BIMM-143: INTRODUCTION TO BIOINFORMATICS

The find-a-gene project assignment

https://bioboot.github.io/bimm143_F21

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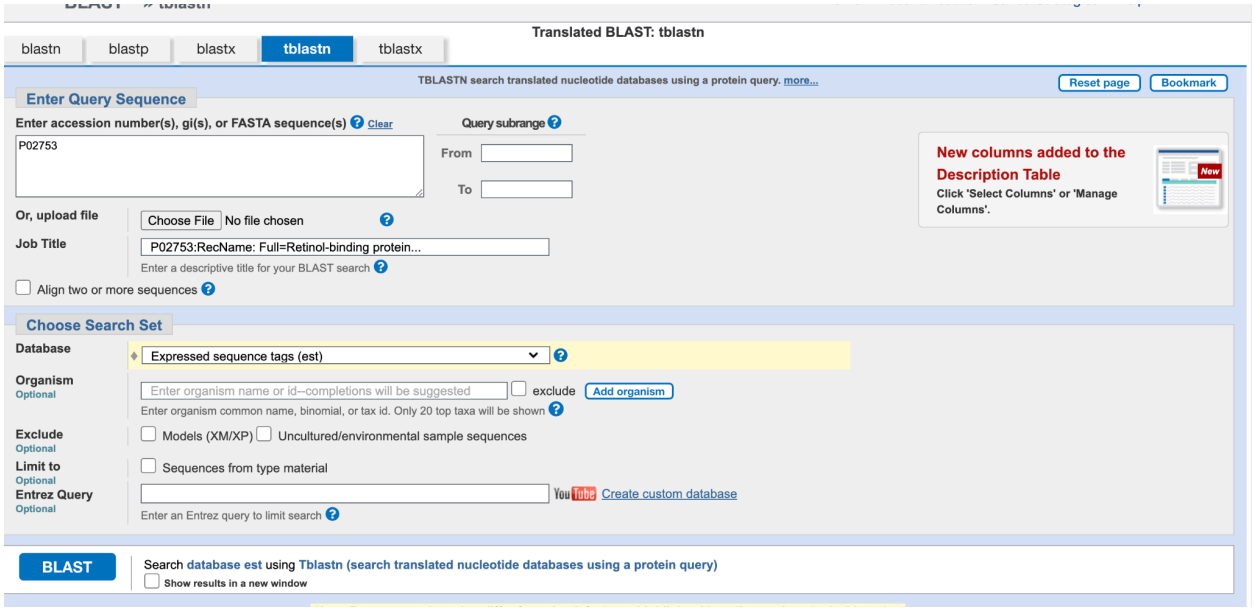
Questions:

[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: Retinol Binding Protein 4
Accession: P02753
Species: Homo Sapiens

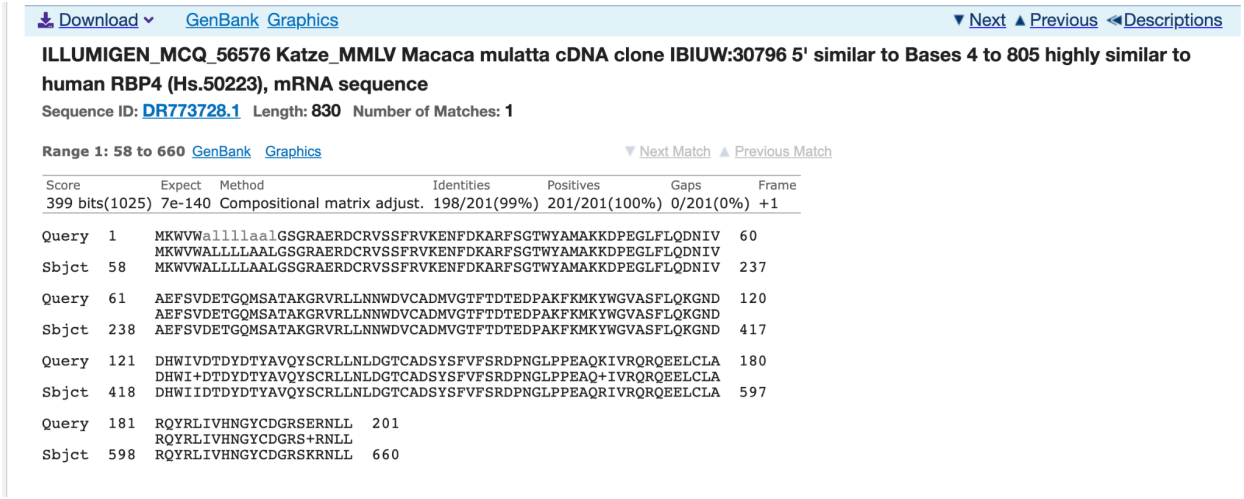
[Q2] Perform a BLAST search against a DNA database, such as a database consisting of genomic DNA or ESTs. The BLAST server can be at NCBI or elsewhere. Include details of the BLAST method used, database searched and any limits applied (e.g. Organism).

