BIMM-143: INTRODUCTION TO BIOINFORMATICS

The find-a-gene project assignment
http://thegrantlab.org/bimm143
Dr. Barry Grant

Overview:

The find-a-gene project is a required assignment for BIMM-143. 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 (note that the example report is from a previous quarter and the questions may differ).

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 12pm on the **Monday of Week 5** (see the Assignments and Grading section of our website for details). Note that these first set of answers can be obtained very quickly (at best within 15 or 20 minutes), so if you don't succeed at first, just keep trying.

The complete assignment, including responses to all questions, is due at 12pm on the **Monday of Week 10**.

Submission instructions:

Your report formatted as a **PDF document** should be uploaded to ***GradeScope***. Please make sure to include your UCSD email and PID number on the first page.

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

Submit your preliminary report with answers to Q1-Q4 as soon as you can 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 (and thus be successful in the later stages of the project).

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

Name: Yi-Hung Lee
PID: A16587141

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 it's 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: albumin preproprotein [Homo sapiens]

Accession: NP_000468

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 2.15.0+ search against nematode ESTs

Database: Expressed Sequence Tags (est)

Organism: Amphibia (taxid:8292)

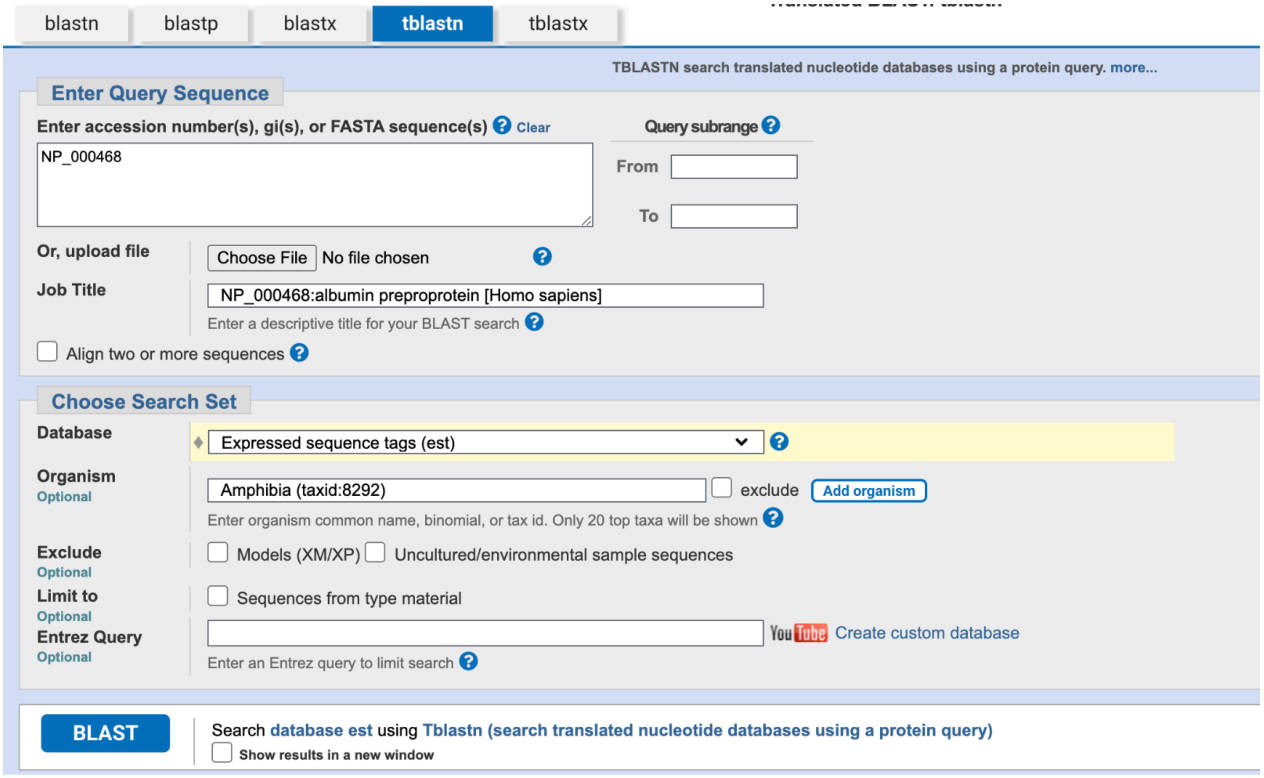
Also include the output of that BLAST search in your document. If appropriate, change the font to `Courier size 10` so that the results are displayed neatly. You can also screen capture a BLAST output (e.g. alt print screen on a PC or on a MAC press ⌘-shift-4. The pointer becomes a bulls eye. Select 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.

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.

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.

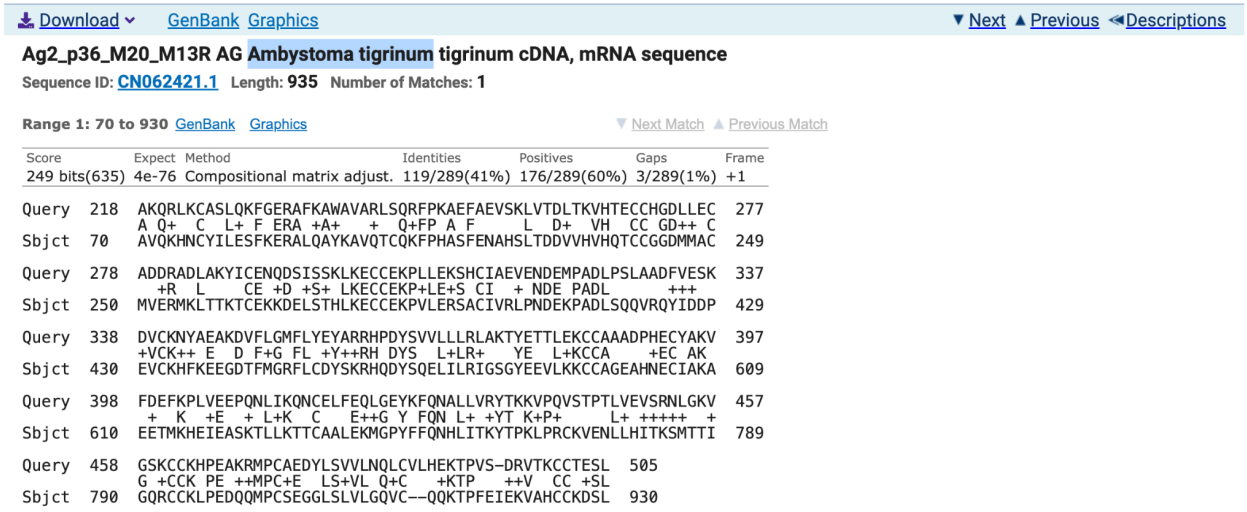
If you are having trouble finding a novel gene try restricting your search to an organism that is

poorly annotated.



