

BGGN-213: FOUNDATIONS OF BIOINFORMATICS

The find-a-gene project assignment

<http://thegrantlab.org/bgg213/>

Dr. Barry Grant

Overview:

The find-a-gene project is a required assignment for BGGN-213. You should prepare a written report in **PDF** format that has responses to each question labeled **[Q1] - [Q10]** below. You may wish to consult the scoring rubric at the end of this document and the example report provided online.

The objective with this assignment is for you to demonstrate your grasp of database searching, sequence analysis, structure analysis and the R environment that we have covered in class.

Due Date:

Your responses to questions Q1-Q4 are due at the beginning of class **Friday May 17th** (05/17/19). Note that these answers can be obtained very quickly (at best within 10 or 15 minutes), so if you don't succeed at first, just keep trying.

The complete assignment, including responses to all questions, is due at the beginning of class **Wednesday June 5th** (06/05/19). Late responses will not be accepted under any circumstances.

Submission instructions:

Email your PDF document as an attachment named BGGN213_w19_[yourUCSDname].pdf to both our IA, Kevin Chau (kkchau@ucsd.edu), and me (bjgrant@ucsd.edu). For example, my document would be named BGGN213_w19_bjgrant.pdf

Be sure to include your UCSD email and PID number on the first page of your report.

Email your preliminary report with answers to Q1-Q4 by **Feb 22nd** so we can determine if you have found a novel gene. Submit this preliminary report as one document with screen shots of the results inserted appropriately.

See the demonstration report linked to on the course website for an example of format. I will email you my decision; proceed with subsequent questions only after we are sure you have found a novel gene.

For the final report add your results for Q5-Q10 to the preliminary report and send a final document containing the results for all questions. Please do not send only Q5-Q10 answers as the final report.

Questions:

[Q1] Tell me the name of a protein you are interested in. Include the species and the accession number. This can be a human protein or a protein from any other species as long as its function is known.

If you do not have a favorite protein, select human RBP4 or KIF11. Do not use beta globin as this is in the worked example report that I provide you with online.

Name: Beta globin

Accession: NP_000509

Species: Homo Sapiens

[Q2] Perform a BLAST search against a DNA database, such as a database consisting of genomic DNA or ESTs. The BLAST server can be at NCBI or elsewhere. Include details of the BLAST method used, database searched and any limits applied (e.g. Organism).

Method: TBLASTN (2.7.1) search against nematode ESTs

Database: Expressed Sequence Tags (est)

Organism: Nematodes (Taxid: 6231)

Also include the output of that BLAST search in your document. If appropriate, change the font to Courier size 10 so that the results are displayed neatly. You can also screen capture a BLAST output (e.g. alt print screen on a PC or on a MAC press ⌘-shift-4. The pointer becomes a bulls eye. Select the area you wish to capture and release. The image is saved as a file called Screen Shot [] .png in your Desktop directory). It is **not** necessary to print out all of the blast results if there are many pages.

BLAST® Basic Local Alignment Search Tool

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Home Recent Results Saved Strategies Help

NCBI/BLAST/ tblastn **Translated BLAST: tblastn**

blastn blastp blastx **tblastn** tblastx

Enter Query Sequence TBLASTN search translated nucleotide databases using a protein query. [more...](#) [Reset page](#) [Bookmark](#)

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#) Query subrange

Or, upload file No file selected.

Job Title Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database

Organism Exclude

Exclude Models (XM/XP) Uncultured/environmental sample sequences

Limit to Sequences from type material

Entrez Query [YouTube](#) [Create custom database](#)

Show results in a new window

BLAST Search database Expressed sequence tags (est) using Tblastn (search translated nucleotide databases using a protein query)

On the BLAST results, clearly indicate a match that represents a protein sequence, encoded from some DNA sequence, that is homologous to your query protein. I need to be able to inspect the pairwise alignment you have selected, including the E value and score. It should be labeled a "genomic clone" or "mRNA sequence", etc. - but include no functional annotation.

Chosen match: Accession JK511422.1, a 559 base pair clone from *Anguillicola crassus*. See below for alignment details.

