

## **A Guide to the stand alone version of MLTreeMap:**

MLTreeMap is a software framework, designed for phylogenetic and functional analysis of metagenomics 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.

### **A) Installation of MLTreeMap**

#### **Step 1**

Unzip and unpack the file `MLTreeMap_package_2_03.tar.gz`.

#### **Step 2**

Enter the directory `MLTreeMap_package_2_03/install/`.

Type `make`.

The `make` program now creates the data structure of MLTreeMap (to be found in `MLTreeMap_package_2_03/mltreemap_2_031/`), 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_03/install/sources/BLAST/`.

Here you will find a collection of binaries for BLAST. Choose the one appropriate for your system and copy it to the directory `MLTreeMap_package_2_03/mltreemap_2_031/sub_binaries/`.

Repeat this for Gblocks (to be found in `MLTreeMap_package_2_03/install/sources/Gblocks/`).

Now MLTreeMap is ready to use. You can copy the directory `mltreemap_2_031` to any place you like (as well as renaming it), the only dependency is that you 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 standard output directory `mltreemap_2_03/output/`.

Further (optional) input options are:

- b activates non-parametric Bootstrapping. Note: usually bootstrapping is not necessary.
- c usage a computer cluster (0 = no cluster (default), s = sun grid). Note: This has only been tested on our cluster, thus further code-adaptations might be necessary to get it running on another cluster.
- f v (Maximum likelihood analysis, default) or p (maximum parsimony analysis).
- g minimal sequence length after Gblocks (default = 50).
- o use this option if you want to write the output to another directory than the default.

- s     minimum bitscore of the blast hits (default = 60).
- t     phylogenetic reference tree (p = MLTreeMap reference tree (default), g = GEBA reference tree).

Note: the mandatory “-q” option of earlier MLTreeMap versions has been abandoned. Instead the handling of output directories has been improved.