Chosen match: Accession CN062421.1, a 935 base pair clone from Ambystoma tigrinum. See below for alignment details.

JGI_CAAR10485.fwd NIH_XGC_tropLiv1 Xenopus tropicalis cDNA clone IMAGE:7742695 5', mRNA sequence	Xenopus tropicalis	250	313	78%	7e-77	40.83%	870	DN034543.1
JGI_CABC6253.fwd NIH_XGC_tropFat1 Xenopus tropicalis cDNA clone IMAGE:7802597 5', mRNA sequence	Xenopus tropicalis	250	312	70%	7e-77	40.07%	883	DR836429.1
JGI_CABC1289.fwd NIH_XGC_tropFat1 Xenopus tropicalis cDNA clone IMAGE:7799341 5', mRNA sequence	Xenopus tropicalis	249	311	73%	7e-77	42.40%	852	DN060715.1
JGI_CAAR11724.fwd NIH_XGC_tropLiv1 Xenopus tropicalis cDNA clone IMAGE:7743774 5', mRNA sequence	Xenopus tropicalis	249	312	78%	8e-77	41.20%	863	DN036844.1
JGI_CAAR3900.fwd NIH_XGC_tropLiv1 Xenopus tropicalis cDNA clone IMAGE:7736489 5', mRNA sequence	Xenopus tropicalis	249	313	78%	8e-77	41.20%	861	DN023018.1
JGI_CABC10616.fwd NIH_XGC_tropFat1 Xenopus tropicalis cDNA clone IMAGE:7805416 5', mRNA sequence	Xenopus tropicalis	250	312	73%	1e-76	41.61%	919	DR841374.1
JGI_CABC1217.rev NIH_XGC_tropFat1 Xenopus tropicalis cDNA clone IMAGE:7799323 3', mRNA sequence	Xenopus tropicalis	249	311	78%	1e-76	40.83%	877	DN060582.1
JGI_CABD10473.fwd NIH_XGC_tropLun1 Xenopus tropicalis cDNA clone CABD10473 5', mRNA sequence	Xenopus tropicalis	249	311	73%	1e-76	40.13%	901	DN077644.1
JGI_CABD14766.rev NIH_XGC_tropLun1 Xenopus tropicalis cDNA clone CABD14766 3', mRNA sequence	Xenopus tropicalis	249	312	74%	1e-76	42.16%	871	DN084472.1
Ag2_p36_M20_M13R AG Ambystoma tigrinum tigrinum cDNA, mRNA sequence	Ambystoma tigrin...	249	249	47%	4e-76	41.18%	935	CN062421.1
JGI_CABD12445.fwd NIH_XGC_tropLun1 Xenopus tropicalis cDNA clone CABD12445 5', mRNA sequence	Xenopus tropicalis	248	310	76%	7e-76	41.64%	898	DN080425.1
JGI_CABC11038.fwd NIH_XGC_tropFat1 Xenopus tropicalis cDNA clone IMAGE:7805714 5', mRNA sequence	Xenopus tropicalis	248	310	72%	7e-76	42.14%	898	DR842130.1
JGI_CAAR4381.fwd NIH_XGC_tropLiv1 Xenopus tropicalis cDNA clone IMAGE:7736945 5', mRNA sequence	Xenopus tropicalis	248	333	84%	8e-76	39.38%	959	DN023916.1
JGI_CABC10416.fwd NIH_XGC_tropFat1 Xenopus tropicalis cDNA clone IMAGE:7805413 5', mRNA sequence	Xenopus tropicalis	247	309	70%	1e-75	40.14%	870	DR841020.1
JGI_CAAR6459.fwd NIH_XGC_tropLiv1 Xenopus tropicalis cDNA clone IMAGE:7738762 5', mRNA sequence	Xenopus tropicalis	246	310	77%	1e-75	40.78%	858	DN027791.1



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Ag2_p36_M20_M13R AG Ambystoma tigrinum tigrinum cDNA, mRNA sequence

Sequence ID: CN062421.1 Length: 935 Number of Matches: 1

Range 1: 70 to 930 GenBank Graphics ▼Next Match ▲Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
249 bits(635)	4e-76	Compositional matrix adjust.	119/289(41%)	176/289(60%)	3/289(1%)	+1

```
Query  218  AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLEC  277
            A Q+  C  L+ F ERA +A+   +  Q+FP A F     L  D+  VH  CC GD++ C
Sbjct  70   AVQKHNCYILESFKERALQAYKAVQTCQKFPHASFENAHSLTDDVVHVHQTCCGGDMMAC  249

Query  278  ADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK  337
              +R  L    CE +D +S+ LKECCEKP+LE+S CI  + NDE PADL      +++
Sbjct  250  MVERMKLTTKTCEKKDELSTHLKECCEKPVLERSACIVRLPNDEKPADLSQQVRQYIDDP  429

Query  338  DVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV  397
            +VCK++ E  D F+G FL +Y++RH DYS  L+LR+   YE  L+KCCA    +EC AK
Sbjct  430  EVCKHFKEEGDTFMGRFLCDYSKRHQDYSQELILRIGSGYEEVLKKCCAGEAHNECIAKA  609

Query  398  FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKV  457
             +  K  +E  + L+K  C   E++G Y FQN L+ +YT K+P+     L+ +++++  +
Sbjct  610  EETMKHEIEASKTLLKTTCAALEKMGPYFFQNHLITKYTPKLPRCKVENLLHITKSMTTI  789

Query  458  GSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVS-DRVTKCCTESL  505
            G +CCK PE ++MPC+E  LS+VL Q+C   +KTP   ++V  CC +SL
Sbjct  790  GQRCCKLPEDQQMPCSEGGLSLVLGQVC--QQKTPFEIEKVAHCCKDSL  930
```