Method: TBLASTN (2.12.0) search with ESTs
Database: Expressed Sequence Tags (est)
Organism: None



Chosen match: Accession DR773728.1, a 830 base pair clone from Macaca mulatta. See below for alignment details.

DC627655 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-24313 5', mRNA sequence	Macaca fascicularis	400	400	100%	5e-140	95.02%	937	DC627655.1
DC643063 macaque kidney cDNA library QreB Macaca fascicularis cDNA clone QreB-29179 5', mRNA sequence	Macaca fascicularis	400	400	100%	6e-140	95.02%	941	DC643063.1
DC626124 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-19200 5', mRNA sequence	Macaca fascicularis	400	400	100%	6e-140	95.02%	942	DC626124.1
DC627041 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-22254 5', mRNA sequence	Macaca fascicularis	400	400	100%	6e-140	95.02%	945	DC627041.1
DC640901 macaque kidney cDNA library QreB Macaca fascicularis cDNA clone QreB-14114 5', mRNA sequence	Macaca fascicularis	400	400	100%	6e-140	95.02%	946	DC640901.1
DC627217 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-23103 5', mRNA sequence	Macaca fascicularis	400	400	100%	7e-140	95.02%	951	DC627217.1
ILLUMIGEN_MCQ_56576 Katze_MMLV Macaca mulatta cDNA clone IBIUW:30796 5' similar to Bases 4 to 805 hi…	Macaca mulatta	399	399	100%	7e-140	94.53%	830	DR773728.1
FS582264 macaque heart cDNA library QhtB Macaca fascicularis cDNA clone QhtB-19211 5', mRNA sequence	Macaca fascicularis	400	400	100%	7e-140	95.02%	955	FS582264.1
DC629401 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-30263 5', mRNA sequence	Macaca fascicularis	400	400	100%	7e-140	95.02%	955	DC629401.1
DC627267 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-23167 5', mRNA sequence	Macaca fascicularis	400	400	100%	7e-140	95.02%	962	DC627267.1
DC628015 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-26021 5', mRNA sequence	Macaca fascicularis	400	400	100%	8e-140	95.02%	972	DC628015.1
DC622200 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-06147 5', mRNA sequence	Macaca fascicularis	400	400	100%	9e-140	95.02%	975	DC622200.1



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ILLUMIGEN_MCQ_56576 Katze_MMLV Macaca mulatta cDNA clone IBIUW:30796 5' similar to Bases 4 to 805 highly similar to human RBP4 (Hs.50223), mRNA sequence

Sequence ID: DR773728.1 **Length:** 830 **Number of Matches:** 1

Range 1: 58 to 660 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
399 bits(1025)	7e-140	Compositional matrix adjust.	198/201(99%)	201/201(100%)	0/201(0%)	+1

```
Query  1    MKWVWallllaalGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV  60
            MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
Sbjct  58   MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV  237

Query  61   AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND  120
            AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Sbjct  238  AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND  417

Query  121  DHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQEELCLA  180
            DHWI+DTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQ+IVRQRQEELCLA
Sbjct  418  DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA  597

Query  181  RQYRLIVHNGYCDGRSERNLL  201
            RQYRLIVHNGYCDGRS+RNLL
Sbjct  598  RQYRLIVHNGYCDGRSKRNLL  660
```

```
>ILLUMIGEN_MCQ_56576 Katze_MMLV Macaca mulatta cDNA clone IBIUW:30796 5' similar to Bases 4 to 805
highly similar to human RBP4 (Hs.50223), mRNA sequence
Sequence ID: DR773728.1 Length: 830
Range 1: 58 to 660

Score:399 bits(1025), Expect:7e-140,
Method:Compositional matrix adjust.,
Identities:198/201(99%), Positives:201/201(100%), Gaps:0/201(0%)

Query  1    MKWVWallllaalGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV  60
            MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
Sbjct  58   MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV  237

Query  61   AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND  120
            AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Sbjct  238  AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND  417

Query  121  DHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQEELCLA  180
            DHWI+DTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQ+IVRQRQEELCLA
Sbjct  418  DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA  597

Query  181  RQYRLIVHNGYCDGRSERNLL  201
            RQYRLIVHNGYCDGRS+RNLL
Sbjct  598  RQYRLIVHNGYCDGRSKRNLL  660
```

[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.

