Q1:

Protein Name: RBP4 retinol binding protein 4

Gene ID: 5950 https://www.ncbi.nlm.nih.gov/gene/5950

Species: Homo Sapiens (Humans)

Accession Number: NP\_006735

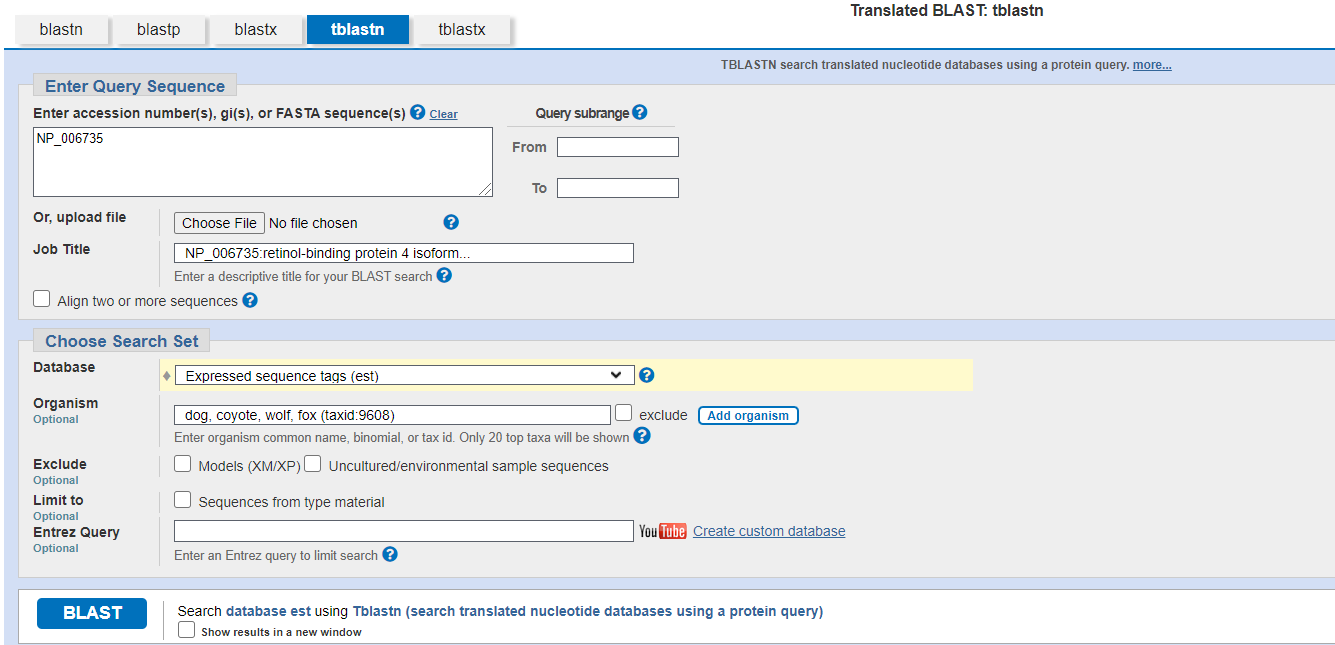
Function: It’s expressed in the liver, a member of the lipocalin family and works as retinol carrier(vitamin A alcohol) in the blood. It delivers retinol from the liver stores to the peripheral tissues. A deficiency of vitamin A blocks secretion of the binding protein post translationally and results in defective delivery and supply to the epidermal cells.