>Ag2_p36_M20_M13R AG Ambystoma tigrinum tigrinum cDNA, mRNA sequence

Sequence ID: CN062421.1 Length: 935

Range 1: 70 to 930

Score:249 bits(635), Expect:4e-76,

Method:Compositional matrix adjust.,

Identities:119/289(41%), Positives:176/289(60%), Gaps:3/289(1%)

```
Query  218  AKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLEC  277
            A Q+  C  L+ F ERA +A+   +  Q+FP A F     L  D+  VH  CC GD++ C
Sbjct  70   AVQKHNCYILESFKERALQAYKAVQTCQKFPHASFENAHSLTDDVVHVHQTCCGGDMMAC  249

Query  278  ADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK  337
              +R  L    CE +D +S+ LKECCEKP+LE+S CI  + NDE PADL      +++
Sbjct  250  MVERMKLTTKTCEKKDELSTHLKECCEKPVLERSACIVRLPNDEKPADLSQQVRQYIDDP  429

Query  338  DVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKV  397
            +VCK++ E  D F+G FL +Y++RH DYS  L+LR+   YE  L+KCCA    +EC AK
Sbjct  430  EVCKHFKEEGDTFMGRFLCDYSKRHQDYSQELILRIGSGYEEVLKKCCAGEAHNECIAKA  609

Query  398  FDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKV  457
             +  K  +E  + L+K  C   E++G Y FQN L+ +YT K+P+     L+ +++++  +
Sbjct  610  EETMKHEIEASKTLLKTTCAALEKMGPYFFQNHLITKYTPKLPRCKVENLLHITKSMTTI  789

Query  458  GSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVS-DRVTKCCTESL  505
```

```
            G +CCK PE ++MPC+E  LS+VL Q+C   +KTP   ++V  CC +SL
Sbjct  790  GQRCCKLPEDQQMPCSEGGLSLVLGQVC--QQKTPFEIEKVAHCCKDSL  930
```

[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.

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.

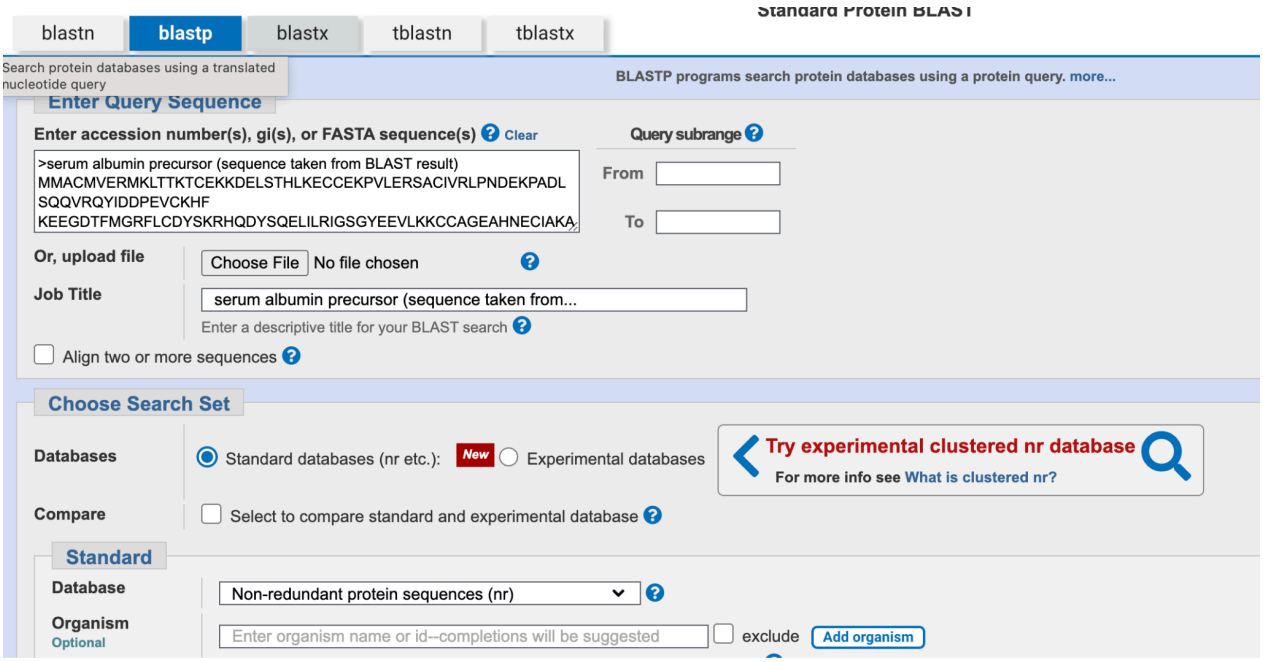
```
Chosen sequence:
>serum albumin precursor(sequence taken from BLAST result)
MMACMVERMKLTTKTCEKKDELSTHLKECCEKPVLERSACIVRLPNDEKPADLSQQVRQYIDDPEVCKHFKEEGDTF
MGRFLCDYSKRHQDYSQELILRIGSGYEEVLKKCCAGEAHNECIAKAEETMKHEIEASKTLLKTTCAALEKMGPYFF
QNHLITKYTPKLPRCKVENLLHITKSMTTIGQRCCKLPEDQQMPCSEGGLSLVLGQVCQQKTPFEIEKVAHCCKDSL
S


Name:serum albumin precursor
Species: Ambystoma tigrinum
      Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
      Amphibia; Batrachia; Caudata; Salamandroidea; Ambystomatidae; Ambystoma
```

