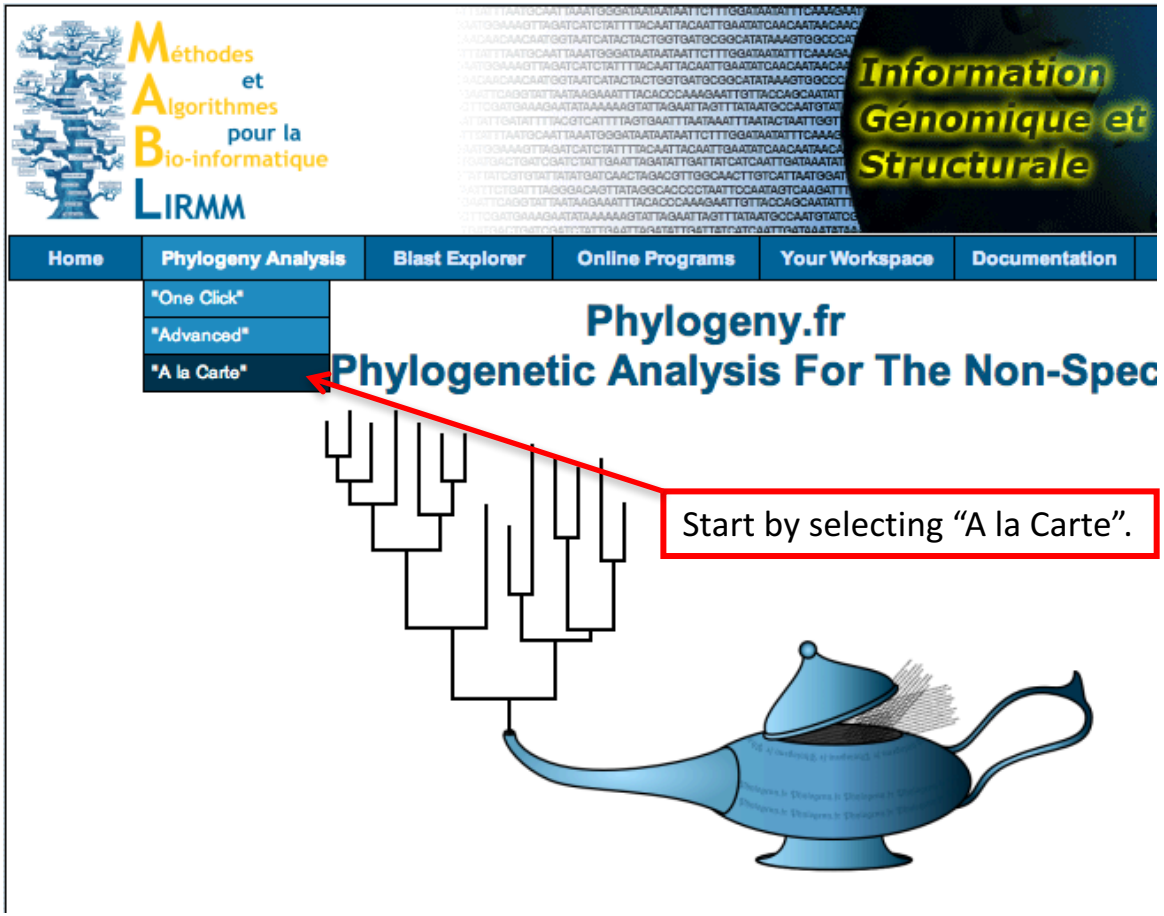
PHYLOGENY: This tutorial explains how to use the online phylogeny website at http://www.phylogeny.fr/ to do distance and parsimony analyses.



Workflow Settings

Name of the analysis (optional): Neighbor-Joining Tutorial

Choose processing steps to run and select software to use:

- [x] Multiple Alignment:
  - ( ) MUSCLE
  - ( ) ProbCons
  - ( ) T-Coffee
  - ( ) 3D-Coffee
  - ( ) ClustalW

This performs a multiple sequences alignment. If you have a set of already aligned sequences Uncheck this box.

- [ ] Alignment curation:
  - ( ) Gblocks
  - ( ) Remove positions with gaps

This feature is an automated check on your alignment. Not necessary in our case.

- [x] Construction of phylogenetic tree:

These 4 methods are the most commonly used to "build" trees.

  - Maximum Likelihood
    - ( ) PhyML
  - Parsimony
    - ( ) TNT
  - Distances
    - ( ) ProtDist/FastDist + BioNJ
    - (•) ProtDist/FastDist + Neighbor
  - Bayesian inference
    - ( ) MrBayes (limit: 30 sequences)

ProtDist calculates distances for proteins, FastDist for DNA.