Q2:

**Method**: TBLASTN (2.7.1) search in dog, cayote and wolf against ESTs

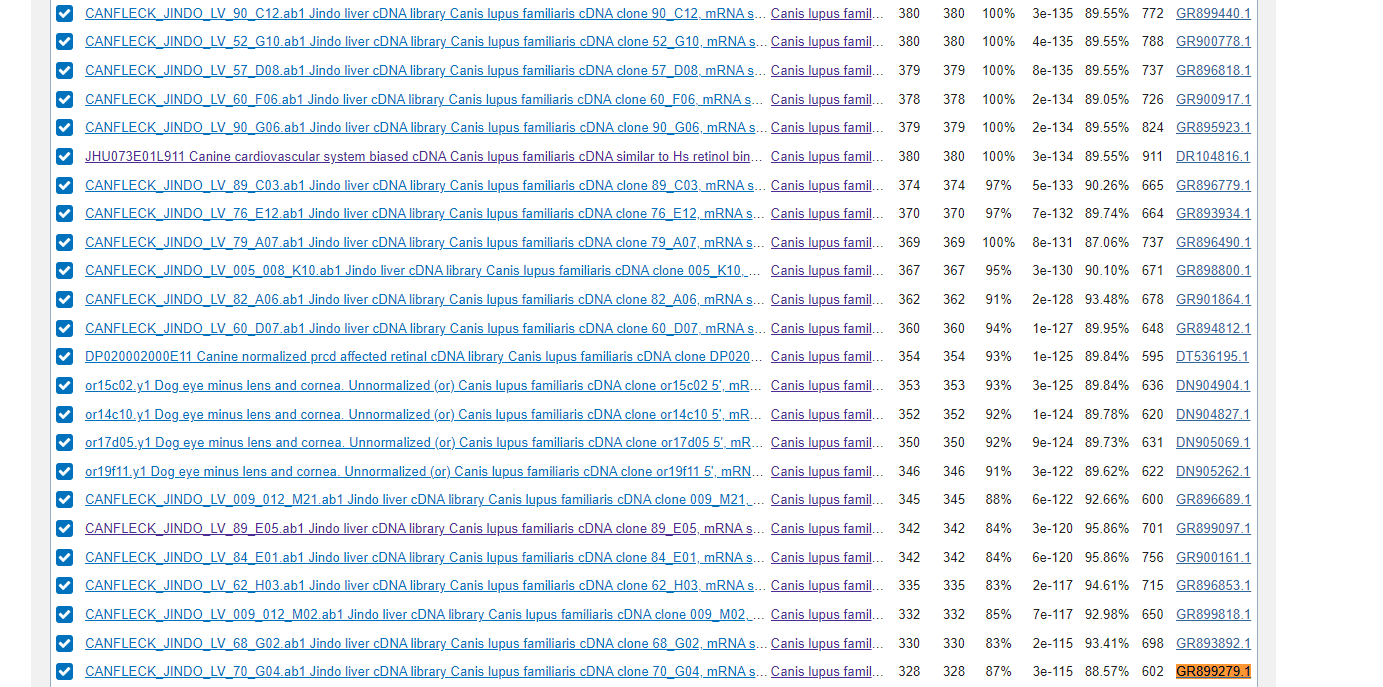
Database: expressed sequence tags(ESTs)

Organism: dog, coyote, wolf (taxid: 9608)



Graphical user interface, text, application, email

Description automatically generated



Chart

Description automatically generated

**Graphical user interface, text, application

Description automatically generated with medium confidence**

**The chosen match is accession GR899279.1, with 602 nucleotides in the sequence, and belongs to the Canis lupus familiaris species.**

**CANFLECK\_JINDO\_LV\_70\_G04.ab1 Jindo liver cDNA library Canis lupus familiaris cDNA clone 70\_G04, mRNA sequence**

**Sequence ID:**[**GR899279.1**](https://www.ncbi.nlm.nih.gov/nucleotide/GR899279.1?report=genbank&log$=nuclalign&blast_rank=28&RID=PBZYFXS0016) **Length: 602 Number of Matches: 1**

Range 1: 76 to 600

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Alignment statistics for match #1** | | | | | | |
| Score | Expect | Method | Identities | Positives | Gaps | Frame |
| 328 bits(840) | 3e-115 | Compositional matrix adjust. | 162/175(93%) | 169/175(96%) | 0/175(0%) | +1 |

