

1001001100001  
0100001110100  
0100001110100

## Hands-On Five

# Comparing Beta Globin Proteins

In this hands-on exercise, we will look at beta globin proteins from different organisms. We learn how to examine evolutionary relationships between different organisms. We learn how to build phylogenetic trees using protein sequences.

### A) Obtaining Beta Globin Sequences

- Open a web browser window and go to Biology Workbench at <http://workbench.sdsc.edu>
- Log in and click on the “Protein Tools” button.
- Highlight the “Ndjinn - Multiple Database Search” from the scrollbar menu and click on the “Run” button.
- Alternatively, scroll down the page and click on “Ndjinn”.

The “Ndjinn - Multiple Database Search” is a feature of the Biology Workbench that allows the user to find information on a topic of interest, using specific databases that may be useful.

- Scroll down the page and click on the small box to the left of the tags labeled “SWISSPROT\_HUMAN”, “SWISSPROT\_MAMMALS”, and “SWISSPROT\_VERTEBRATES”.
- Scroll up again above the database selections to make more choices.
- Choose “Exact Match” from the window that has “Contains”. If that window already has “Exact Match”, do not change it.
- In the text box right next to “Exact Match” type the two separate words: “beta globin”.
- Select “All” from “Hits per page”.
- Keep the default of “Full” for “Display mode”.
- Submit your query by clicking on the “Search” button.

### B) CLUSTAL-W Sequence Alignments

In the scroll menu, you will see that a large number of sequences were found with your query.

- Scroll through the list and choose the following entries by selecting (in other words, clicking the small box to the left of):
  - ❖ SWISSPROT\_HUMAN:HBB\_HUMAN (Rank = 0)
  - ❖ SWISSPROT\_VERTEBRATES:HBB\_CHICK (Rank = 3)
  - ❖ SWISSPROT\_MAMMALS:HBB\_SHEEP (Rank = 7)
  - ❖ SWISSPROT\_VERTERBRATES:HBB\_SALSA (Rank = 35)
  - ❖ SWISSPROT\_MAMMALS:HBB\_MACFU (Rank = 155)
  - ❖ SWISSPROT\_MAMMALS:HBB\_TURTR (Rank = 239)

The ranks between parentheses might have changed.

- Import the sequences by clicking the button “Import Sequence(s)” found at the bottom of the page.

You will get a new page.

- Select each one of the 6 sequences by clicking in the small boxes next to the sequences. We are now ready to align them.
- Aligning is accomplished by scrolling to the bottom of the page and by clicking on “CLUSTALW”.

The next screen presents an assortment of settings for the CLUSTALW program. Just use the default settings:

- Click on the “Submit” button.
- When the results are returned, scroll down until you get to the “Clustal W dendrogram”.

A phylogenetic tree is a graphical means to depict the evolutionary relationships of a group of organisms. The branch length of the tree represents time, and an inner node represents a hypothetical common ancestor. Computer programs can be used to construct these trees.

According to the unrooted tree you see on the screen:

- 1) Which two species have the most similar beta globins?
- 

- 2) Are the beta globins of the salmon and chicken more similar than those of the salmon and dolphin?
- 

- Import the multiple sequence alignment to the workbench by clicking on the “Import Alignment(s)” button found at the top (or bottom) of the page.

### **C) Comparing the Protein Sequences**

- Select the aligned sequences by clicking in the small box next to “CLUSTALW - Protein”.
- Click on “BOXSHADE” at the bottom of the page.
- Accept the default settings from the new page and click the “Submit” button.

BOXSHADE can be used on groups of proteins that have been aligned (as by CLUSTALW) to determine their similarities. It produces a color-coded output of the protein sequences.

- Green is for amino acids that are the same (conserved) in all the proteins examined.

- Yellow is for amino acids that are the same in nearly all proteins examined.
- Cyan means that the amino acid has the similar structure and charge but is a different amino acid.
- An unshaded region means a very high variability within comparisons.

Look at your aligned sequences.

3) Which species have the most similar beta globins?

---

4) Are the beta globins of the salmon and chicken more similar than those of the salmon and dolphin?

---

5) Why do you think the 6 sequences are quite similar?

---

- Click on "Return" at the bottom of the page.

We can also construct a rooted tree.

- Select the imported multiple sequence alignment by checking the white box next to "CLUSTALW- protein".
- Scroll down and click on "DRAWGRAM".
- Accept all the default values and click on "Submit" on the next screen.
- Now scroll down to see the rooted tree.

6) Which species have the most similar beta globins?

---

7) Are the beta globins of the salmon and chicken more similar than those of the salmon and dolphin?

---