- [x] Visualisation of phylogenetic tree:
  - (•) TreeDyn
  - ( ) Drawgram
  - ( ) Drawtree

These programs draw the tree.

Run workflow:

(•) all at once ( ) step by step

Create workflow

Click “Create workflow” to continue. This will take you to the Neighbor-Joining workflow where you upload or paste in your data.

Input Data

Upload your set of sequences in FASTA, EMBL or

Choose File No file chosen

Or paste it here (load example of sequences)

Here, I have copy and pasted in a DNA sequence alignment in FASTA format.

```
>STLSOA
---ATGGTCAGGTAGGGTGGAGGGTCTCGCC-AGCCCTTATACCCACATGGCGCAACG--
TGGGCACCAGTAACTCCTATGCTATAATACC---TGCTCTTCG-AGATC-CCAGTCTAAC
TATGATCATCGCCCGACGGGGCGAGATAGTCGTGGGTTCCCTTTCTGGAGGGAGAGGGAA
TTCCACGTTGACCGGGGGAACCGGCCAGGCCCGGAAGGGAGCAACCGTGCCCGGCTATCC
GCGTTCGTCGGTCTCCGATAGGAGGA---AGACTGGGGGTAAATCTCGGGGAGTAAGGGT
TATGGCATAGGGGAGCTGACCA---------T----
>STLSOB
---GGGGTCAGGGAGGGTGGGGGATCTCGCCAATCCCTATTACCCGCAAGGCGTAATG--
CGGGCACCAGTAACTCCTACCCTATGGTGTC---TCCTATCTGTAGGTC-CCAGTGGAGC
GATGAAGCCTGCCCAGCGGGGCTTGGCGGTCATGGGCTTTCTCTCCGGAGGGAGAGAAAG
TACCATGATAGCTGGGGGAATCGGCGAGGCCCGGAAGGGAGCAGCCGTGCCTGGACGCCA
GCGTTCGCTGGTCAACAGCCAGAGTG---AAACTGGGGTAAACCTATAGATAGGTA-GGC
CATGGGGTAGGGGGTCTGGCCC---------CAT--
>PYROCC
```

Maximum number of sequences is 200 for proteins and 200 for nucleic acids.
Maximum length of sequences is 2000 for proteins and 6000 for nucleic acids.

Phylogeny: Neighbor

Settings

For this alignment, we will build a tree using the default distance calculations. But these can be easily changed.

Number of bootstraps: 0

Substitution model: Default

☐ Gamma distribution parameter (for amino-acid substitution models): 1

☐ Transition / transversion ratio (for nucleic acids): 2

Tree Rendering: TreeDyn

Settings

Tree can be customized using the dynamic tree editing interface.

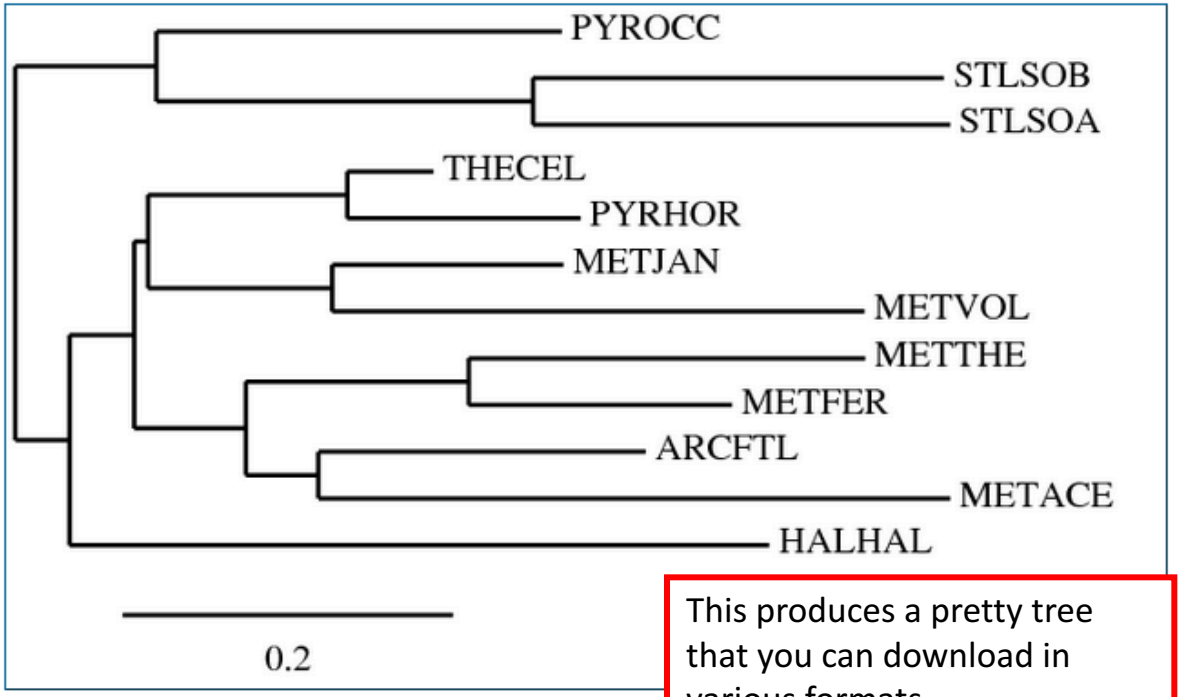
Email is optional, but is good for slower methods such as Maximum Likelihood and Bayesian.

