### 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\_03/), 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 03/data/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\_03 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 analyis).
- -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.