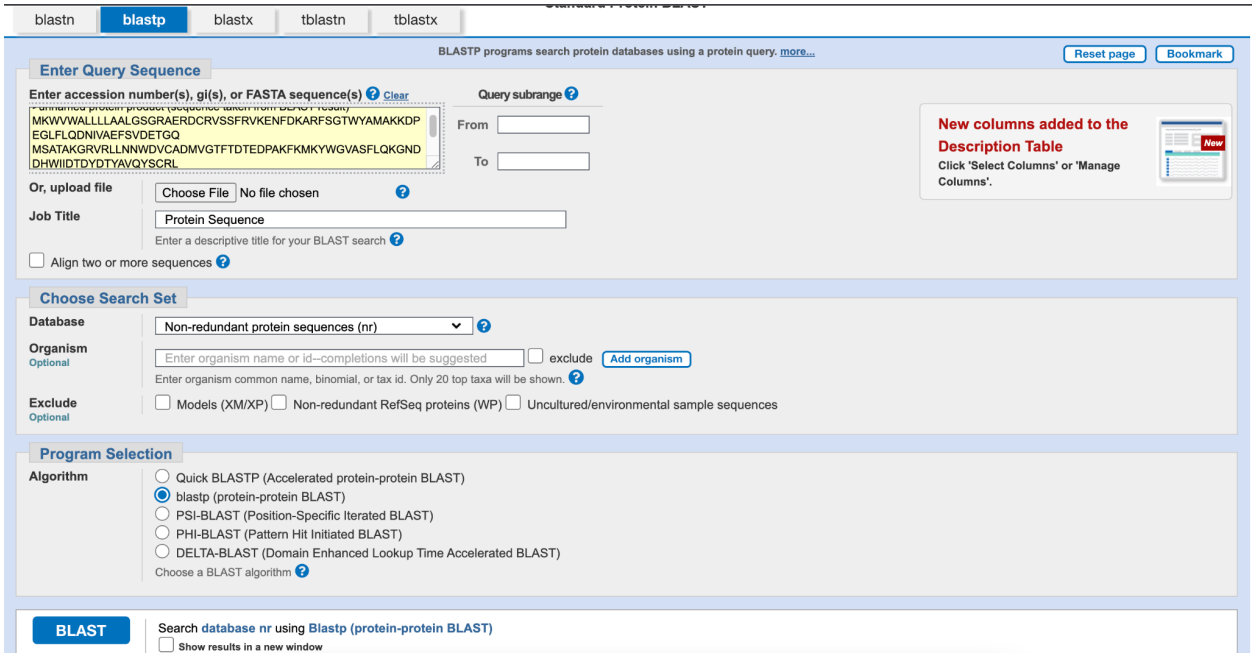
Chosen sequence:
>ILLUMIGEN_MCQ_56576 Katze_MMLV Macaca mulatta cDNA clone IBIUW:30796 5' similar to Bases 4 to 805 highly similar to human RBP4 (Hs.50223), mRNA sequence (sequence taken from BLAST result)
MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAE
FSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHW
IIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLARQYRLIVH
NGYCDGRSKRNLL