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Home Recent Results Saved Strategies Help

NCBI BLAST/blastn/ Formatting Results - Y9CBA1JU015

Your search is limited to records matching entrez query: txid6231 [ORGN].
[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#) [YouTube](#) [How to read this page](#) [Blast report description](#)

ref|NP_000509| (147 letters)

RID [Y9CBA1JU015](#) (Expires on 09-02 03:56 am)

Query ID [gi|4504349|ref|NP_000509.1](#) Database Name est
 Description hemoglobin subunit beta [Homo sapiens] Description Database of GenBank+EMBL+DBJ sequences from EST Divisions
 Molecule type amino acid Program TBLASTN 2.2.32+ [Citation](#)
 Query Length 147

Other reports: [Search Summary](#) [Taxonomy reports](#)

Graphic Summary

Distribution of 9 Blast Hits on the Query Sequence

Mouse-over to show define and scores, click to show alignments

Descriptions

Legend for links to other resources: [U](#) UniGene [E](#) GEO [G](#) Gene [S](#) Structure [M](#) Map Viewer [X](#) PubChem BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Ident	Links
JK511422.1	Ac_EH1r_01A07_M13 Adult Anguillicola crassus Anguillicola crassus cDNA clone Ac_EH1r_01A07, mRNA sequence.	149	149	100%	7e-44	47%	
EX545299.1	AIAC-aaa96h11.g1 Ancylostoma caninum_EST_Male_pSMART Ancylo	33.9	33.9	17%	0.42	50%	
AA294483.1	SWOV3MCA690SK Onchocerca volvulus molting L3 larva cDNA (SL96M	32.7	32.7	17%	0.68	54%	
AA294588.1	SWOV3MCA825SK Onchocerca volvulus molting L3 larva cDNA (SL96M	32.7	32.7	17%	0.78	54%	

Alignments

Select All [Get selected sequences](#)

```
>gb|JK511422.1| Ac_EH1r_01A07_M13 Adult Anguillicola crassus Anguillicola crassus
cDNA clone Ac_EH1r_01A07, mRNA sequence.
Length=559

Score = 149 bits (375), Expect = 7e-44, Method: Compositional matrix adjust.
Identities = 69/148 (47%), Positives = 97/148 (66%), Gaps = 1/148 (1%)
Frame = +1

Query 1  MVHLTPEEKSAVTALWGKVNVDVEVGGGALGRLLVVYPWTQRRFFESFGDLSTPDAVMGNPK 60
          MV I E +A+ +LW K+NV+E+G +A+ RLL+V PWTQR F +FG+LST A+M N K
Sbjct 40  MVEWTDAEHTAII LSLWKKINVEEIGPQAMRRLIVCPWTQRHFANFGNLSTAAAIMNNEK 219

Query 61  VKAHGKKVLGAFSDGLAHLNLTGTFATLSELHCDKLVDPENFRLLGNVLCVLAHHFG 120
          V HG V+G + ++D++K + IS +H +KLVDP+NFRLL + +A FG
Sbjct 220  VAKHGTTVMGGLDRAIQNMDDIKNAYRELSVMHSEKLVDPDNFRLLSEHITLCMAAKFG 399

Query 121 -KEFTPPVQAAYQKVVAGVANALAHKYH 147
           EFT VQ A+QK + V +AL +YH
Sbjct 400  PTEFTADVQEAWQKFLMAVTSALGRQYH 483
```

Alignment details:

```
>gb|JK511422.1| Ac_EH1r_01A07_M13 Adult Anguillicola crassus Anguillicola crassus  
cDNA clone Ac_EH1r_01A07, mRNA sequence.  
Length=559
```

Score = 149 bits (375), Expect = 7e-44, Method: Compositional matrix adjust.

Identities = 69/148 (47%), Positives = 97/148 (66%), Gaps = 1/148 (1%)
Frame = +1

```
Query 1 MVHLTPEEKSAVTALWGKVNVEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK 60  
MV T E +A+ +LW K+NV+E+G +A+ RLL+V PWTQR F +FG+LST A+M N K  
Sbjct 40 MVEWTD AEHTAILSLWKKINVEEIGPQAMRRL LIVCPWTQRHFANFGNLSTAAAIMNNEK 219  
  
Query 61 VKAHGKKVLGAFSDGLAHL DNLKGT FATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG 120  
V HG V+G + ++D++K + LS +H +KLHVDP+NFRL L + +A FG  
Sbjct 220 VAKHGTTVMGGLDRAIQNMDDIKNAYRELSVMHSEKLVDPDNFRLLSEHITLCMAAKFG 399  
  
Query 121 -KEFTPPVQAAYQKVVAGVANALAHKYH 147  
EFT VQ A+QK + V +AL +YH  
Sbjct 400 PTEFTADVQEAWQKFLMAVTSALGRQYH 483
```

In general, [Q2] is the most difficult for students because it requires you to have a “feel” for how to interpret BLAST results. You need to distinguish between a perfect match to your query (i.e. a sequence that is not “novel”), a near match (something that might be “novel”, depending on the results of [Q4]), and a non-homologous result.

