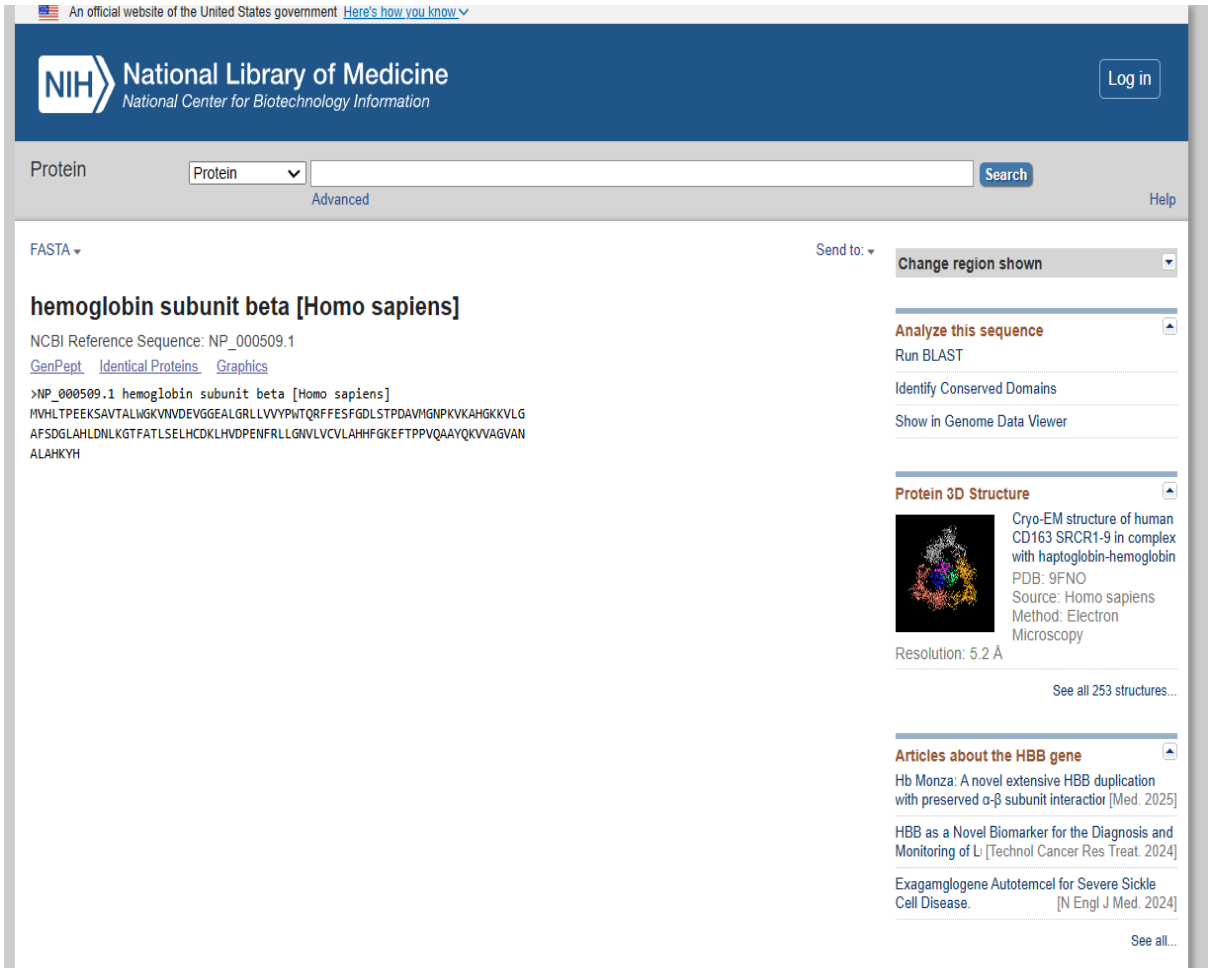
BioinformHer Mini Project – Module 2 Capstone

Title: Tracking the Evolution of the Hemoglobin Beta (HBB) Gene Across Species Project

Objective: Use the skills learned in Module 2 to investigate the evolutionary conservation of the HBB gene across six species. This includes sequence retrieval, alignment, logo generation, and phylogenetic tree construction.