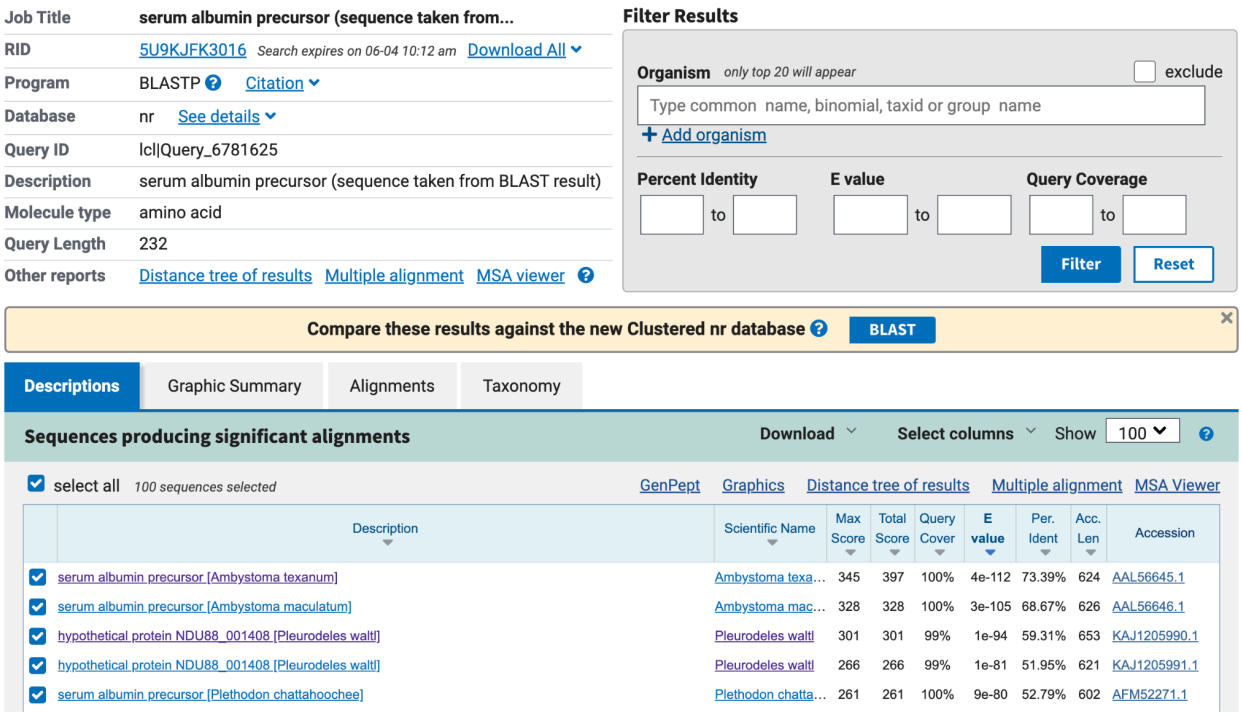
[Q4] Prove that this gene, and its corresponding protein, are novel. For the purposes of this project, "novel" is defined as follows. Take the protein sequence (your answer to [Q3]), and use it as a query in a blastp search of the nr database at NCBI.

- 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.



The top result is to a protein from Ambystoma texanum, see second screen shot below for alignment details:



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serum albumin precursor [Ambystoma texanum]

Sequence ID: AAL56645.1 Length: 624 Number of Matches: 2

Range 1: 283 to 507 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
345 bits(885)	4e-112	Compositional matrix adjust.	171/233(73%)	190/233(81%)	9/233(3%)

```
Query  1    MMACMVERMKLTTKTCEKKDELSTHLKECCEKPVLERSACIVRLPNDEKPADLSQQVRQY  60
            MMACM ERMKLTT+TCEKK         +CCEKPVLERS CIVRLPNDEKPADLS +VR Y
Sbjct  283  MMACMAERMKLTTQTCEKK--------KCCEKPVLERSECIVRLPNDEKPADLSPEVRYY  334

Query  61   IDDPEVCKHFKEEGDTFMGRFLCDYSKRHQDYSQELILRIGSGYEEVLKKCCAGEAHNEC  120
             DDPEVCK FKEEGD FMGRFLCDY+K H ++S EL LRI SG E+  K CCAGEAHNEC
Sbjct  335  FDDPEVCKRFKEEGDAFMGRFLCDYAKIHPEHSAELNLRIASGLEKAYKTCCAGEAHNEC  394

Query  121  IAKAEETMKHEIEASKTLLKTTCAALEKMGPYFFQNHLITKYTPKLPRCKVENLLHITKS  180
            IAK EET++HEIEASKT LKTTC ALEK+GPY FQN +I +YT  LPR     LL+ITK+
Sbjct  395  IAKEEETLRHEIEASKTKLKTTCGALEKLGPYHFQNIMIVRYTGILPRSSDAFLLYITKT  454

Query  181  MTTIGQRCCKLPEDQQMPCSEGGLSLVLGQVCQ-QKTPFEIEKVAHCCKDSLS  232
            +T IGQ+CCKLPEDQQMPCSEGGL +V  Q+CQ QKTPFE EK+AHCCKDSLS
Sbjct  455  LTNIGQKCCKLPEDQQMPCSEGGLGMVFAQICQNQKTPFENEKLAHCCKDSLS  507
```

Range 2: 121 to 279 GenPept Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Method	Identities	Positives	Gaps
52.4 bits(124)	0.002	Compositional matrix adjust.	38/163(23%)	64/163(39%)	6/163(3%)

```
Query  29   CCEKPVLERSACIVRLPNDEKPADLSQQVRQYIDDPE-VCKHFKEEGDTFMGRFLCDYSK  87
            CC+K   ER  C +    + K  D  ++       PE +CK   E  D F+G ++   +
Sbjct  121  CCKKEDPERHKCFI----EHKSTDPKERTEYVKPSPEQICKDHAENRDEFLGHYIHKVAS  176

Query  88   RHQDYSQELILRIGSGYEEVLKKCCAGEAH-NECIAKAEETMKHEIEASKTLLKTTCAAL  146
             H       IL     ++ ++  CC  EA   +C+++     K E+E    + K  C  L
Sbjct  177  SHTTMYPPAILSFTLHFDGIVSHCCKDEATVGQCLSEKMPAHKEEVEHVCAVQKHNCYIL  236

Query  147  EKMGPYFFQNHLITKYTPKLPRCKVENLLHITKSMTTIGQRCC  189
            +       +         K P    EN+  +T  +  + Q CC
Sbjct  237  QNFNERALRASKAAHACSKFPHASFENVQRLTDGIVHLHQTCC  279
```

[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 an alignment for building a phylogenetic tree that illustrates species divergence.

>NP_000468.1 albumin preproprotein [Homo sapiens]
MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEV
TEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLV
RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELR
DEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADD
RADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVF
LGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFE
QLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEK
TPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHK
PKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL

>serum albumin precursor [Ambystoma texanum](sequence taken from BLAST result)
MMACMVERMKLTTKTCEKKDELSTHLKECCEKPVLERSACIVRLPNDEKPADLSQQVRQYIDDPEVCKHFKEEGDTFM
GRFLCDYSKRHQDYSQELILRIGSGYEEVLKKCCAGEAHNECIAKAEETMKHEIEASKTLLKTTCAALEKMGPYFFQN
HLITKYTPKLPRCKVENLLHITKSMTTIGQRCCKLPEDQQMPCSEGGLSLVLGQVCQQKTPFEIEKVAHCCKDSLS