If you are having trouble finding a novel gene try restricting your search to an organism that is poorly annotated.

[Q3] Gather information about this “novel” **protein**. At a minimum, show me the protein sequence of the “novel” protein as displayed in your BLAST results from [Q2] as FASTA format (you can copy and paste the aligned sequence subject lines from your BLAST result page if necessary) or translate your novel DNA sequence using a tool called EMBOSS Transeq at the EBI. Don’t forget to translate all six reading frames; the ORF (open reading frame) is likely to be the longest sequence without a stop codon. It may not start with a methionine if you don’t have the complete coding region. Make sure the sequence you provide includes a header/subject line and is in traditional FASTA format.

Chosen sequence:

```
>A. crassus protein (sequence taken from BLAST result)  
MVEWTD AEHTAILSLWKKINVEEIGPQAMRRL LIVCPWTQRHFANFGNLSTAAAIMNNEKVAKH  
GTTVMGGLDRAIQNMDDIKNAYRELSVMHSEKLVDPDNFRLLSEHITLCMAAKFGPTEFTADV  
QEAWQKFLMAVTSALGRQYH
```

Here, tell me the name of the novel protein, and the species from which it derives. It is very unlikely (but still definitely possible) that you will find a novel gene from an organism such as *S. cerevisiae*, human or mouse, because those genomes have already been thoroughly annotated. It is more likely that you will discover a new gene in a genome that is currently being sequenced, such as bacteria or plants or protozoa.

Name: *Anguillicola* globin

Species: *Anguillicola crassus*

Eukaryota; Metazoa; Ecdysozoa; Nematoda; Chromadorea; Spirurida;
Dracunculoidea; Anguillicolidae; Anguillicola.

[Q4] Prove that this gene, and its corresponding protein, are novel. For the purposes of this project, “novel” is defined as follows. Take the protein sequence (your answer to [Q3]), and use it as a query in a blastp search of the nr database at NCBI.

- If there is a match with 100% amino acid identity to a protein in the database, from the same species, then your protein is NOT novel (even if the match is to a protein with a name such as “unknown”). Someone has already found and annotated this sequence, and assigned it an accession number.
- If the top match reported has less than 100% identity, then it is likely that your protein is novel, and you have succeeded.
- If there is a match with 100% identity, but to a different species than the one you started with, then you have likely succeeded in finding a novel gene.
- If there are no database matches to the original query from [Q1], this indicates that you have partially succeeded: yes, you may have found a new gene, but no, it is not actually homologous to the original query. You should probably start over.

Details:

A BLASTP search against NR database (see setup in first screen-shot below) yielded a top hit result is to a protein from *Anguilla anguilla* (European eel).

See additional screen shots below for top hits and selected alignment details:

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NCBI/BLAST/blastp suite **Standard Protein BLAST**

blastn blastp **blastx** tblastn tblastx

Enter Query Sequence BLASTP programs search protein databases using a protein query. [more...](#) [Reset page](#) [Bookmark](#)

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

>A. crassus protein (sequence taken from BLAST result)
 MVWFDASHIALLSLWKKINVEIGQAMRPLLIVCPWTRHFANFQNLSTAAAIMNNEKVAKH
 GTTVNGSLDRAIQMDIKRVRKLSNRSEKLVDPDNFRLLSEHITLCMAAKFGPTEFTADV
 DEANCKFLMAVTSALGRQYH

From

To

Or, upload file No file selected.

