

DETERMINING MAMMALIAN EVOLUTIONARY RELATIONSHIPS

Lab Protocol

PART A

FINDING AMINO ACID SEQUENCES

1. First we need to get the sequence data for the hemoglobin protein from our marine and land animals: seals, whales, dogs, cows.

- Go to <http://www.ncbi.nlm.nih.gov/protein>

2. In the search box, type in HBB (signifying the hemoglobin protein we are comparing) and then the name of the first species in the following list:

- [harbor seal](#)
 - [minke whale](#)
 - [Canis familiaris](#) (dog)
 - [Bos Taurus](#) (cow).
- Click "Search".

3. If the screen that pops up contains a list of choices, like a google search, choose the listing with an Accession "P0####". Or else you will be brought to this page automatically. The page should look like this:

NCBI Resources How To Sign In

Protein Protein Search

Limits Advanced

Display Settings: GenPept Send to: Change region shown

RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Hemoglobin beta chain

UniProt/Swiss-Prot: P09909.1

[FASTA](#) [Graphics](#)

Analyze this sequence

4., Click on the "FASTA" link near the top of the page to see the actual amino sequence for this protein in a coded format using single letters to represent each of the 20 amino acids (A=alanine, M=methionine, P=proline,etc.)

5. Copy the **entire** sequence, from before (>) to the last letter. *You **must** include the header line, starting from the greater than symbol (>). THIS IS IMPORTANT.*

6. Paste this sequence into a word document that you will save as "FASTA text file."

7. Now search for the HBB (hemoglobin) sequences for the rest of the species in your list (#2 in these instructions). Paste these amino acid sequences into the same FASTA text file.

8. EDIT your file by deleting the header of each sequence and adding the species name instead. **You MUST preserve the ">" symbol.**

For example:

The harbor seal sequence begins like this:

>gi|122664|sp|P09909.1|HBB_PHOVI RecName: Full=Hemoglobin subunit beta

This can be edited to simply say this:

>Harbor_seal

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Also, make sure there is a blank line left in between each sequence.

This is what it should look like when it is edited:

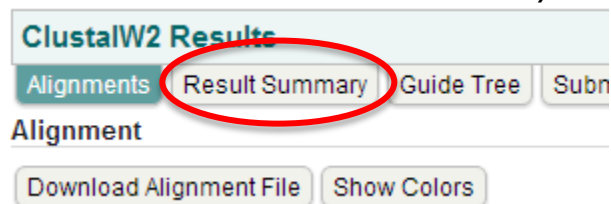
```
>Harbor_seal  
VHLTGEEKSAVTALWGKVNVDVEVGGEALGRLLVVYPWTQRFFDSFGDLSSADAIMGNPKVKAHGKKVLNS  
FSDGLKNLDNLKGTFAKLSEKHLCDKLVDPENFKLLGNVLVLCVLAHFGKEFTPQVQAAYQKVVAGVANA  
LAHKYH
```

ALIGNING AND ANALYZING YOUR SEQUENCES

9. Google search: **ClustalW2**. Choose the program with the website:
www.ebi.ac.uk/Tools/msa/clustalw2

10. Copy and paste your FASTA text file (all 4 sequences at the same time) into the "Step 1" box and submit this data.

11. Click on the "results summary" tab under the Results heading.



The screenshot shows the ClustalW2 Results interface. The 'Result Summary' tab is highlighted with a red circle. Below the tabs, there are buttons for 'Download Alignment File' and 'Show Colors'.

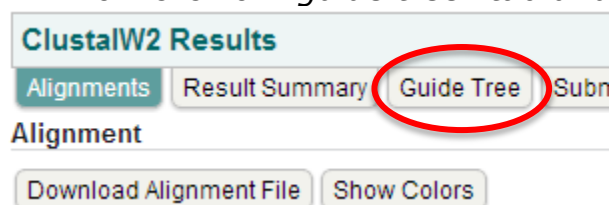
Observe the last column titled **Score** on the Scores Table. Click the double arrow once to see the scores in ascending order.

View Output File

SeqA	Name	Length	SeqB	Name	Length	Score
1	Harbor_seal	146	2	minke_whale	146	88.0
1	Harbor_seal	146	3	Canis_familiaris	146	93.0
1	Harbor_seal	146	4	Bos_taurus	145	84.0
2	minke_whale	146	3	Canis_familiaris	146	86.0
2	minke_whale	146	4	Bos_taurus	145	86.0
3	Canis_familiaris	146	4	Bos_taurus	145	82.0

These scores show the percent similarities between the species' hemoglobin sequence, and therefore, their **EVOLUTIONARY RELATIONSHIP**. The more amino acids they have in common, the more closely related the species! Use this to fill in the table in Part A of your lab handout.

12. Now click on "guide tree" tab under the Results heading.



The screenshot shows the ClustalW2 Results interface. The 'Guide Tree' tab is highlighted with a red circle. Below the tabs, there are buttons for 'Download Alignment File' and 'Show Colors'.

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Observe the phylogenetic tree and *copy this onto the designated spot in Part A your lab handout.*

PART B

15. Below are the representative marine mammals for your new cladogram:

- minke whale (baleen whales)
- dolphin (toothed whales)
- harbor seal
- walrus
- river otter
- manatee

16. In this exercise, we will compare the hemoglobin proteins of these marine mammals to some representatives of the major taxa of land mammals:

carnivora: dog, *Canis familiaris*

rodentia: rat, *Rattus norvegicus*

herbivore: cow, *Bos Taurus*

primates: human, *Homo sapiens*

proboscidea: African elephant, *Loxodonta africana*

marsupials: red kangaroo, *Macropus rufus*

17. Reminder: We are testing the hypothesis that all marine mammals have a common land mammal ancestor.

18. Once again, use the hemoglobin beta protein sequence to test this hypothesis. You will be discovering a tree with all the marine mammals and all the land mammals listed above.

19. Follow the complete process we used in Part A to obtain your amino acid sequences from GenBank, align them in ClustalW2, and build the evolutionary tree

20. Then use your phylogenetic tree to determine the evolutionary relationships amongst marine mammals and the representative land mammals. Determine whether your hypothesis was supported by the molecular data.