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

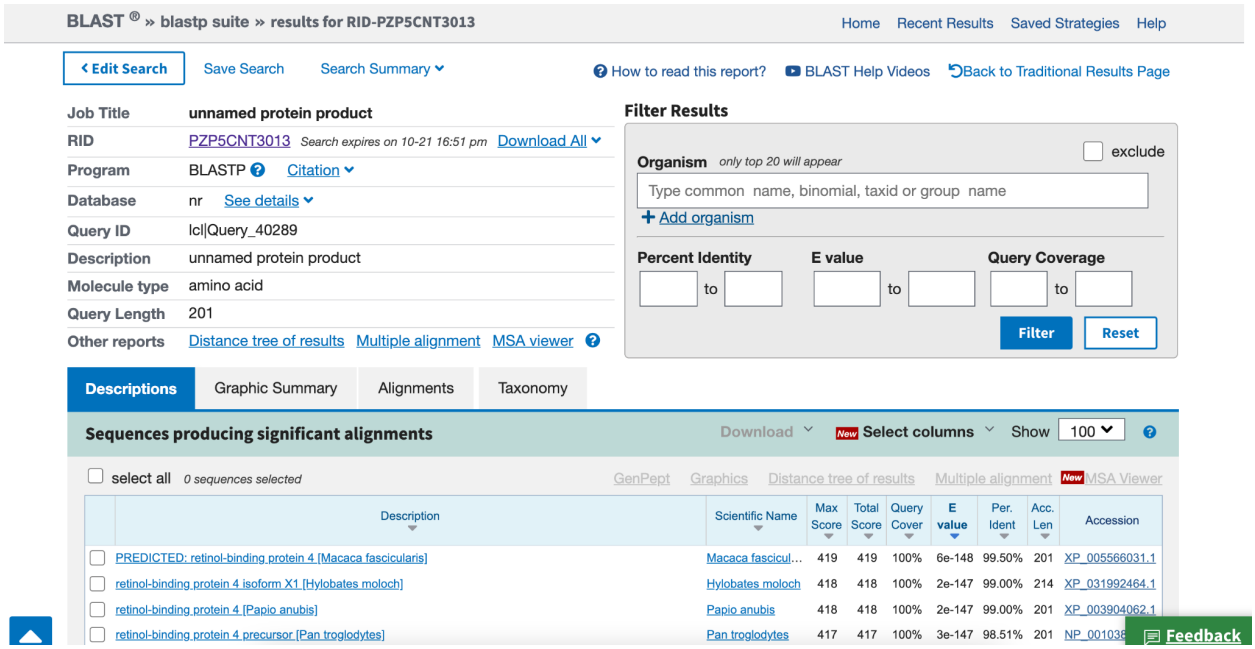
Name: Macaca retinol binding protein 4
Species: Macaca mulatta
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Cercopithecidae; Cercopithecinae; Macaca.

[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 to a protein from Macaca fascicularis (Crab-eating macaque). See additional screenshots below for top hits and selected alignment details:



The top result is to a protein from Macaca fascicularis (Crab-eating macaque), see second screen shot below for alignment details:



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PREDICTED: retinol-binding protein 4 [Macaca fascicularis]

Sequence ID: XP_005566031.1 **Length:** 201 **Number of Matches:** 1

See 5 more title(s) See all Identical Proteins(IPG)

Range 1: 1 to 201 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
419 bits(1077)	6e-148	Compositional matrix adjust.	200/201(99%)	201/201(100%)	0/201(0%)

```
Query  1    MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV  60
            MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
Sbjct  1    MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV  60

Query  61   AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND  120
            AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Sbjct  61   AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND  120

Query  121  DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA  180
            DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA
Sbjct  121  DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA  180

Query  181  RQYRLIVHNGYCDGRSKRNLL  201
            RQYRLIVHNGYCDGRS+RNLL
Sbjct  181  RQYRLIVHNGYCDGRSERNLL  201
```

Related Information

Gene - associated gene details

Genome Data Viewer - aligned genomic context

Identical Proteins - Identical proteins to XP_005566031.1

[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:

>sp|P02753|RET4_HUMAN Retinol-binding protein 4 OS=Homo sapiens OX=9606 GN=RBP4 PE=1 SV=3 RBP-4
Human_RBP4
MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
DHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQEELCLA
RQYRLIVHNGYCDGRSERNLL

>ILLUMIGEN_MCQ_56576 Katze_MMLV Macaca mulatta cDNA clone IBIUW:30796 5' similar to Bases 4 to 805 highly similar to human RBP4 (Hs.50223), mRNA sequence (sequence taken from BLAST result)
Macaca_mulatta
MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAE
FSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHW
IIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLARQYRLIVH
NGYCDGRSKRNLL

>ILLUMIGEN_MCQ_54653 Katze_MNLV Macaca nemestrina cDNA clone IBIUW:31443 5' similar to Bases 5 to 854 highly similar to human RBP4 (Hs.50223), mRNA sequence
Macaca_nemestrina
MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA
RQYRLIVHNGYCDGRSERNLL

>DC629429 macaque liver cDNA library QlvC Macaca fascicularis cDNA clone QlvC-30299 5', mRNA sequence
Macaca_fascicularis
MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA

RQYRLIVHNGYCDGRSERNLL

>HX496860 full-length enriched common marmoset liver cDNA library Callithrix jacchus cDNA clone MLI-189E17, mRNA sequence
Callithrix_jacchus
GISRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIIAEFSVDETGQMSA
TAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYA
VQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIIRQRQEELCLARQYRLIVHNGYCD
GKSERNLL