Job Title Enter a descriptive title for your BLAST search

Align two or more sequences

Choose Search Set

Database

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

Exclude Models (XM/XP) Uncultured/environmental sample sequences

Entrez Query [Create custom database](#) Enter an Entrez query to limit search

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

Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST) Show results in a new window

The top result is to a protein from *Anguilla anguilla* (European eel), see second screen shot below for alignment details:

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

Alignments

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	RecName: Full=Hemoglobin anodic subunit beta; AltName: Full=Hemoglobin anodic beta chain	289	289	99%	6e-99	93%	P80946.1
<input type="checkbox"/>	RecName: Full=Hemoglobin anodic subunit beta; AltName: Full=Hemoglobin anodic beta chain	261	261	99%	7e-88	84%	P84206.1
<input type="checkbox"/>	RecName: Full=Hemoglobin subunit beta-3; AltName: Full=Beta-3-globin; AltName: Full=Hemoglobin subunit beta-3	253	253	99%	2e-84	80%	Q7LZC1.1
<input type="checkbox"/>	PREDICTED: hemoglobin subunit beta-A [Lates calcarifer]	245	245	100%	3e-81	76%	XP_018560551.1
<input type="checkbox"/>	PREDICTED: hemoglobin subunit beta-A-like [Paralichthys olivaceus]	244	244	100%	5e-81	75%	XP_019950077.1
<input type="checkbox"/>	PREDICTED: hemoglobin subunit beta-A-like [Lates calcarifer]	243	243	100%	1e-80	76%	XP_018560550.1

Alignments

Download ▾ GenPept Graphics ▾ Next ▲ Previous ▲ Descriptions

RecName: Full=Hemoglobin anodic subunit beta; AltName: Full=Hemoglobin anodic beta chain
 Sequence ID: [P80946.1](#) Length: 147 Number of Matches: 1

Range 1: 1 to 147 [GenPept](#) [Graphics](#) ▾ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
289 bits(740)	6e-99	Compositional matrix adjust.	136/147(93%)	141/147(95%)	0/147(0%)

Query 2 VEWTDAEHTAILS~~L~~WKKINVEEIGPQAMRRLIVCPW~~T~~QRHFANFGNLSTAAAIMNNEKV 61
 VEWT+ E TAI S W KIN+EEIGPQAMRRLIVCPW~~T~~QRHFANFGNLSTAAAIMNN+KV
 Sbjct 1 VEWTEDETAIKSKWLKINIEEIGPQAMRRLIVCPW~~T~~QRHFANFGNLSTAAAIMNNDKV 60

Query 62 AKHGTTVMGGLDRAIQNMDDIKNAYRELSVMHSEKLVDPDNFRLLEHITLCMAAKFGP 121
 AKHGTTVMGGLDRAIQNMDDIKNAYR+LSVMHSEKLVDPDNFRLLEHITLCMAAKFGP
 Sbjct 61 AKHGTTVMGGLDRAIQNMDDIKNAYRQLSVMHSEKLVDPDNFRLLEHITLCMAAKFGP 120

Query 122 TEFTADVQEA~~W~~QKFLMAVTSALGRQYH 148
 TEFTADVQEA~~W~~QKFLMAVTSAL ROYH
 Sbjct 121 TEFTADVQEA~~W~~QKFLMAVTSALARQYH 147

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RecName: Full=Hemoglobin anodic subunit beta; AltName: Full=Hemoglobin anodic beta chain
 Sequence ID: [P84206.1](#) Length: 147 Number of Matches: 1

Range 1: 1 to 147 [GenPept](#) [Graphics](#) ▾ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
261 bits(668)	7e-88	Compositional matrix adjust.	124/147(84%)	133/147(90%)	0/147(0%)

Query 2 VEWTDAEHTAILS~~L~~WKKINVEEIGPQAMRRLIVCPW~~T~~QRHFANFGNLSTAAAIMNNEKV 61
 VEWTD E TAIL+LWKKINVEEIG QAM RLLIV PWT RHFA+FGNLST +AIM+N+KV
 Sbjct 1 VEWTDGERTAILTLWKKINVEEIGAQAMGRLLIVYPWTHRFASFNGNLSTFSAIMSNKV 60

Query 62 AKHGTTVMGGLDRAIQNMDDIKNAYRELSVMHSEKLVDPDNFRLLEHITLCMAAKFGP 121
 AKHG TVMGGLD+AI+NMDDIKNAYR+LSVMHSEKLVDPDNFRLLE I TLC+AAKFGP
 Sbjct 61 AKHGATVMGGLDKAIKNMDDIKNAYRDLVSMHSEKLVDPDNFRLLESECITLCVAAKFGP 120

