
A guide to the stand-alone version of MLTreeMap

MLTreeMap is a software framework, designed for phylogenetic and functional analysis of metagenomic data. It searches for instances of marker genes on nucleotide sequences and deduces their most likely origin in a set of reference phylogenies. The current version of MLTreeMap can be downloaded and installed individually. MLTreeMap runs on Mac and Linux systems.

This guide can be downloaded from http://mltreemap.org/treemap_cgi/show_download_page.pl.

A) Installation of MLTreeMap

Step 1

Unzip and unpack the file 'MLTreeMap_package_2_061.tar.gz'.

Step 2

Enter the directory 'MLTreeMap_package_2_061/install/'.

Type 'make'.

The make program now creates the data structure of MLTreeMap (to be found in 'MLTreeMap_package_2_061/mltreemap_2_06/') and compiles most needed sub-programs (hmmalign, Genewise and RAxML).

Step 3

BLAST and Gblocks have to be added manually. For this enter the directory 'MLTreeMap_package_2_061/install/sources/BLAST/'. Here you will find a collection of BLAST binaries. Choose the one appropriate for your system and copy it to the directory 'MLTreeMap_package_2_061/mltreemap_2_06/sub_binaries/'.

Repeat this for Gblocks
(to be found in 'MLTreeMap_package_2_061/install/sources/Gblocks/').

MLTreeMap is now ready to use. You can copy the directory mltreemap_2_06 to any place you like (as well as renaming it). The only dependency of MLTreeMap is that you must have Perl installed on your system.

B) Usage of MLTreeMap

MLTreeMap has to be accessed on the command line.

An example for a valid input command is:
`./mltreemap.pl -i example_input/rubisco.txt`

This will analyze the sequence in the file rubisco.txt and write the result to the output directory 'mltreemap_2_06/output/'.

Further (optional) input parameters are:

- b** number of bootstrap replicates (default: 0 i.e. no bootstrapping).
- c** usage a computer cluster (0 = no cluster (default), s = sun grid).
- f** RAxML algorithm (v = maximum likelihood (default), p = maximum parsimony).
- g** minimal sequence length after Gblocks (default: 50).
- l** long input files will be split into files of n sequences each (default: 2000).
- o** output directory (default: output/).
- s** minimum bitscore for the blast hits (default: 60).
- t** phylogenetic reference tree (p = MLTreeMap tree (default), g = GEBA tree, i = Fungi tree. Note: if the 'i' option is chosen, MLTreeMap searches for fungi sequences and maps them on a detailed fungi phylogeny. All other sequences are discarded).

C) The MLTreeMap output

MLTreeMap searches for phylogenetic and functional marker genes. As soon as the results can be assigned to a specific marker gene, they are labelled accordingly by the first character of the output files.

Phylogenetic analysis:

- a** 16S rRNA reference tree
- b** 18S rRNA reference tree
- g** GEBA reference tree
- i** Fungi reference tree
- p** MLTreeMap reference tree

Functional analysis:

- c** Photolyase & cryptochrome
- d** Reverse dissimilatory sulfite reductase (DsrAB)
- e** HZO/HAO
- h** NifH (K02588)
- m** Methane & ammonia monooxygenase
- n** NifD (K02586)
- r** RuBisCo (COG1850)
- w** Fumarate adding enzymes (bssA-nmsA-assA)
- y** Methyl coenzyme M reductase alpha subunit (mcrA)

Additionally, each output file after the RAxML step gets a header line, providing this information in words. In case of the RuBisCO example from above, the final output file would look as follows:

Functional analysis, RuBisCO:

Placement weight 100%: Assignment of query to Acry 1067 (*Acidiphilium cryptum*) (6).