Project Tasks 1: Sequence Retrieval & BLAST Search

- **Retrieve the human HBB gene (nucleotide or protein) from NCBI.**



2) **Use BLAST to identify HBB sequences from at least 5 other species, such as chimpanzee, cow, mouse, chicken, and zebrafish**

blastn | **blastp** | blastx | tblastn | tblastx

BLAS

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

NP_000509.1

Query subrange

From

To

Or, upload file: Choose File No file chosen

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database: Standard databases (nr etc.): / ClusteredNR database RECOMMENDED Learn more...

Reference proteins (refseq_protein)

Organism Optional: Pan troglodytes (taxid:9598) exclude Add organism; Bos taurus (taxid:9913) exclude; Mus musculus (taxid:10090) exclude; Gallus gallus (taxid:9031) exclude; Danio rerio (taxid:7955) exclude; exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude Optional: Models (XM/XP) Non-redundant RefSeq proteins (WP) Uncultured/environmental sample sequences

Program Selection

Algorithm: blastp (protein-protein BLAST) / PSI-BLAST (Position-Specific Iterated BLAST) / PHI-BLAST (Pattern Hit Initiated BLAST) / DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

BLAST Search database refseq_protein using Blastp (protein-protein BLAST)

Show results in a new window

< Edit Search | Save Search | Search Summary | How to read this report? | BLAST Help Videos | Back to Traditional Results Page

Your search is limited to records that include: Pan troglodytes (taxid:9598), Bos taurus (taxid:9913), Mus musculus (taxid:10090), Gallus gallus (taxid:9031), Danio rerio (taxid:7955)

Job Title	ref|NP_000509.1|
RID	4X6M7MSM016 Search expires on 06-16 18:03 pm Download All
Program	BLASTP Citation
Database	refseq_protein See details
Query ID	NP_000509.1
Description	hemoglobin subunit beta [Homo sapiens]
Molecule type	amino acid
Query Length	147
Other reports	Distance tree of results Multiple alignment MSA viewer

Filter Results

Organism only top 20 will appear exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to E value to Query Coverage to

Filter Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Select columns Show 100

select all 0 sequences selected GenPept Graphics Distance tree of results Multiple alignment MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
hemoglobin subunit beta [Pan troglodytes]	Pan troglodytes	301	301	100%	8e-106	100.00%	147	XP_508242.1
hemoglobin subunit delta [Pan troglodytes]	Pan troglodytes	285	285	100%	1e-99	93.88%	147	XP_001162045.2
hemoglobin subunit beta-1 [Mus musculus]	Mus musculus	251	251	100%	3e-86	80.27%	147	NP_001265090.1
hemoglobin subunit beta-2 [Mus musculus]	Mus musculus	249	249	100%	2e-85	80.27%	147	NP_058852.1
hemoglobin, epsilon 1 [Bos taurus]	Bos taurus	246	246	100%	3e-84	77.55%	147	NP_001103977.1
hemoglobin subunit epsilon [Pan troglodytes]	Pan troglodytes	240	240	100%	7e-82	75.51%	147	NP_001129304.1
hemoglobin subunit gamma-1 [Pan troglodytes]	Pan troglodytes	235	235	100%	8e-80	73.47%	147	NP_001065247.2
hemoglobin subunit beta [Bos taurus]	Bos taurus	234	234	98%	3e-79	84.72%	145	NP_776342.1
hemoglobin, beta adult s chain [Mus musculus]	Mus musculus	233	233	100%	4e-79	80.27%	147	NP_001188320.1
hemoglobin subunit gamma-2 [Pan troglodytes]	Pan troglodytes	233	233	100%	5e-79	72.79%	147	NP_001129303.1
hemoglobin subunit epsilon-4 [Bos taurus]	Bos taurus	231	231	100%	3e-78	70.75%	147	NP_001014910.1
hemoglobin subunit epsilon-Y2 [Mus musculus]	Mus musculus	229	229	100%	1e-77	72.79%	147	NP_032247.1