Query 122 TEFTADVQEA~~W~~QKFLMAVTSALGRQYH 148
 EF ADV EAW KFLMAVTSAL ROYH
 Sbjct 121 KEFNADVHEAWYKFLMAVTSALARQYH 147

[Q5] Generate a multiple sequence alignment with your novel protein, your original query protein, and a group of other members of this family from different species. A typical number of proteins to use in a multiple sequence alignment for this assignment purpose is a minimum of 5 and a maximum of 20 - although the exact number is up to you. Include the multiple sequence alignment in your report. Use Courier font with a size appropriate to fit page width.

Side-note: Indicate your sequence in the alignment by choosing an appropriate name for each sequence in the input unaligned sequence file (i.e. edit the sequence file so that the species, or short common, names (rather than accession numbers) display in the output alignment and in the subsequent answers below). The goal in this step is to create an interesting alignment for building a phylogenetic tree that illustrates species divergence.

Re-labeled sequences for alignment:

```
>Human_HBB gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDVEVGGELGRLLVYVPWTQRFVFESFGDLSTPDVAVMGNPKVKAHGKKVLGAFSDGLA
HLDNLIKGTFTATLSELHCDKLVDPENFRLLGNVLCVLAHHFGKEFTTPPVQAAYQKVVAGVANALAHKYH
```

```
>Anguillicola_globin (sequence taken from BLAST result)
```


MVEWTDAEHTAILSLWKKINVEEIGPQAMRRLIVCPWTRHFANFGNLSTAAAIMNNEKVAKHGTTVMGGLDRAIQ
NMDDIKNAYRELSVMHSEKLVDPDNFRLLSEHITLCMAAKFGPTEFTADVQEAWQKFLMAVTSALGRQYH

>Eel gi|2494788|sp|P80946.1|HBBA_ANGAN RecName: Full=Hemoglobin anodic subunit beta; AltName: Full=Hemoglobin anodic beta chain
VEWTEDEKTAIKSKWLKINIEEIGPQAMRRLIVCPWTRHFANFGNLSTAAAIMNNDKVAKHGTTVMGGLDRAIQ
MDDIKNAYRQLSVMHSEKLVDPDNFRLLAEHITLCMAAKFGPTEFTADVQEAWQKFLMAVTSALARQYH

>Atlantic_salmon gi|469832338|gb|AGH92521.1| hemoglobin subunit beta [Salmo salar]
MVDWTDAERSAIVGLWGKISVDEIGPQALARLLIVSPWTRHFSTFGNLSTPAAIMGNPAVAKHGKTMHGLDRAVQ
NLDDIKNAYTALSVMHSEKLVDPDNFRLLADCITVCAAKLGPVFSADIQEAQKFLAVVVSALGRQYH

>Rainbow_trout gi|1183021|dbj|BAA11632.1| beta-globin [Oncorhynchus mykiss]
MVDWTDPERSAIVGLWGKISVDEIGPQALARLLIVSPWTRHFSTFGNLSTPAAIMGNPAVAKHGKTMHGLDRAVQ
NLDDIKNTYTALSVMHSEKLVDPDNFRLLADCITVCAAKLGPVFSADTQEAQKFLAVVVSALGRQYH

>Zebrafish gi|18858329|ref|NP_571095.1| hemoglobin subunit beta-1 [Danio rerio]
MVEWTDARTAILGLWGKLNIDEIGPQALSRLIVYPWTRQYFATFGNLSPPAAIMGNPKVAAHGRTVMGGLERAIK
NMDNVKNTYAALSVMHSEKLVDPDNFRLLADCITVCAAMKFGQAGFNADVQEAQKFLAVVVSALCRQYH

>Channel_catfish gi|318171215|ref|NP_001187115.1| hemoglobin-beta [Ictalurus punctatus]
>gi|38606322|gb|AAR25199.1| hemoglobin-beta [Ictalurus punctatus]
MVHWTDAERHIIADLWGKINHDEIGGQALARLLIVYPWTRQYFSSFGNLSNAAAIIGNPKVAAHGKVVGLGGLTKAVQ
NLDNIKGIYTLSTLHSEKLVDPNSFTLLGDTFTVTLAANFGPSVFTPEVHETWQKFLNVVVAALGKQYH