>AAL56645.1:283-507 serum albumin precursor [Ambystoma texanum]
MMACMAERMKLTTQTCEKKKCCEKPVLERSECIVRLPNDEKPADLSPEVRYYFDDPEVCKRFKEEGDAFMGRFLCDYA
KIHPEHSAELNLRIASGLEKAYKTCCAGEAHNECIAKEEETLRHEIEASKTKLKTTCGALEKLGPYHFQNIMIVRYTG
ILPRSSDAFLLYITKTLTNIGQKCCKLPEDQQMPCSEGGLGMVFAQICQNQKTPFENEKLAHCCKDSLS

>KAJ1205990.1:309-539 hypothetical protein NDU88_001408 [Pleurodeles waltl]
MMGCMIERLHLTTRTCEKKDRISKHLKDCCDKDVIERSACIVKMENDEKPADLSPQVREYLEGPDVCKHYADEKDLYL
AKFSCDYAKRHPEFSLQLLLRVSKGYQDLLTKCCAEENSHDCLIKGEEALKKEIESSTTLLKTTCAAFEKLGPYSFQN
ELLVKYTRNIPQLTDESLLHITSGMTRIGQKCCKIPEEKQMPCSEGSLSLVIGEMCEKMPANFPNEKVTHCCSDT

>AFM52292.1:266-498 serum albumin precursor, partial [Plethodon yonahlossee]
MIACMEDRLALTTKTCAKKDELSSKLAACCEKPVVERSACIVKMDNDDRPADLSPQVREYIDDVSVCKRFEDDKNELL
NHFLYDYSRRHPEMSTEMLLKIVIGYDGVLVKCCHKEDKLACLGKAEGEMKKEVQSSVELLKTNCAALEKVGSYHFEV
MLLGKYTLTMPQVTTPTLIHLIDDMTHVGEYCCKVPAEKQLPCSEGALGLIIGSMCQKQEGHFVNNQVAHCCSDSYA

>AFM52295.1:266-498 serum albumin precursor, partial [Plethodon ouachitae]
MMACMEDRLALTTKTCAKKDELSSKLAACCEKPIVERSACIVKMDNDDRPADLSPQVREYIDDVSVCKRFEDDKNELL
SHFLYDYSRRHPEMSTEMLLKIVIGYDGLLVKCCHEEDKLACLGKAEGEMKKEVQSSVELLKTNCAALEKVGSYHFEV
MLLGKYTTRMPQVTTPTLIHLIDDMTHVGEYCCKVPAEKQLPCSEGALGLIIGSMCEKQEGHFVNNQVAHCCSDSYA

>AFM52300.1:266-498 serum albumin precursor, partial [Plethodon kentucki]
MMACMEDRLALTTKTCAKKDELSSKLAACCXKPVVERSACIVKMDNDDRPADLSPQVREYIDDVSVCKRFEDDKNELL
SHFLYDYSRRHPEMSTEMLLKIVIGYDGLLVKCCHEEDKLACLGKAEGEMKKEVQSSVELLKTNCAALEKVGSYHFEV
MLLGKYTPTMPQVTTPTLIHLIDDMTHVGEYCCKVPAEKQLPCSEGALGLIIGSMCEKQEGHFVNNQVAHCCSDSYA

Alignment:

Obtained from CLUSTAL 1.7 multiple sequence alignment from Seaview

Query_10001: albumin preproprotein [Homo sapiens]

