BIMM-143: INTRODUCTION TO BIOINFORMATICS

Professor Barry J. Grant

Find A Gene Final Project

Garrett Cole | A15988021
g1cole@ucsd.edu

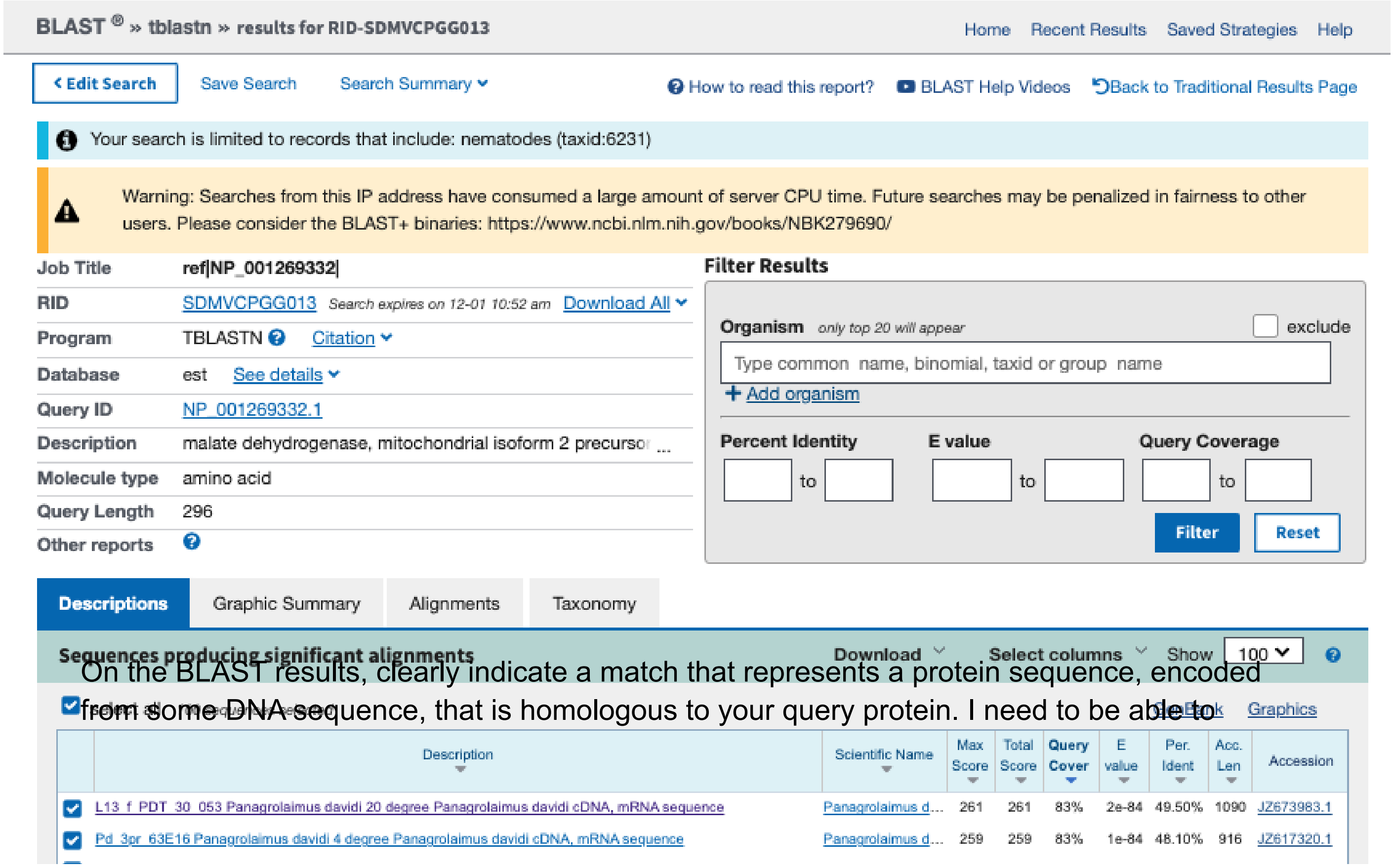
[Q1] Tell me the name of a protein you are interested in. Include the species and the accession number. This can be a human protein or a protein from any other species as long as it's function is known. If you do not have a favorite protein, select human RBP4 or KIF11. Do not use beta globin as this is in the worked example report that I provide you with online.