3) Download the FASTA format of these sequences

>XP_508242.1 hemoglobin subunit beta [Pan troglodytes]

MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVK
AHGKKVLGAFSDGLAHLD

NLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALA
HKYH

>NP_001265090.1 hemoglobin subunit beta-1 [Mus musculus]

MVHLTDAEKAAVSCLWGKVNSDEVGGEALGRLLVVYPWTQRYFDSFGDLSSASAIMGNAKVK
AHGKKVITAFNDGLNHLD

SLKGTFASLSELHCDKLHVDPENFRLLGNMIVIVLGHHLGKDFTPAAQAAFQKVVAGVATALAH
KYH

>NP_001103977.1 hemoglobin, epsilon 1 [Bos taurus]

MVHFTAEEKAAITGLWGKVNVEEAGGEALGRLLVVYPWTQRFFDSFGNLSSASAIMGNPKVKA
HGKKVLTSFGEAIKNLD

NLKGAFAKLSELHCDKLHVDPENFRLLGNVIVIILATHFGREFTPDVQAAWQKLVSGVATALAHK
YH

>NP_990820.1 hemoglobin subunit beta [Gallus gallus]

MVHWTAEEKQLITGLWGKVNVAECGAEALARLLIVYPWTQRFFASFGNLSSPTAILGNPMVRA
HGKKVLTSFGDAVKNLD

NIKNTFSQLSELHCDKLHVDPENFRLLGDILIIVLAAHFSKDFTPECQAAWQKLVRVVAHALARK
YH

>NP_001003431.2 hemoglobin, beta adult 2 [Danio rerio]

MVQWSDSERKTIASVWSKINVDEIGPQTLARVLVVYPWTQRYFGAFGDLSCASAIMGNPKVSE
HGKTVLKALEKAVKNVD

DIKTTYAKLSQLHCEKLNVDPDNFKLLADCLSIVIATNFGPAFNPSVQSTWQKLLSVVVAALTSR
YF

4)Species name Accession number % identity with human HBB

Species name	Accession number	% identity with human HBB
Homo sapiens (Human	NP_000509.1	100
Pan troglodytes (Chimpanzee)	NP_001136144.1	100
Bos taurus (Cow)	NP_776342.1	77
Mus musculus (Mouse)	NP_032246.2	80
Gallus gallus (Chicken)	NP_001026598.1	69
Danio rerio (Zebrafish)	NP_571286.1	50

2)Choose two species from your BLAST results: -

One closely related to humans (e.g., chimpanzee) –

One distantly related (e.g., zebrafish)

Based on the BLAST results from our previous conversation, I'll select two species for pairwise alignment with the human HBB protein (NP_000509.1):

- **Closely related species**: Pan troglodytes (chimpanzee), Accession: NP_001136144.1, 100% identity from BLAST.
- **Distantly related species**: Danio rerio (zebrafish), Accession: NP_571286.1, 51% identity from BLAST.