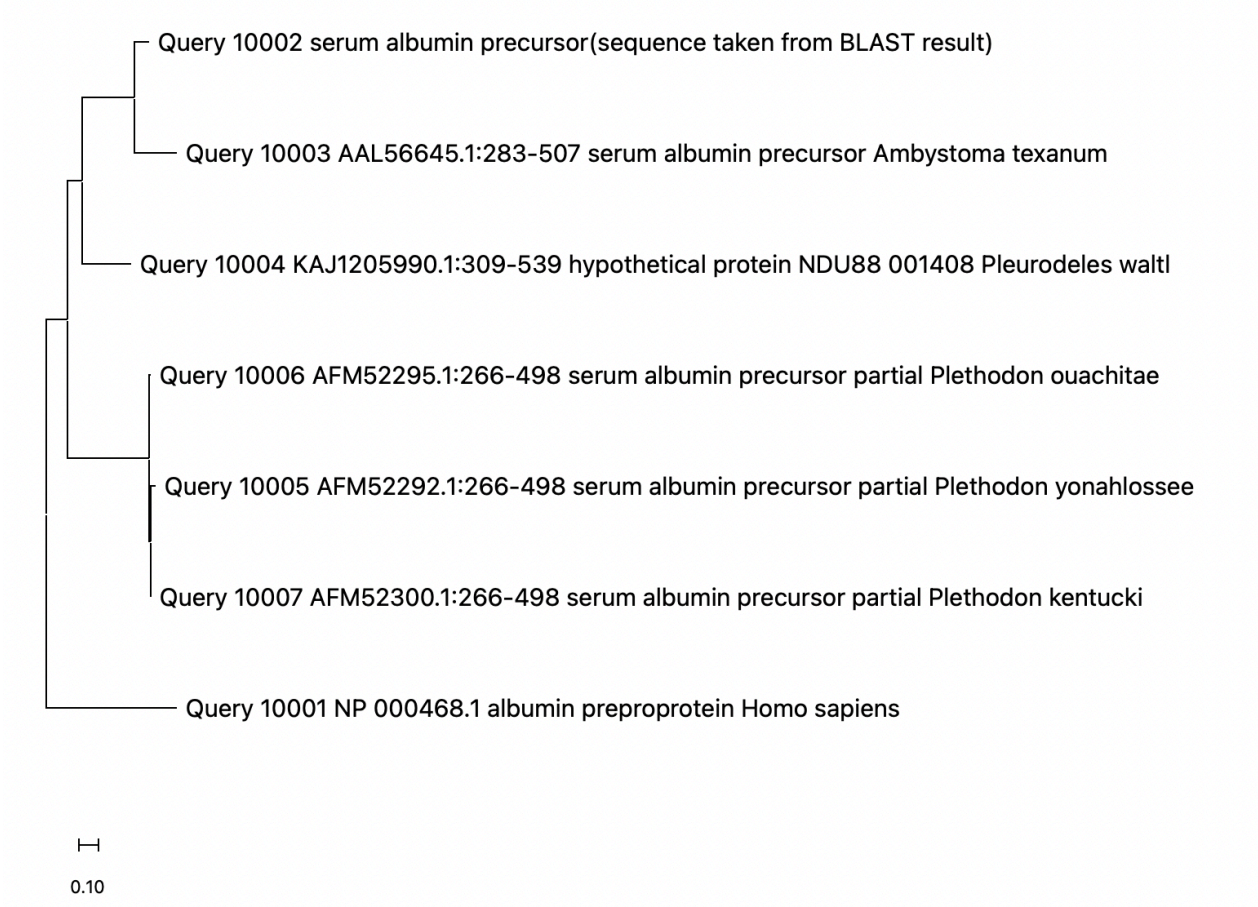
Query_10002: serum albumin precursor [Ambystoma texanum](sequence taken from BLAST result)
Query_10003: serum albumin precursor [Ambystoma texanum]
Query_10004: hypothetical protein NDU88_001408 [Pleurodeles waltl]
Query_10005: serum albumin precursor [Plethodon yonahlossee]
Query_10006: serum albumin precursor [Plethodon ouachitae]
Query_10007: serum albumin precursor [Plethodon kentucki]

```
CLUSTAL W (1.7) multiple sequence alignment


Query_10001  LLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADF
Query_10002  MMACMVERMKLTTKTCEKKDELSTHLKECCEKPVLERSACIVRLPNDEKPADLSQQVRQY
Query_10003  MMACMAERMKLTTQTCEKK--------KCCEKPVLERSECIVRLPNDEKPADLSPEVRYY
Query_10004  MMGCMIERLHLTTRTCEKKDRISKHLKDCCDKDVIERSACIVKMENDEKPADLSPQVREY
Query_10005  MIACMEDRLALTTKTCAKKDELSSKLAACCEKPVVERSACIVKMDNDDRPADLSPQVREY
Query_10006  MMACMEDRLALTTKTCAKKDELSSKLAACCEKPIVERSACIVKMDNDDRPADLSPQVREY
Query_10007  MMACMEDRLALTTKTCAKKDELSSKLAACCXKPVVERSACIVKMDNDDRPADLSPQVREY


Query_10001  VESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHEC
Query_10002  IDDPEVCKHFKEEGDTFMGRFLCDYSKRHQDYSQELILRIGSGYEEVLKKCCAGEAHNEC
Query_10003  FDDPEVCKRFKEEGDAFMGRFLCDYAKIHPEHSAELNLRIASGLEKAYKTCCAGEAHNEC
Query_10004  LEGPDVCKHYADEKDLYLAKFSCDYAKRHPEFSLQLLLRVSKGYQDLLTKCCAEENSHDC
Query_10005  IDDVSVCKRFEDDKNELLNHFLYDYSRRHPEMSTEMLLKIVIGYDGVLVKCCHKEDKLAC
Query_10006  IDDVSVCKRFEDDKNELLSHFLYDYSRRHPEMSTEMLLKIVIGYDGLLVKCCHEEDKLAC
Query_10007  IDDVSVCKRFEDDKNELLSHFLYDYSRRHPEMSTEMLLKIVIGYDGLLVKCCHEEDKLAC


Query_10001  YAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRN
Query_10002  IAKAEETMKHEIEASKTLLKTTCAALEKMGPYFFQNHLITKYTPKLPRCKVENLLHITKS
Query_10003  IAKEEETLRHEIEASKTKLKTTCGALEKLGPYHFQNIMIVRYTGILPRSSDAFLLYITKT
Query_10004  LIKGEEALKKEIESSTTLLKTTCAAFEKLGPYSFQNELLVKYTRNIPQLTDESLLHITSG
Query_10005  LGKAEGEMKKEVQSSVELLKTNCAALEKVGSYHFEVMLLGKYTLTMPQVTTPTLIHLIDD
Query_10006  LGKAEGEMKKEVQSSVELLKTNCAALEKVGSYHFEVMLLGKYTTRMPQVTTPTLIHLIDD
Query_10007  LGKAEGEMKKEVQSSVELLKTNCAALEKVGSYHFEVMLLGKYTPTMPQVTTPTLIHLIDD
```

```
Query_10001   LGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV
Query_10002   MTTIGQRCCKLPEDQQMPCSEGGLSLVLGQVCQ-QKTPFEIEKVAHCCKDSLS
Query_10003   LTNIGQKCCKLPEDQQMPCSEGGLGMVFAQICQNQKTPFENEKLAHCCKDSLS
Query_10004   MTRIGQKCCKIPEEKQMPCSEGSLSLVIGEMCEKMPANFPNEKVTHCCSDT--
Query_10005   MTHVGEYCCKVPAEKQLPCSEGALGLIIGSMCQKQEGHFVNNQVAHCCSDSYA
Query_10006   MTHVGEYCCKVPAEKQLPCSEGALGLIIGSMCEKQEGHFVNNQVAHCCSDSYA
Query_10007   MTHVGEYCCKVPAEKQLPCSEGALGLIIGSMCEKQEGHFVNNQVAHCCSDSYA
```