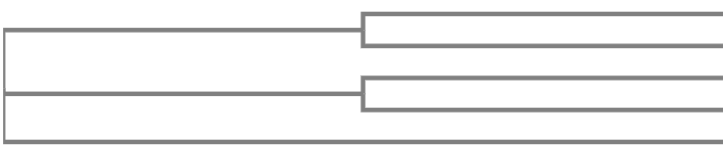
Alignment:

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

```
Callithrix_jacchus      ------------GISRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNII
Human_RBP4              MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
Macaca_mulatta          MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
Macaca_nemestrina       MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
Macaca_fascicularis     MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV
                                    * .*****************************************:

Callithrix_jacchus      AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Human_RBP4              AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Macaca_mulatta          AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Macaca_nemestrina       AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
Macaca_fascicularis     AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND
                        ************************************************************

Callithrix_jacchus      DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIIRQRQEELCLA
Human_RBP4              DHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQEELCLA
Macaca_mulatta          DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA
Macaca_nemestrina       DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA
Macaca_fascicularis     DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQRIVRQRQEELCLA
                        ****:*****************************************.*:**********

Callithrix_jacchus      RQYRLIVHNGYCDGKSERNLL
Human_RBP4              RQYRLIVHNGYCDGRSERNLL
Macaca_mulatta          RQYRLIVHNGYCDGRSKRNLL
Macaca_nemestrina       RQYRLIVHNGYCDGRSERNLL
Macaca_fascicularis     RQYRLIVHNGYCDGRSERNLL
                        **************.*:****
```

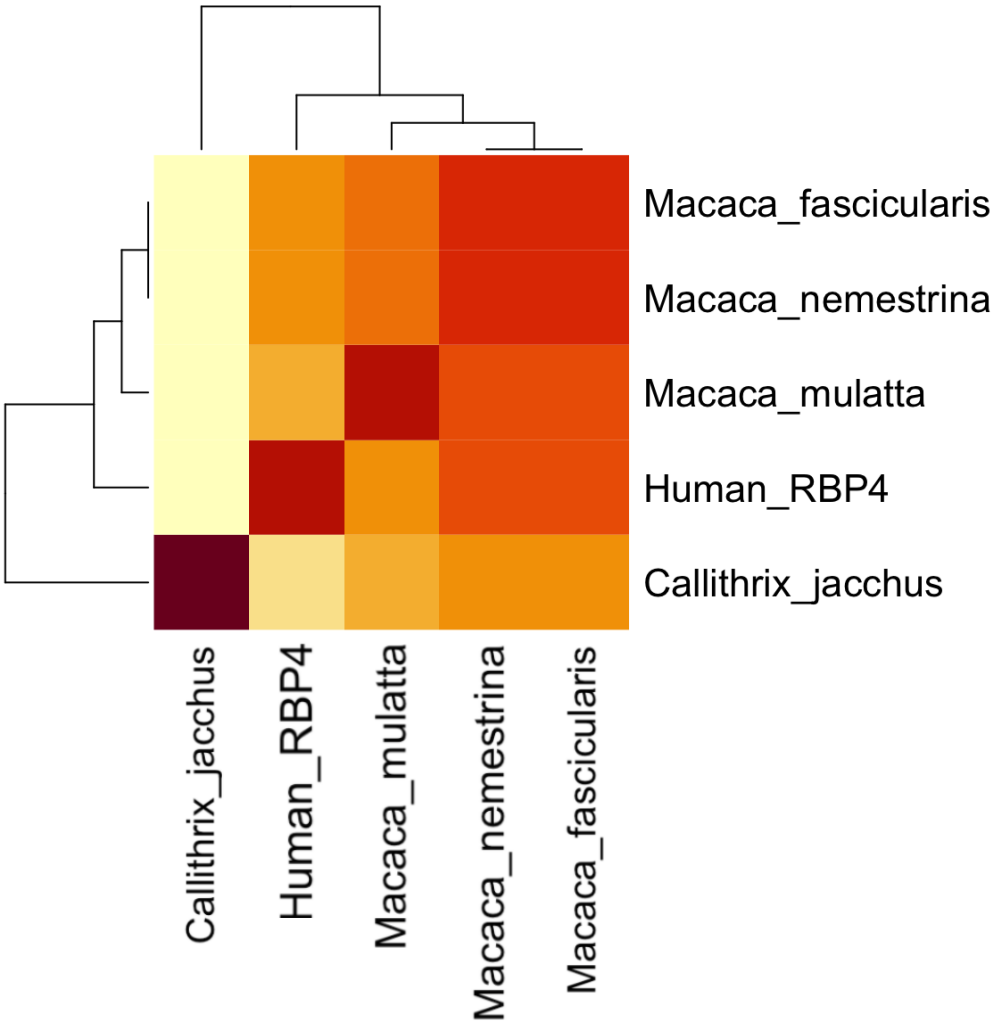
[Q6] Create a phylogenetic tree, using either a parsimony or distance-based approach. Bootstrapping and tree rooting are optional. Use “simple phylogeny” online from the EBI or any respected phylogeny program (such as MEGA, PAUP, or Phylip). Paste an image of your Cladogram or tree output in your report.