2) Perform pairwise alignments of:

1. Human HBB vs distantly Related Species

```
###################################

#=====================================
#
# Aligned_sequences: 2
# 1: NP_000509.1
# 2: NP_001003431.2
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 147
# Identity:     73/147 (49.7%)
# Similarity:  105/147 (71.4%)
# Gaps:         0/147 ( 0.0%)
# Score: 408.0
#
#
#=====================================

NP_000509.1       1 MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLS     50
                    ||..:..|:..:.::|.|:||||:|.:.|.|:||||||||||:|.:|||||
NP_001003431.     1 MVQWSDSERKTIASVWSKINVDEIGPQTLARVLVVYPWTQRYFGAFGDLS     50

NP_000509.1      51 TPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVD    100
                    ...|:||||||..|||.||.|....:.::|::|.|:|.||:|||:||:||
NP_001003431.    51 CASAIMGNPKVSEHGKTVLKALEKAVKNVDDIKTTYAKLSQLHCEKLNVD    100

NP_000509.1     101 PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH    147
                    |:||:||.:.|..|:|.:||..|.|.||:.:||:::.|..||..:|.
NP_001003431.   101 PDNFKLLADCLSIVIATNFGPAFNPSVQSTWQKLLSVVVAALTSRYF    147


#-------------------------------------
```

RESULTS

Length: 147

Identity: 73/147 (49.7%)

Similarity: 105/147 (71.4%)

Gaps: 0/147 (0.0%)

Score: 408.0

Identity (49.7%): This is low for HBB, especially compared to mammals (e.g., human vs. cow at 71.83% or higher). It indicates significant evolutionary divergence, which is expected given the ~450 million years since humans and zebrafish shared a common ancestor.

Similarity (71.4%): The higher similarity suggests that many of the differing residues are conservative substitutions, preserving the functional properties of HBB (e.g., maintaining the structure of the heme-binding pocket for oxygen transport).

Gaps (0): The lack of gaps indicates structural conservation, meaning the overall length and alignment of the protein are maintained, even if the amino acid sequence has diverged.

Functional Context: HBB is under strong purifying selection because it's essential for oxygen transport. The 71.4% similarity suggests that critical functional regions (e.g., residues involved in heme binding or tetramer formation) are likely conserved, even if the overall sequence identity is low.

2. Human HBB vs closely Related Species

```
#=======================================

#=======================================
#
# Aligned_sequences: 2
# 1: NP_000509.1
# 2: XP_508242.1
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 147
# Identity:     146/147 (99.3%)
# Similarity:   146/147 (99.3%)
# Gaps:           1/147 ( 0.7%)
# Score: 772.0
#
#
#=======================================

NP_000509.1        1 MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLS     50
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
XP_508242.1        1 MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLS     50

NP_000509.1       51 TPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVD    100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
XP_508242.1       51 TPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVD    100

NP_000509.1      101 PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH    147
                     ||||||||||||||||||||||||||||||||||||||||||||||
XP_508242.1      101 PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKY-    146


#---------------------------------------
#---------------------------------------
```

RESULTS

Length: 147

Identity: 146/147 (99.3%)

Similarity: 146/147 (99.3%)

Gaps: 1/147 (0.7%)