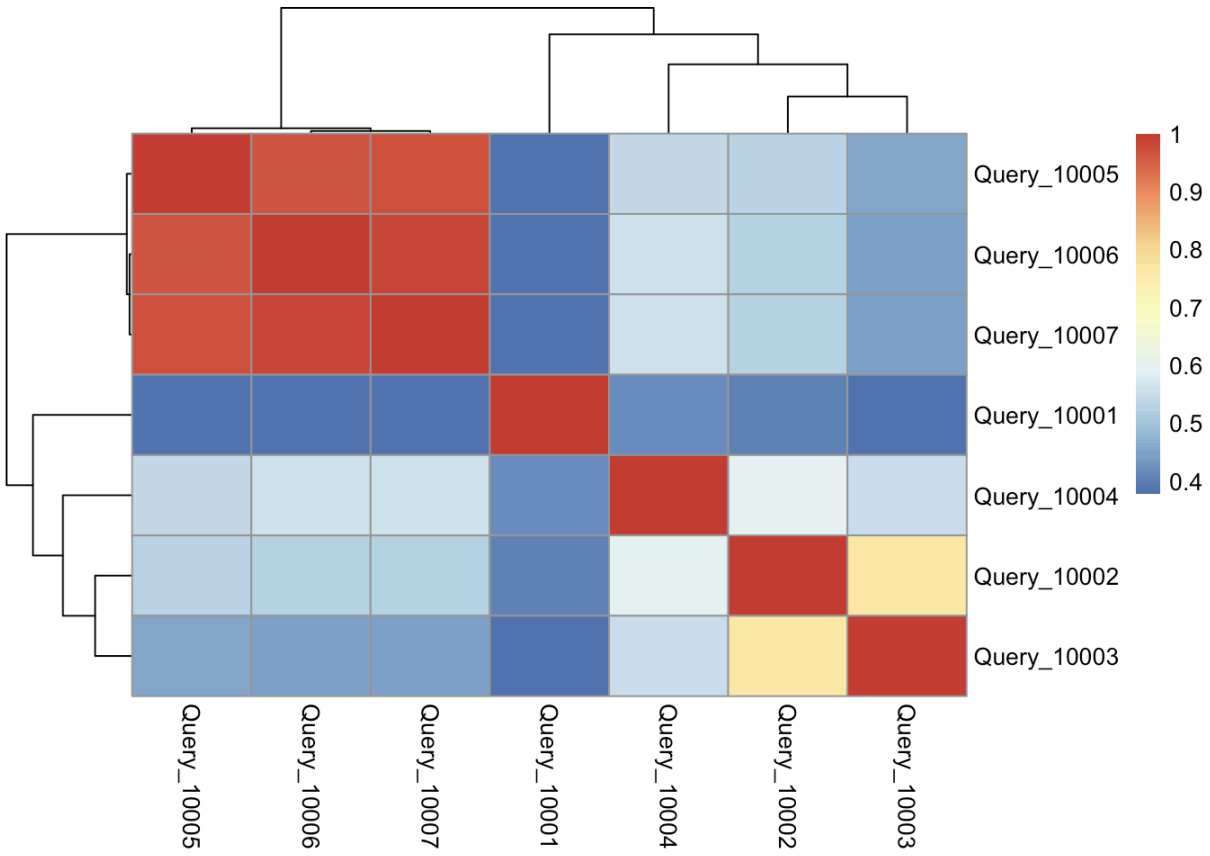
[Q6] Create a phylogenetic tree, using either a parsimony or distance-based approach. Bootstrapping and tree rooting are optional. Use “simple phylogeny” online from the EBI or any respected phylogeny program (such as MEGA, PAUP, or Phylip). Paste an image of your Cladogram or tree output in your report.

Import the sequences into MEGA, align with CLUSTAL, 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.



Query_10001: albumin preproprotein [Homo sapiens]

Query_10002: serum albumin precursor [Ambystoma texanum](sequence taken from BLAST result)

Query_10003: serum albumin precursor [Ambystoma texanum]

Query_10004: hypothetical protein NDU88_001408 [Pleurodeles waltl]

Query_10005: serum albumin precursor [Plethodon yonahlossee]

Query_10006: serum albumin precursor [Plethodon ouachitae]

Query_10007: serum albumin precursor [Plethodon kentucki]

[**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 chose the sequence with the highest identity to all others in your alignment by calculating the row-wise maximum from your sequence identity matrix.

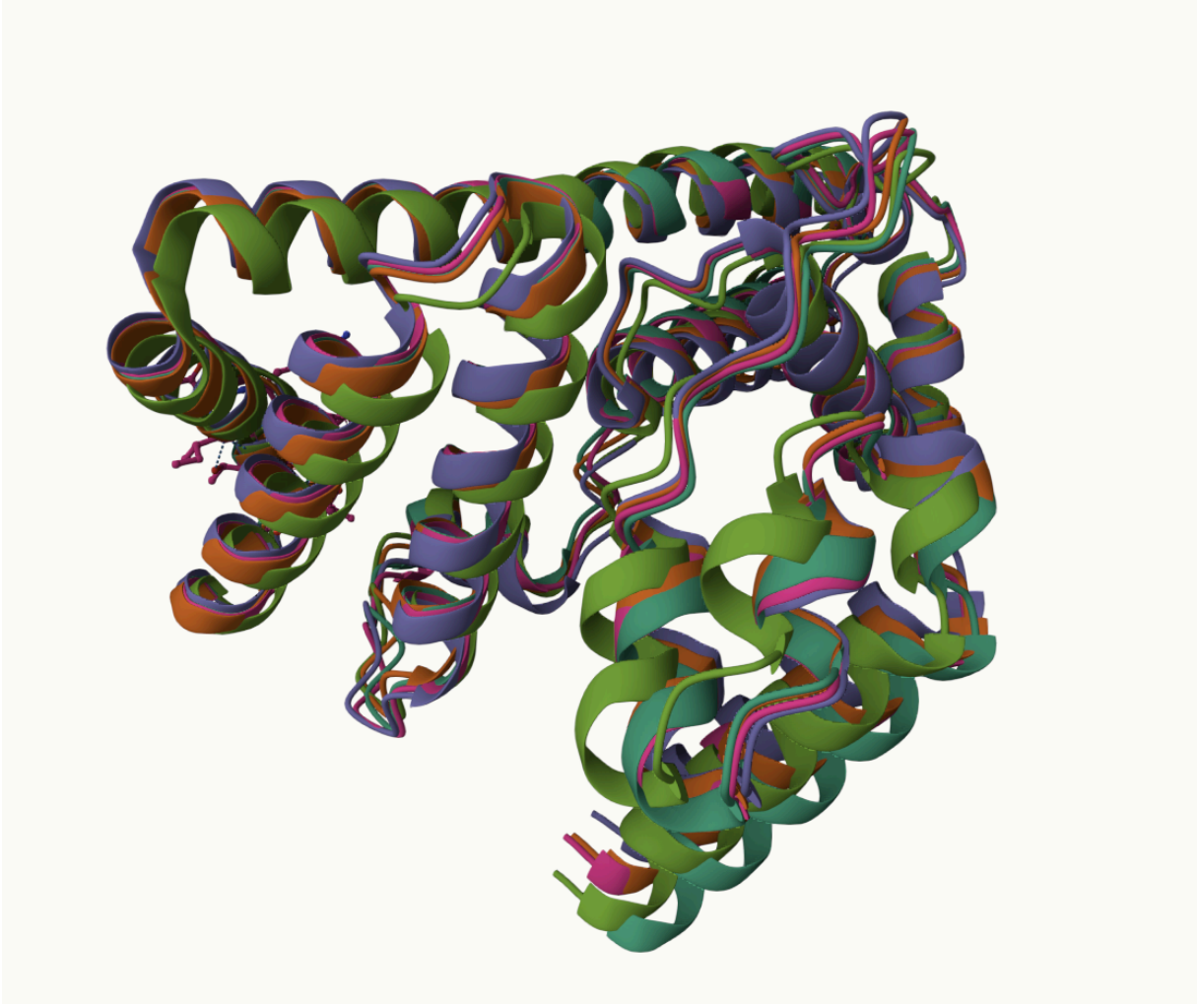
ID	Technique	Resolution	Source	E-value	Identity
4F5V	X-RAY DIFFRACTION	2.27	Oryctolagus cuniculus	1.02e-62	42.489
3V09	X-RAY DIFFRACTION	2.27	Oryctolagus cuniculus	1.16e-62	42.489
5Z0B	X-RAY DIFFRACTION	2.17	Homo sapiens	7.60e-62	41.631

[**Q9**] Using AlphaFold notebook generate a structural model using the default parameters for your novel protein sequence.

