

Ihre Namen: \_\_\_\_\_ Gruppe: \_\_\_\_\_

## Evolutionsbiologie 2, WS2016/2017: Bioinformatik – Übung 2-2

**Erstellen Sie bitte vor Beginn der Übung einen Ordner auf dem Desktop, in dem Sie alle benötigten Dateien speichern können (z.B. „Uebung\_2“).**

### 2-2 Web-based Analysis

#### **A: Multiple sequence alignments (MSA)**

1.1 Finde the file `sequences.fasta` on the desktop.

**Note:** DROME = *Drosophila melanogaster*, HUMAN = *Homo sapiens*, CHICK = *Gallus gallus*, DARIO = *Danio rerio*, MOUSE = *Mus musculus*

1.2 Open the following web page with an online interface to ClustalO (Clustal Omega).

<http://www.ebi.ac.uk/Tools/msa/clustalo>

Enter the sequences (copy it from your editor or upload the saved file), use the default settings and create the multiple alignment by hitting the button “Submit”.

1.3 Download and save the alignment file; you will need it later to create the phylogeny.

1.4 Guide tree: Save the guide tree in Newick format for later comparison with the trees you will create with PHYLIP. To do this, right click on “Download Phylogenetic Tree File” and save the file.

#### **B: Phylogeny**

**Caution:** In the next section you will produce several output files and it might be difficult to differentiate between them after some analyses if you do not name them unambiguously.

2.1 You will use the output of ClustalO from the previous step.

2.2 Open the web page for PHYLIP.

<http://emboss.toulouse.inra.fr>

On the left side, go to “Phylogeny Molecular Sequence” and choose `fprotdist`. Upload your multiple sequence alignment from ClustalO and hit the button “Run” (with default settings). Save and check the resulting output alignment.

**(Q1) What are the numbers in the resulting file?**

**(Q2) Why are some numbers “0.0”?**

2.3 Neighbor joining: Make sure that every gene entry in the protein distance matrix is in only one line by manually changing the saved output from 2.2. On the left side, go to “Phylogeny Distance Matrix” and use the output of `fprotdist` (protein distance matrix) to create the neighbor-joining tree with `fneighbor` (with default settings).

2.4 Open the webpage

[www.phylogeny.fr](http://www.phylogeny.fr)

and visualize the tree. At the bottom of the webpage, you find the section “TreeViewers”. For example, use `TreeDyn` for plotting the tree.

**(Q3) What is an outgroup?**

**(Q4) Where is the outgroup in your tree?**

**(Q5) Which species from your set would be an appropriate outgroup for the other species in your dataset? Why?**

2.4 Protein parsimony: use the PHYLIP program `fprotpars` to create the maximum parsimony tree from your multiple sequence alignment (`ClustalO`). Visualize as above and save the tree.

**Note:** Since Maximum Likelihood methods (`fproml`) usually take too long to compute, we will not use it in this practical. However, keep in mind that it is one of the most used methods in phylogeny.

2.5 You have now 3 trees:

- (i) the guide tree from `ClustalO`,
- (ii) the neighbor-joining tree,
- (iii) the protein maximum parsimony tree.

Compare the obtained phylogenies with each other, with the `ClustalO` guide tree and with the species tree.

**(Q6) Which trees are similar?**

**(Q7) Why do the human sequences not group together? (hint: what kind of homologs do you may have here?)**

2.6 Bootstrapping: We will perform a bootstrapping analysis with the maximum parsimony procedure.

On the left side, navigate to “Phylogeny Molecular Sequence” and choose `fseqboot`. Enter your alignment file (`ClustalO`), enter 10 bootstrap repeats, a random odd seeding number (= 1) and run the program with all other options left at default settings. Save the resulting 10 bootstrapped alignments in a single file.

Use the `fprotpars` program to create maximum parsimony trees and save them. Using `fprotdist` and `fneighbor` you can also produce a bootstrapped neighbor-joining tree (not required in this practical though).

On the left side, go to “Phylogeny Consensus” and use the 10 trees from above to create a consensus tree using `fconsense`.

**(Q8) Draw the consensus tree.**

**(Q9) Do you have a rooted or unrooted tree?**

**(Q10) What are the numbers?**

Use `TreeDyn` to root the tree with an outgroup (the same outgroup as above).

**(Q11) Draw the tree.**

**Please delete all files that you generated after finishing the practical.**