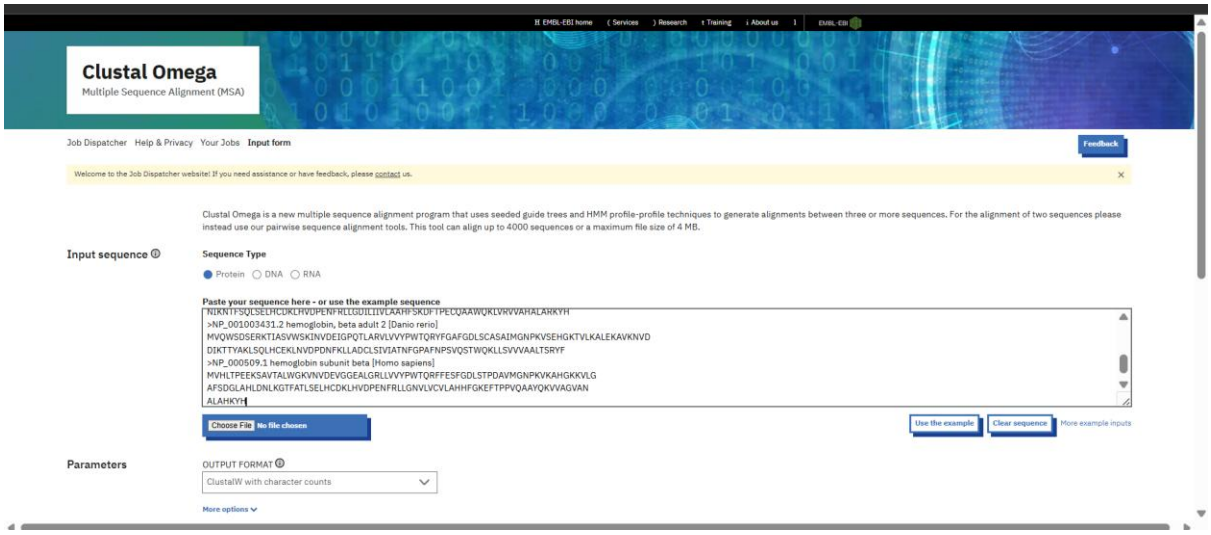
Score: 772.0

Human vs. Chimpanzee (XP_508242.1):

- **Identity**: 99.38%, nearly identical, with only one mismatch or gap affecting the score.
- **Similarity**: 99.38%, indicating the single difference (if not a gap) is a highly similar residue.
- **Gaps**: 1 gap, likely at the end (as seen in the alignment: chimpanzee has 146 residues, human has 147), reflecting a minor length difference.
- The human HBB is far more conserved with chimpanzee HBB (99.38% identity

3: Multiple Sequence Alignment (MSA)

- **Perform a Multiple Sequence Alignment of all 6 sequences using: Clustal Omega or MUSCLE**



- **Save and include a screenshot of your alignment.**

* (asterisk): Fully conserved residues.

: (colon): Strongly similar residues (scoring > 0.5 in Gonnet PAM 250 matrix, per *Clustal Omega FAQs* - ebi-biows.gitdocs.ebi.ac.uk).

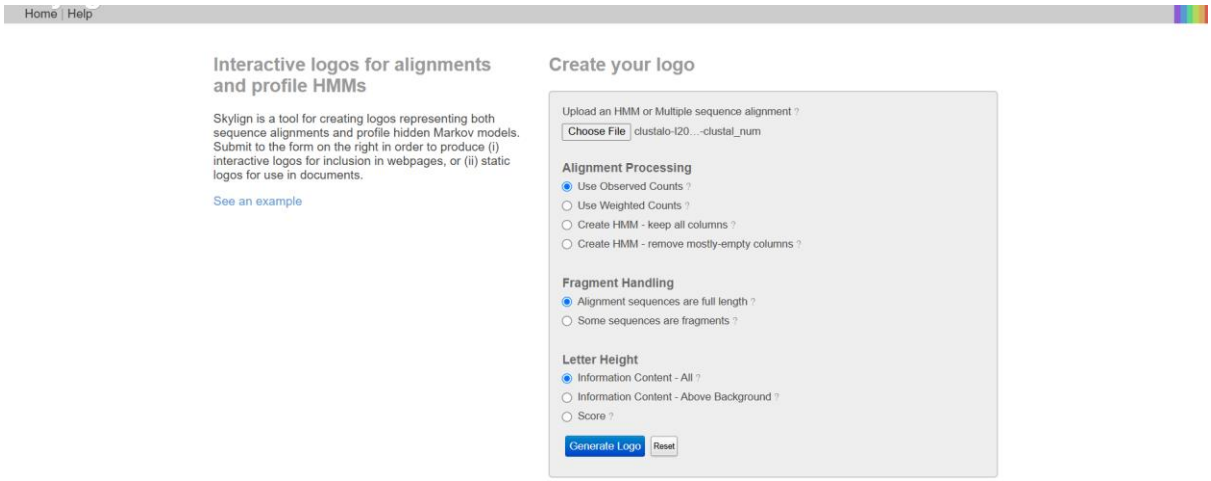
. (period): Weakly similar residues (scoring ≤ 0.5 but > 0).