Alignment:

Obtained using MUSCLE (version 3.8) at EBI:

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

```
Human_HBB      MVHLTPEEKSAVTALWGKVVNDEVGGEALGRLLVVPWTQRFVFESFGDLSTPDVAVMGNPK
Channel_catfish MVHWTDAERHIIADLWGKINHDEIGGQALARLLIVYPWTRQYFSSFGNLSNAAAIIGNPK
Anguillicola   MVEWTDAEHTAILSLWKKINVEEIGPQAMRRLIVCPWTRHFANFGNLSTAAAIMNNEK
Eel             -VEWTEDEKTAIKSKWLKINIEEIGPQAMRRLIVCPWTRHFANFGNLSTAAAIMNNDK
Zebrafish      MVEWTDARTAILGLWGKLNIDEIGPQALSRLIVYPWTRQYFATFGNLSPPAAIMGNPK
Atlantic_salmon MVDWTDAERSAIVGLWGKISVDEIGPQALARLLIVSPWTRHFSTFGNLSTPAAIMGNPA
Rainbow_trout  MVDWTDPERSAIVGLWGKISVDEIGPQALARLLIVSPWTRHFSTFGNLSTPAAIMGNPA
                *  *  *.  :  *  *:.  :*:  :*:  *  *:*  *****.*  .**:*..  *::.*
```

```
Human_HBB      VKAHGKVKVLAFAVSDGLAHLNLSKGTATLSELHCDKLVDPENFRLLGNVLCVLAHFFG
Channel_catfish VAAHGKVVGLGGLTKAVQNLNLIKGIYTLSTLHSEKLVDPNSFTLLGDTFTVTLAANFG
Anguillicola   VAKHGTTVMGGLDRAIQNMDDIKNAYRELSVMHSEKLVDPDNFRLLSEHITLCMAAKFG
Eel             VAKHGTTVMGGLDRAIQNMDDIKNAYRQLSVMHSEKLVDPDNFRLLAEHITLCMAAKFG
Zebrafish      VAAHGRTVMGGLERAIKNDNVKNTYAALSVMHSEKLVDPDNFRLLADCITVCAAMKFG
Atlantic_salmon VAKHGKTMHGLDRAVQNLDDIKNAYTALSVMHSEKLVDPDNFRLLADCITVCAAKLG
Rainbow_trout  VAKHGKTMHGLDRAVQNLDDIKNTYTALSVMHSEKLVDPDNFRLLADCITVCAAKLG
                *  **  *:.  .:  .:  ::*:  :  *  *:.  :*****.*  **:.  :.  *  :*
```