To receive the results by e-mail, enter your address(es): my_email@gmail.com

Submit

And, of course, Submit!

Tree Rendering results



This produces a pretty tree that you can download in various formats.

Figure 1: *Phylogenetic tree.*

==> **Download the tree:** PNG - PDF - SVG - TGF (Treedyn format) - Newick - Text

The TreeDyn Program has a lot of options to make your tree as you like it.

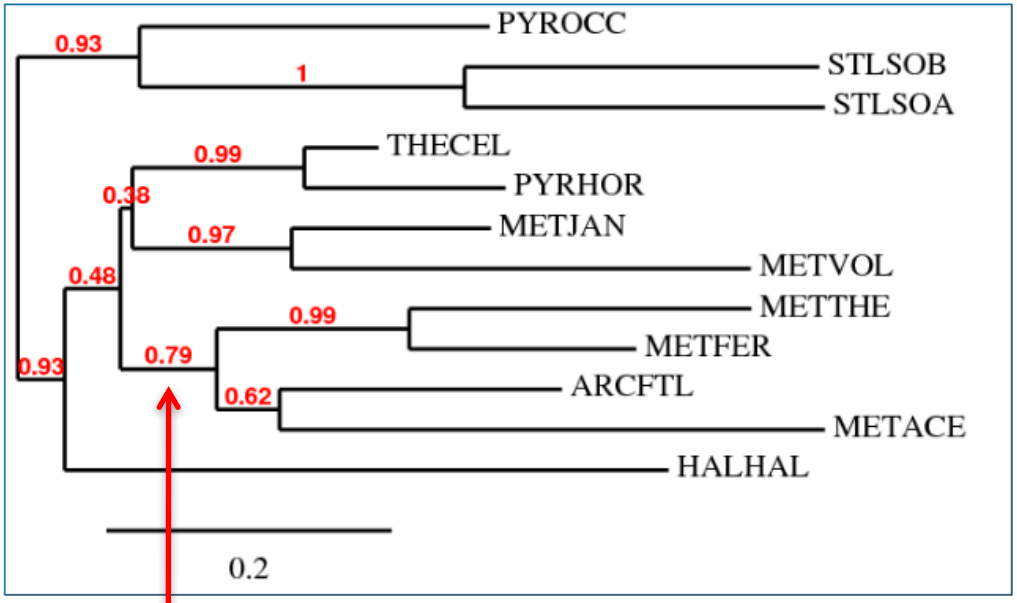
Select an action:

Reset (cancel all changes)
Mid-point rooting
Use Genbank information to automatically rename leaves by: species and gi species only colorize
Collapse branches having branch support value smaller than 50 % or a number of bootstraps smaller than

Select an action and click leaf or internal branch:

Colorize leaf branch *choose a color* blue *and a legend label*
Reroot (**outgroup**)
Flip (flip an entire tree at a node)
Swap (flip two branches at a node)
Change **leaf name**
Add **leaf annotations** *color* red

Results of a bootstrap (100 replicates) using same dataset and Neighbor-Joining.



The numbers in RED indicate in what proportion of the 100 bootstrap replicates , this particular node was present. 0.79 = 79% of the time (79times out of the 100 bootstrap replicates)

To use the TNT program for Parsimony Analysis, you have to first agree to some “terms of service”. Then you can enter your data.