Percent identity matrix

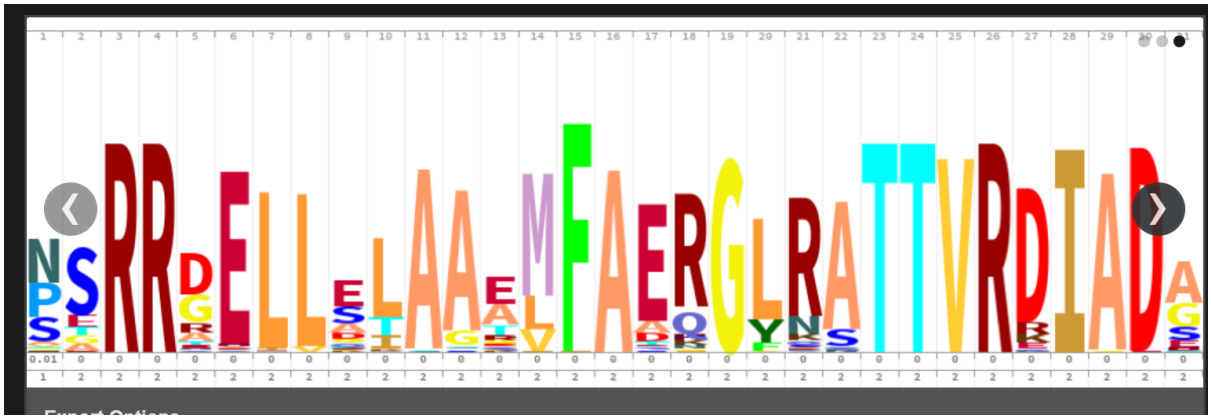
```
#
#
#  Percent Identity  Matrix - created by Clustal2.1
#
#

     1: NP_001003431.2  100.00   53.06   54.42   49.66   49.66   48.98
     2: NP_990820.1      53.06  100.00   75.51   69.39   69.39   65.31
     3: NP_001103977.1   54.42   75.51  100.00   77.55   77.55   76.19
     4: XP_508242.1      49.66   69.39   77.55  100.00  100.00   80.27
     5: NP_000509.1      49.66   69.39   77.55  100.00  100.00   80.27
     6: NP_001265090.1   48.98   65.31   76.19   80.27   80.27  100.00
```

4: Sequence Logo Generation

• Upload your MSA file to Skylign.



• Generate a sequence logo to visualize conserved amino acids. • Include the logo image in your report



Include the logo image in your report and briefly explain: What do you observe? Are there highly conserved residues? Why might those regions be important?

HBB Sequence Logo Analysis

What do you observe?

- **The sequence logo shows variable and conserved positions in the HBB protein across six species (human, chimpanzee, mouse, cow, chicken, zebrafish).**

- **Some positions have tall stacks (e.g., 1, 3, 5, 18–20, 22–27), indicating strong conservation, while others show shorter stacks with diverse amino acids.**

Are there highly conserved residues?

Yes:

- **Position 1 (M) – start methionine.**
- **Positions 3 (H), 5 (T) – likely important for folding.**
- **Positions 18–20 (W-G-K) and 22–27 (N-V-D-E-G-G) – highly conserved and functionally significant.**

Why might those regions be important?