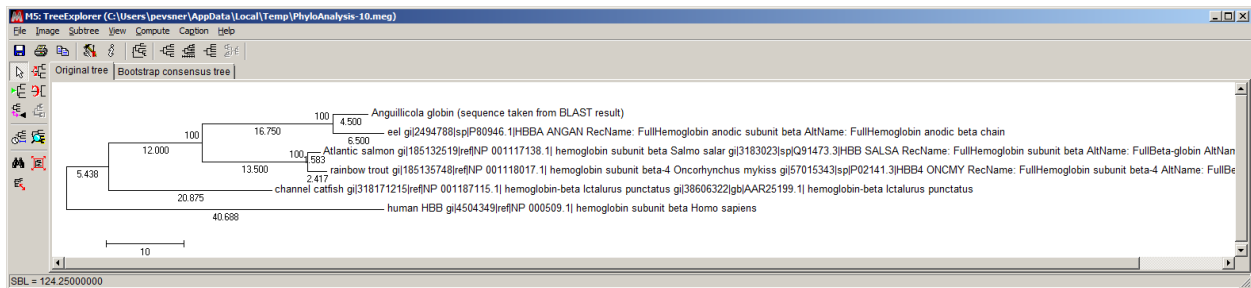
```

Human_HBB      KE-FTPPVQAAYQKVVAGVANALAHKYH
Channel_catfish PSVFTPEVHETWQKFLNVVVAALGKQYH
Anguillicola   PTEFTADVQEAWQKFLMAVTSALGRQYH
Eel            PTEFTADVQEAWQKFLMAVTSALARQYH
Zebrafish      QAGFNADVQEAWQKFLAVVVSALCRQYH
Atlantic_salmon PTVFSADIQEAFAQKFLAVVVSALGRQYH
Rainbow_trout  PAVFSADTQEAFAQKFLAVVVSALGRQYH
               *.. : :*:.*: *. ** .:***

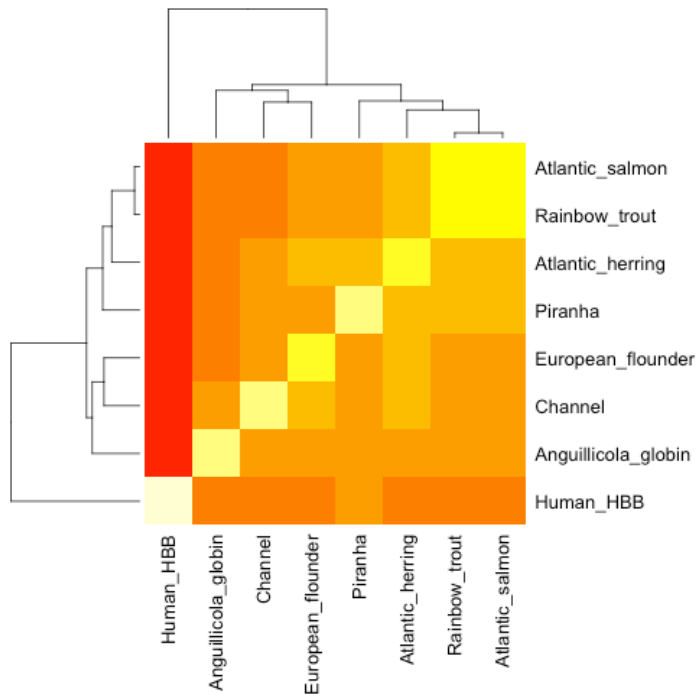
```

[Q6] Create a phylogenetic tree, using either a parsimony or distance-based approach. Bootstrapping and tree rooting are optional. Use “simple phylogeny” online from the EBI or any respected phylogeny program (such as MEGA, PAUP, or Phylip). Paste an image of your Cladogram or tree output in your report.

Import the sequences into MEGA, align with MUSCLE, and create a neighbor-joining tree:



[Q7] Generate a sequence identity based **heatmap** of your aligned sequences using R. If necessary convert your sequence alignment to the ubiquitous FASTA format (Seaview can read in clustal format and “Save as” FASTA format for example). Read this FASTA format alignment into R with the help of functions in the **Bio3D** package. Calculate a sequence identity matrix (again using a function within the Bio3D package). Then generate a heatmap plot and add to your report. Do make sure your labels are visible and not cut at the figure margins.



[Q8] Using R/Bio3D (or an online blast server if you prefer), search the main protein structure database for the most similar atomic resolution structures to your aligned sequences.

List the top 3 *unique* hits (i.e. not hits representing different chains from the same structure) along with their Evalue and sequence identity to your query. Please also add annotation details of these structures. For example include the annotation terms PDB identifier (structureId), Method used to solve the structure (experimentalTechnique), resolution (resolution), and source organism (source).

HINT: You can use a single sequence from your alignment or generate a consensus sequence from your alignment using the Bio3D function `consensus()`. The Bio3D functions `blast.pdb()`, `plot.blast()` and `pdb.annotate()` are likely to be of most relevance for completing this task. Note that the results of `blast.pdb()` contain the hits PDB identifier (or `pdb.id`) as well as Evalue and identity. The results of `pdb.annotate()` contain the other annotation terms noted above.