Use “simple phylogeny” and import the sequences into EBI, and create a neighbor-joining tree:



Callithrix_jacchus 0.02677
Macaca_nemestrina -0.00017
Human_RBP4 0.00999
Macaca_mulatta 0.00493
Macaca_fascicularis -0.00013

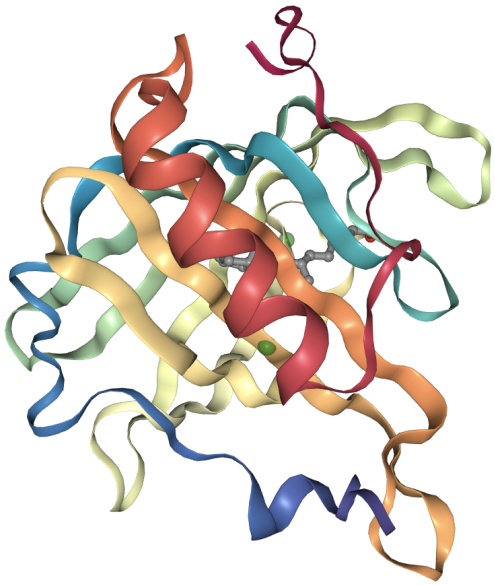
[Q7] Generate a sequence identity based heatmap of your aligned sequences using R. If necessary convert your sequence alignment to the ubiquitous FASTA format (Seaview can read in clustal format and “Save as” FASTA format for example). Read this FASTA format alignment into R with the help of functions 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).

ID	Technique	Resolution	Source	E-value	Identity
1AQB	X-ray diffraction	2.5	Sus scrofa domesticus	7e-131	92.35
1HBQ	X-ray diffraction	2.5	Bos taurus	3e-130	91.80
1RLB	X-ray diffraction	3.1	Gallus gallus	4e-123	91.38

[Q9] Generate a molecular figure of one of your identified PDB structures using the NGL viewer online (or VMD/PyMol). 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?



It is very likely to be similar in structure to *Macaca* retinol binding protein given the high sequence similarity (>90%). In the figure above, the alpha globulin unit is colored in yellow and corresponds to the *Macaca* retinol binding protein subject of this report.

[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?

A Target search on ChEMBEL on my novel protein details 1 binding assay (CHEMBL3100), 5 Functional Assays, 1 unclassified protein, but no ligand efficiency data.

https://www.ebi.ac.uk/chembl/g/#blast_search_results/eyJzZXF1ZW5jZSI6Ik1LV1ZXQUxMTExBQUxHU0dSQUVSRENSVlNTRlJWS0VORkRLQVJGU0dUV1lBTUFLS0RQRUdMRkxRRE5JVkFFRlNWREVUR1FNU0FUQUtHUlZSTExOTldEVkNBRE1WR1RGVERURURQQUtGS01LWVdHVkFTRkxRS0dORERIV0lJRFREWURURUWUFWUVITQ1JMTE5MREdUQ0FEU1ITRlZGU1JEUE5HTFBQRUFRUklWUlFSUUVFTENMQVJRWVJMSVZITkdZQ0RHUlNLUk5MTCJ9

The gene that encodes this binding assay “catalyzes the conversion of prostaglandin H2 (PGH2) to prostaglandin D2 (PGD2)”. PGD2 functions as a neuromodulator as well as a trophic factor in the central nervous system. PGD2 is also involved in “smooth muscle contraction/relaxation and is a potent inhibitor of platelet aggregation”.

> Wang, P. (2021, November 23). *PTGDS prostaglandin D2 synthase [Homo Sapiens (human)] - gene - NCBI*. National Center for Biotechnology Information. Retrieved December 6, 2021, from https://www.ncbi.nlm.nih.gov/gene/5730.

https://www.ncbi.nlm.nih.gov/gene/5730