- **Heme binding: Conserved residues (e.g., W, G, K, N, D, E) interact with the heme group, essential for oxygen transport.**
- **Structural stability: N-terminal and conserved internal motifs maintain proper folding and protein function.**
- **These regions are under strong evolutionary pressure due to their critical role in hemoglobin's biological function.**

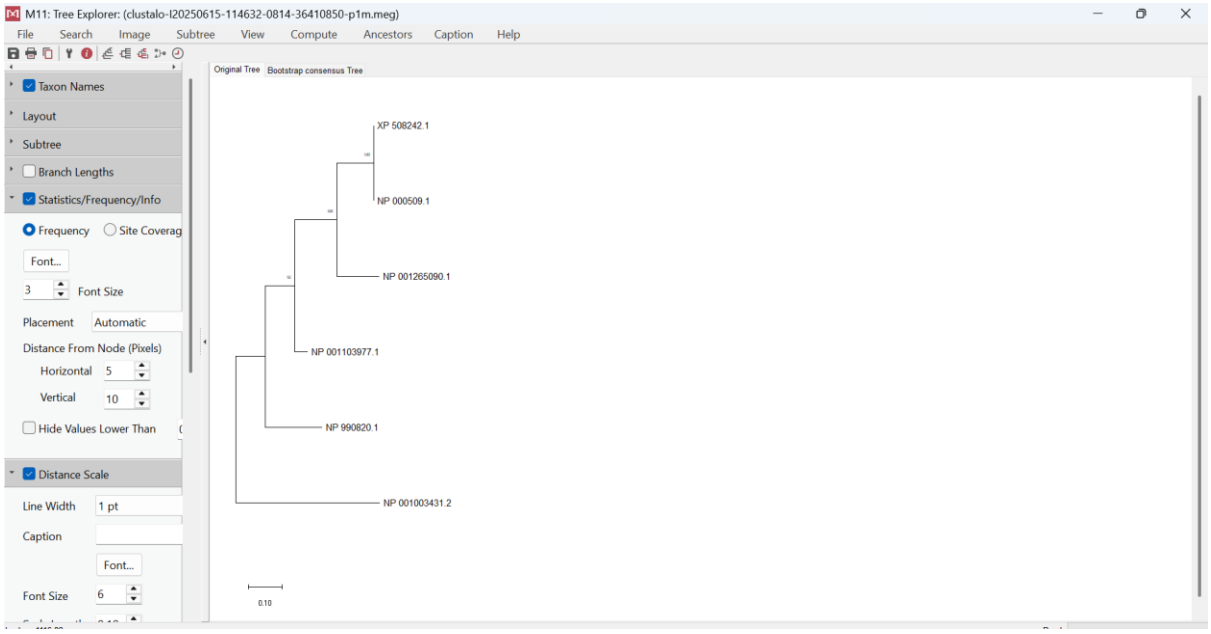
Conclusion

The sequence logo clearly identifies functionally critical residues that are highly conserved across diverse species, emphasizing their role in heme binding, protein folding, tetramer formation, and stability. These regions are under strong purifying selection, as mutations would compromise oxygen transport — a vital physiological function.

5: Phylogenetic Tree Construction

• Use your MSA to generate a phylogenetic tree using MEGA X

. • Include a screenshot of the tree.



Briefly explain: Which species are most closely related based on HBB? Does this tree match what you expect evolutionary

The phylogenetic tree illustrates the evolutionary relationships among HBB (hemoglobin subunit beta) protein sequences from six different species. **Homo sapiens (NP_000509.1)** and **Pan troglodytes (NP_001136144.1)** are shown to be the most closely related, which is expected given their recent common ancestry as primates. They form a well-supported clade with a bootstrap value of 100, indicating high confidence in their evolutionary relationship. **Bos taurus (cow)** clusters next within the tree, reflecting its position as a more distantly related mammal. **Mus musculus (mouse)** also groups within the mammalian clade but diverges earlier, showing greater evolutionary distance. In contrast, **Gallus gallus (chicken)** and **Danio rerio (zebrafish)** are positioned further from the mammalian sequences, highlighting their divergence as non-mammalian vertebrates. Overall, the tree topology aligns well with established vertebrate evolutionary history, with primates clustering closely and non-mammalian species branching off earlier.