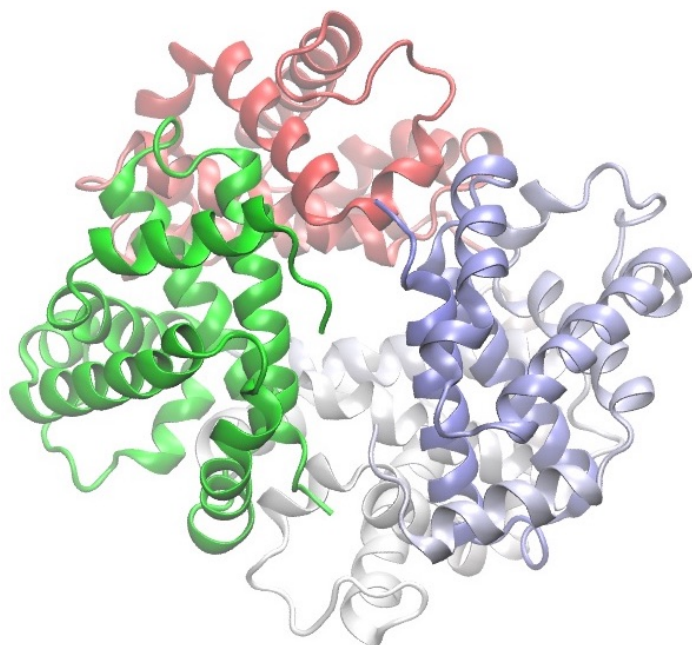
Note that if your consensus sequence has lots of gap positions then it will be better to use an original sequence from the alignment for your search of the PDB. In this case you could choose the sequence with the highest identity to all others in your alignment by calculating the row-wise maximum from your sequence identity matrix.

ID	Technique	Resolution	Source	Evalue	Identity
3BOM	X-RAY DIFFRACTION	1.35	Oncorhynchus mykiss	6.59E-63	81.4
1SPG	X-RAY DIFFRACTION	1.95	Leiostomus xanthurus	3.16E-58	75.9
3BCQ	X-RAY DIFFRACTION	2.4	Brycon cephalus	5.11E-57	77.2

[Q9] Generate a molecular figure of one of your identified PDB structures using **VMD**. You can optionally highlight conserved residues that are likely to be functional. Please use a white or transparent background for your figure (i.e. not the default black).

Based on sequence similarity. How likely is this structure to be similar to your “novel” protein?

Very likely to be similar in structure to *Anguillicola* globin given the high sequence similarity (>80%). In the figure below the beta globin chain B is colored green and corresponds to the *Anguillicola* globin subject of this report.



[Q10] Perform a “Target” search of ChEMBL (<https://www.ebi.ac.uk/chembl/>) with your novel sequence. Are there any **Target Associated Assays** and **ligand efficiency**

data reported that may be useful starting points for exploring potential inhibition of your novel protein?

CHEMBL details 1 Binding Assay (CHEMBL695842) and 5 Functional Assays; No ligand efficiency data.

<https://www.ebi.ac.uk/chembl/target/inspect/11544>

Binding assay linked manuscript tested a set of 2-(N,N-dimethylamino)ethyl isothiocyanate compounds with results suggesting “substantial promise as lead compounds for development of therapeutic agents for sickle cell disease”.

Park S, Hayes BL, Marankan F, Mulhearn DC, Wanna L, Mesecar AD, Santarsiero BD, Johnson ME, Venton DL. Regioselective covalent modification of hemoglobin in search of antisickling agents. *J Med Chem.* 2003 Mar;46(6) 936-953. doi:10.1021/jm020361k. PMID: 12620071.

<http://europepmc.org/abstract/MED/12620071>

Scoring Rubric:

[45 total points available]

Q1 (4 points)

Protein name	1
Species	1
Accession number	1
Function known	1

Q2 (6 points)

Blast method	1
Database searched	1
Limits applied	1
Search output list (top hits)	1
Alignment of choice	1
Evalue and other alignment stats	1

Q3 (3 points)

Protein sequence of choice matches Subject above	1
Name in header	1
Species	1

Q4 (3 point)

Blastp output list with identities & Evalue	1
Top alignment shown with alignment statistics	1
Results indicates a “novel” gene found	1

Q5 (3 points)

MSA labeled with useful names	1
MSA trimmed appropriately (i.e. no gap overhangs)	1
Pasted MSA fits report page width (i.e. font, format)	1

Q6 (1 point)

Figure illustrates sequence clustering pattern	1
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Q7 (10 points)

Heatmap figure included in report	5
Heatmap is legible (i.e. no labels obscured)	5

Q8 (10 points)

PDB identifiers from multiple species reported	5
Annotation of PDB source, resolution and technique	4
Annotation of Evalue and Sequence Identity	1

Q9 (4 points)

Structure figure provided	2
Uses white background for molecular figure	1
Figure of high resolution (i.e. not just snapshot)	1

Q10 (1 point)

Evidence of ChEMBL searches	1
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