Query 1 MKWVWallllaalGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV 60

M+WVWAL LLAALGS RAE DCRVS+F+VK+ FDKARF+GTWYAMAKKDPEGLFLQDNIV

Sbjct 76 MEWVWALELLAALGSARAESDCRVSNFQVKKTFDKARFAGTWYAMAKKDPEGLFLQDNIV 255

Query 61 AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND 120

AEFSVDE G+MSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND

Sbjct 256 AEFSVDENGRMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND 435

Query 121 DHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQE 175

DHWI+DTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLP EAQKIVRQRQE

Sbjct 436 DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPLEAQKIVRQRQE 600

Q3: The sequence is :

>dog| GR899279.1|C. lupus familiaris liver protein(sequence taken from BLAST result)

MEWVWALELLAALGSARAESDCRVSNFQVKKTFDKARFAGTWYAMAKKDPEGLFLQDNIVAEFSVDENGRMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPLEAQKIVRQRQE

Name: Canis lupus familiaris Liver protein

Animalia, Chordata, Mammalia, Carnivora, Canidae, Canis, Lupus, familiaris

Q4:

**A BLAST-P search against NR database (see setup in first screen-shot below) yielded a**

**top hit result is to a protein from Canis lupus familiaris.**

**Since the percent identity is 98.9% less than 100%, it’s likely to be a novel protein.**

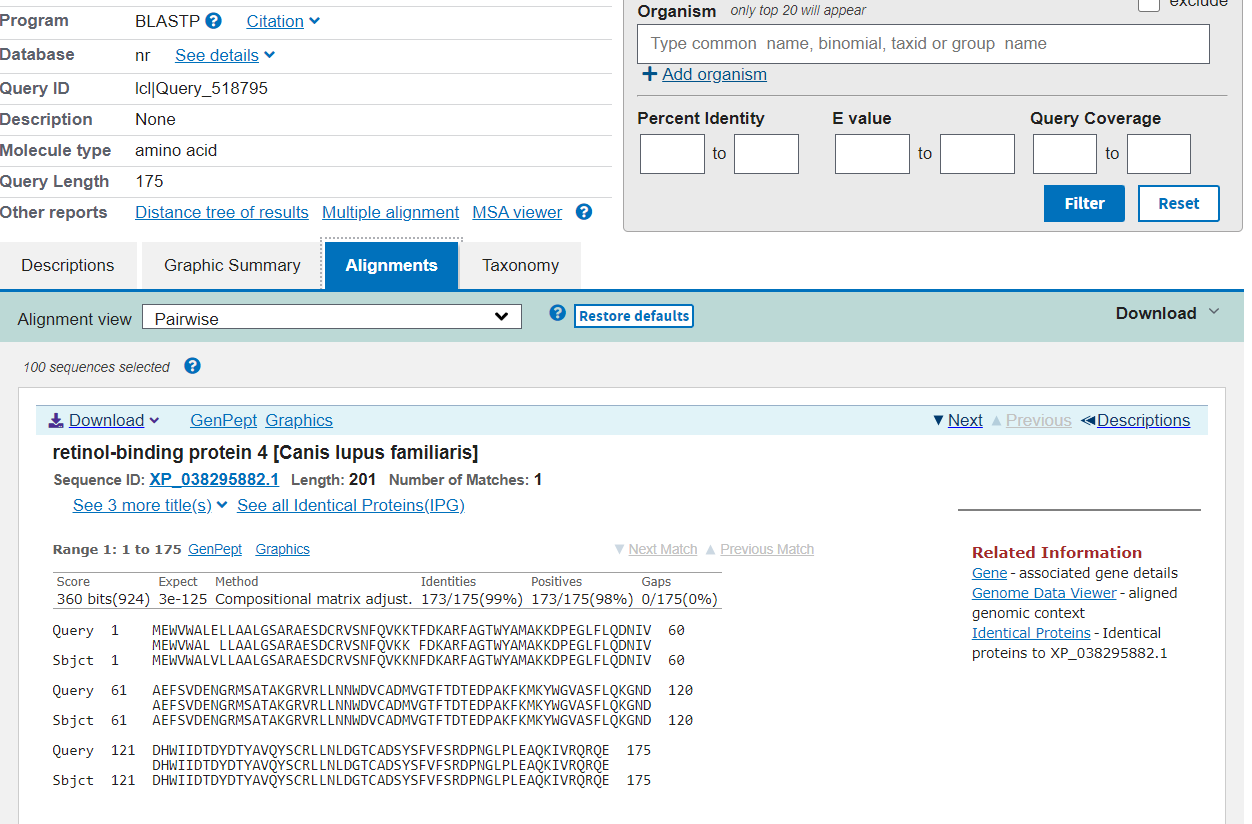
**Graphical user interface, text, application, email