Input Data

Upload your alignment (FASTA, Phylip, Clustal, EMBL or NEXUS format) from a file:

Choose File | No file chosen

Or paste it here (load example of alignment)

```
#NEXUS

BEGIN DATA;
 DIMENSIONS NTAX=94 NCHAR=486;
 FORMAT DATATYPE=PROTEIN INTERLEAVE MISSING=?

MATRIX
oOmpU_Van       ---------- ----------
MNKTLIALAVSAAAVVTGVN AG----------ELYNQDG TSLEMGGRAEARLSLKDGK-
oOmpU_Vch       ---------- ----------
MNKTLIALAVSAAAVATGAY ADGINQSGDKAGSTVYSAKG TSLEVGGRAEARLSLKDGK-
ompCTIGRPpr1    ---------- ----------MIINK
MRKSTIALSIFSALLVNTAN AA----------KVFNDGE SELNIHGRVQGMYYVSDDKE
phoETIGRPpr1    ---------- ----------MIINK
MRKSTIALSIFSALLVNTAN AA----------KVFNDGE SELNIHGRVQGMYYVSDDKE
```

Clear

Maximum number of sequences: 200;
Maximum alignment length: 6000.

In this example, we are using a protein sequence alignment in NEXUS format.

Phylogeny: TNT

Settings

We left everything except the Search type as default. No bootstrap this time.

Search type:

- (•) Traditional search
- ( ) New Technology search
  - [x] Sectorial search (with RSS and CSS)
  - [ ] Ratchet
  - [ ] Drift
  - [x] Tree fusing

Costs:

Amino-acids stepmatrix: Disabled

Please note that TNT is not very efficient (ie. slow) when amino-acids substitution stepmatrices are used.

Nucleic acids transversion cost: 1 (integer above 0)

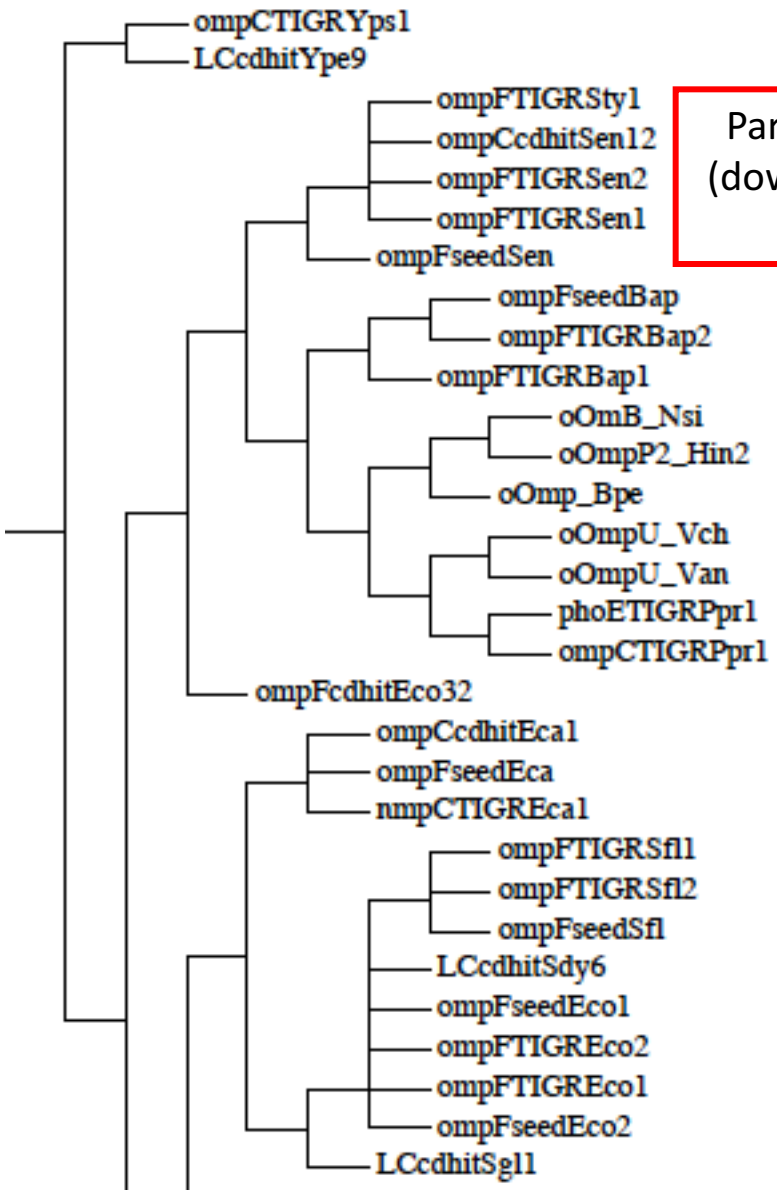
Resampling:

- (•) No resampling
- ( ) Standard bootstrap
- ( ) Jacknife (with 36% removal probability)
- ( ) Symmetric resampling (with 33% change probability)

Number of replicats: 0

You know what to do now.

Submit



Part of the Tree (downloaded as a PDF file)