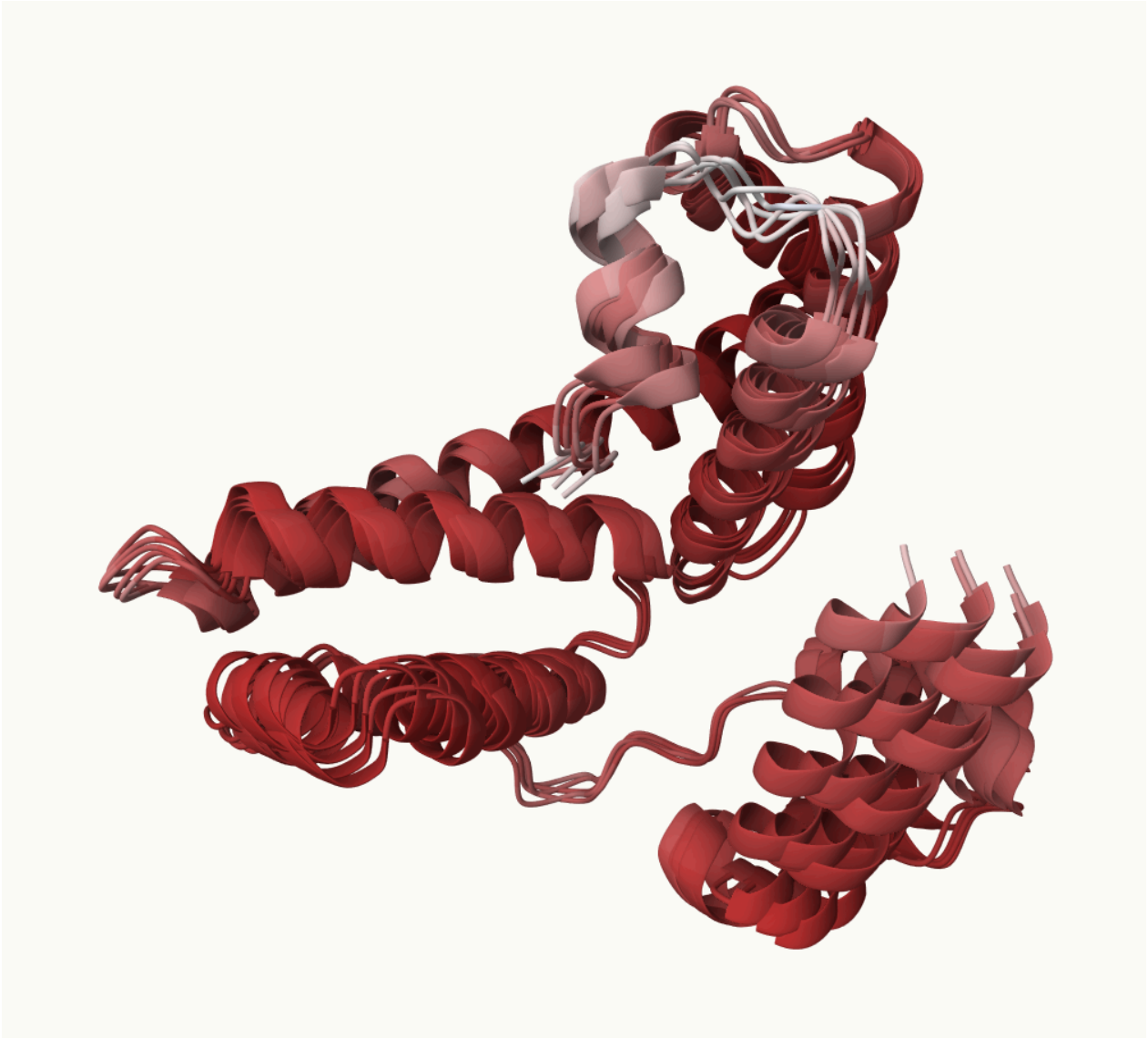
Note that this can take some time depending upon your sequence length. If your model is taking many hours to generate or your input sequence yields a “too many amino acids” (i.e. length) error you can focus on a single domain from your

sequence - identify region by searching for PFAM domain matches.

Once complete save the resulting PDB format file for your records. Finally, generate a molecular figure of your generated PDB structure using the **Mol* viewer** online (or VMD/PyMol/Chimera if you prefer). To complete your analysis you can optionally highlight *conserved residues* that are likely to be functional as **spacefill** and the protein as **cartoon** colored by local alpha fold *pLDDT quality score*. This score is contained in the B-factor column of your PDB downloaded file. Please use a white or transparent background for your figure (i.e. not the default black in PyMol/VMD/Chimera etc.).



Annotated by pLDDT quality score:



[**Q10**] Perform a “Target” search of ChEMBEL (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? If there are no assays listed here simply list “non available as of [date]”.

CHEMBL details 1 Binding Assay (CHEMBL1055715) and 3 Functional Assays; No ligand efficiency data.

https://www.ebi.ac.uk/chembl/assay_report_card/CHEMBL1055715/

Binding assay linked manuscript tested a potent 3,4-disubstituted benzofuran P1' MMP-13 inhibitors. By replacing a backbone benzene with a pyridine and valine with threonine, compounds (e.g., 44) with greatly reduced plasma protein binding were also obtained.

W. Li, et al., 3,4-Disubstituted benzofuran P1′ MMP-13 inhibitors: Optimization of selectivity and reduction of protein binding. Bioorganic & Medicinal Chemistry Letters 19, 4546–4550 (2009).

Scoring Rubric: [50 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

Q7 (10 points)

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

Q8 (9 points)

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

Q9 (10 points)

Structure figure provided 2 Uses white background for molecular figure 1 Figure of high resolution (i.e. not just snapshot) 1 Conserved residues as spacefill 3 Protein cartoon colored by pLDDT quality score 3

Q10 (1 point)

Evidence of ChEMBEL searches 1