Description automatically generated**

**Graphical user interface, text, application, email

Description automatically generated**

This protein seems to be a novel protein as it has 98.9% identity with the top hit.

The e-value is 3\*10^(-125)****

Q4’s Fasta result:

<https://www.ebi.ac.uk/Tools/services/web/toolresult.ebi?jobId=fasta-I20211104-184820-0908-48368064-p2m>

Q5.

The re-labeled sequences

>Human|NP\_006735.2|Homo sapiens|RBP4

MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQEELCLARQYRLIVHNGYCDGRSERNLL

>dog|GR899279.1|C. lupus familiaris liver protein

MEWVWALELLAALGSARAESDCRVSNFQVKKTFDKARFAGTWYAMAKKDPEGLFLQDNIVAEFSVDENGRMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPLEAQKIVRQRQE

>Lynx|XP\_030190934.1|Lynx canadensis|RBP4

MAWVWALVLLAALGSARAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDENGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDSAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFARDPNGLPLDVQKIVRQRQD

>Lesser Egyptian Jerboa|XP\_044986072.1|Jaculus jaculus|RBP4

MEWMWALVLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAIAKKDPEGLFLQDNIIAEFAVDENGHMSATAKGRVRLLSNWEVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYALQYSCRLLNLDGTCADSYSFVFSRDPNGLPPETRKLVRQRQE

>horses|XP\_023498127.1|Equus caballus|RBP4 isoform X1

MEWVWALVVLAALGSARAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDEYGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFARDPNGFPPEVQRIVRRRQE

>yak|QDZ58630.1|Bos grunniens|RBP4

MEWVWALVLLAAPGSAQAERDCRVSSFRVKENFDKARFAGTWYAMAKKDPEGLFLQDNIVAEFSVDENGHMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIIDTDYETFAVQYSCRLLNLGGTCADSYSFVFARDPSGFSPEVQKIVRQRQE

>panther|XP\_019300661.1|Panthera pardus|RBP4

MAWVWALVLLAALGSARAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDENGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDSAKFKMKYWGVASFLQKGNDDHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFARDPNGLPPDVQKIVRQRQD

Alignment: Obtained using MUSCLE (version 3.8) at EBI

EBI MUSCLE results:

Jerboa MEWMWALVLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAIAKKDPEGLFLQDNII

yak|QDZ58630.1|Bos MEWVWALVLLAAPGSAQAERDCRVSSFRVKENFDKARFAGTWYAMAKKDPEGLFLQDNIV

dog|GR899279.1|C. MEWVWALELLAALGSARAESDCRVSNFQVKKTFDKARFAGTWYAMAKKDPEGLFLQDNIV

Human|NP\_006735.2|Homo MKWVWALLLLAALGSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV

horses|XP\_023498127.1|Equus MEWVWALVVLAALGSARAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV

Lynx|XP\_030190934.1|Lynx MAWVWALVLLAALGSARAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV

panther|XP\_019300661.1|Panthera MAWVWALVLLAALGSARAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIV

\* \*:\*\*\* :\*\*\* \*\*..\*\* \*\*\*\*\*.\*.\*\*:.\*\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*\*\*\*\*\*\*\*\*\*:

Jerboa AEFAVDENGHMSATAKGRVRLLSNWEVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND

yak|QDZ58630.1|Bos AEFSVDENGHMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND

dog|GR899279.1|C. AEFSVDENGRMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND

Human|NP\_006735.2|Homo AEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND

horses|XP\_023498127.1|Equus AEFSVDEYGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGND

Lynx|XP\_030190934.1|Lynx AEFSVDENGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDSAKFKMKYWGVASFLQKGND

panther|XP\_019300661.1|Panthera AEFSVDENGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDSAKFKMKYWGVASFLQKGND

\*\*\*:\*\*\* \*.\*\*\*\*\*\*\*\*\*\*\*\*.\*\*:\*\*\*\*\*\*\*\*\*\*\*\*\*\*.\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Jerboa DHWIIDTDYDTYALQYSCRLLNLDGTCADSYSFVFSRDPNGLPPETRKLVRQRQE

yak|QDZ58630.1|Bos DHWIIDTDYETFAVQYSCRLLNLGGTCADSYSFVFARDPSGFSPEVQKIVRQRQE

dog|GR899279.1|C. DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPLEAQKIVRQRQE

Human|NP\_006735.2|Homo DHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQE

horses|XP\_023498127.1|Equus DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFARDPNGFPPEVQRIVRRRQE

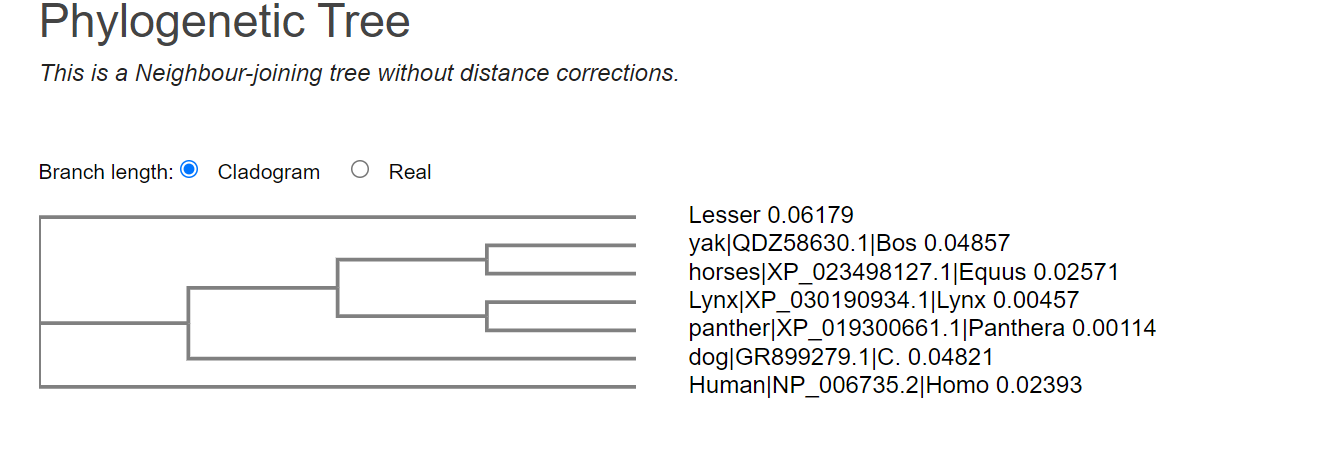
Lynx|XP\_030190934.1|Lynx DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFARDPNGLPLDVQKIVRQRQD

panther|XP\_019300661.1|Panthera DHWIIDTDYDTYAVQYSCRLLNLDGTCADSYSFVFARDPNGLPPDVQKIVRQRQD

\*\*\*\*:\*\*\*\*:\*:\*:\*\*\*\*\*\*\*\*\*.\*\*\*\*\*\*\*\*\*\*\*:\*\*\*.\*:. :...:\*\*.\*\*:

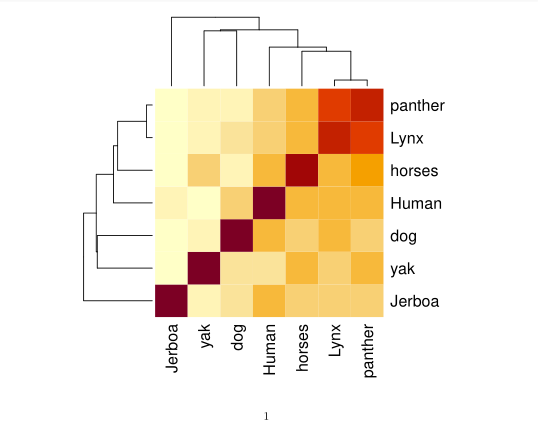
Q6

Used Simple-Phylogeny function of EBI



Q7

Using the heatmap function of R:

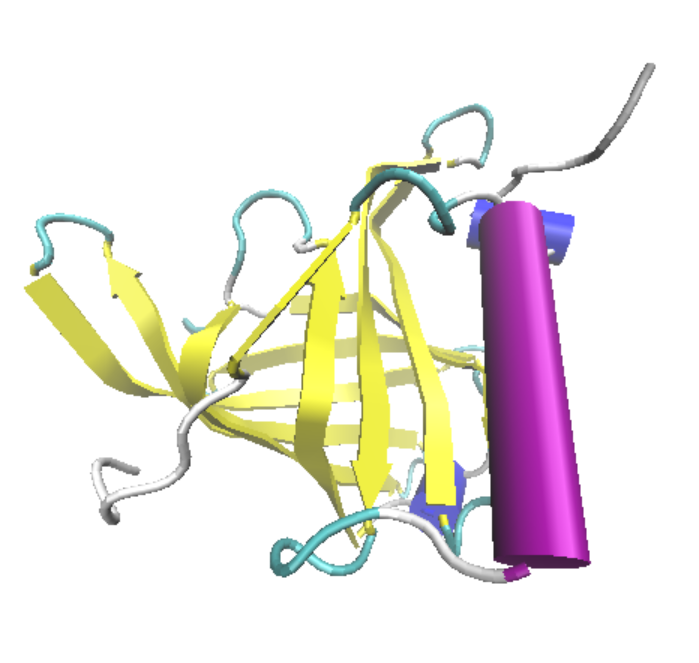


Q8.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ID | Methods | Sources | Resolution | e-value | Identity |
| 1ERB | X-RAY DIFFRACTION | Bos taurus | 1.9 | 8.30e-107 | 91.083 |
| 1IIU | X-RAY DIFFRACTION | Gallus gallus | 2.5 | 1.63e-101 | 85.714 |
| 6QBA | X-RAY  DIFFRACTION | Saccharolobus solfataricus | 1.8 | 3.46e-110 | 93.631 |

Q9

The human RBP4 structure is very similar to the structure of lupus familiaris liver protein because they have high sequence similarity (>90%).



Human RBP4

Q10

29 assays, including 16 functional assays and 12 binding assays, 1 ADME assay were found,

<https://www.ebi.ac.uk/chembl/g/#search_results/targets/query=RBP4>

One of the top binding techniques, CHEMBL4304746, is retinol-induced MBP-tagged RBP4, testing for the activity of the RBP4 protein, suggesting that RBP4 antagonists may also have therapeutic utility for the treatment of NAFLD(nonalcoholic fatty liver disease).

Cioffi CL, Racz B, Varadi A, Freeman EE, Conlon MP, Chen P, Zhu L, Kitchen DB, Barnes KD, Martin WH, Pearson PG, Johnson G, Blaner WS, Petrukhin K. Design, Synthesis, and Preclinical Efficacy of Novel Nonretinoid Antagonists of Retinol-Binding Protein 4 in the Mouse Model of Hepatic Steatosis. J Med Chem (2019) 62:5470-5500.

[10.1021/acs.jmedchem.9b00352](http://dx.doi.org/10.1021/acs.jmedchem.9b00352)