Name: Malate Dehydrogenase (mitochondrial isoform 2 precursor)
Accession: NP_001269332
Species: Homo Sapiens
Functions in Catalytic Activity using both NADP+ or NAD+ as cofactors to increase the catalyzation rate of interconversion among the acids oxaloacetate and malate

[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 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.



Your search is limited to records that include: nematodes (taxid:6231)

Warning: Searches from this IP address have consumed a large amount of server CPU time. Future searches may be penalized in fairness to other users. Please consider the BLAST+ binaries: https://www.ncbi.nlm.nih.gov/books/NBK279690/

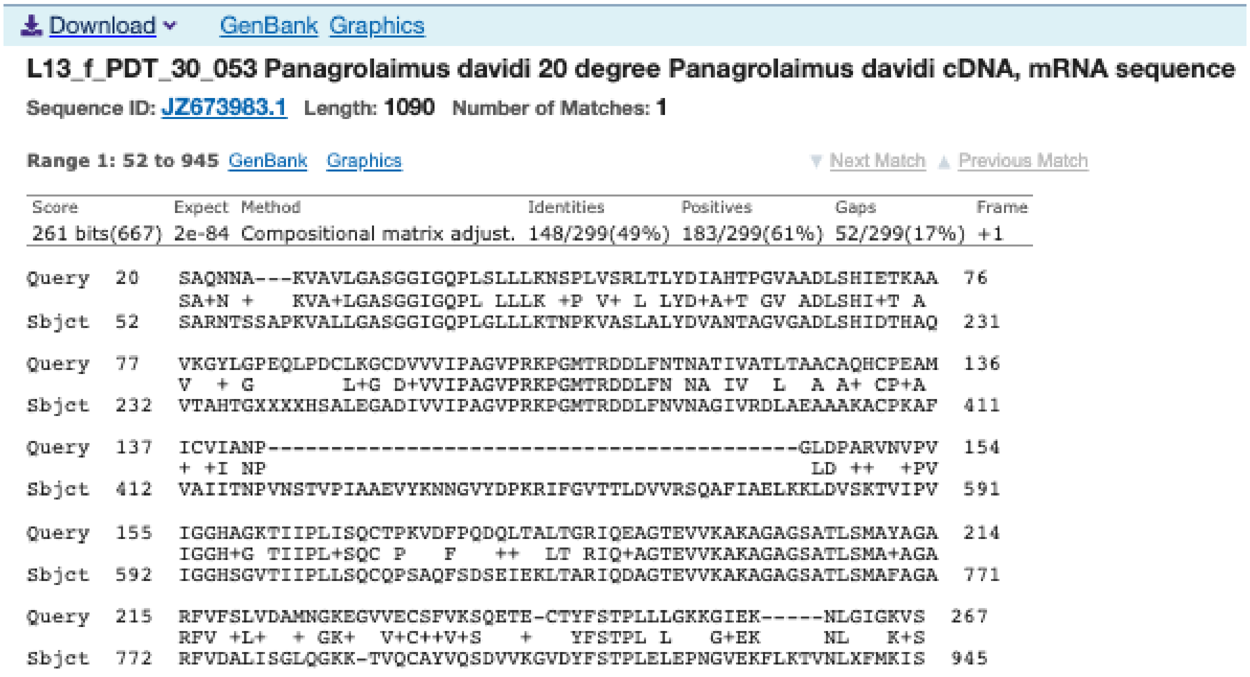
Job Title	ref|NP_001269332|
RID	SDMVCPGG013 *Search expires on 12-01 10:52 am* Download All ⌄
Program	TBLASTN Citation ⌄
Database	est See details ⌄
Query ID	NP_001269332.1
Description	malate dehydrogenase, mitochondrial isoform 2 precursor ...
Molecule type	amino acid
Query Length	296
Other reports	

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	L13_f_PDT_30_053 Panagrolaimus davidi 20 degree Panagrolaimus davidi cDNA, mRNA sequence	Panagrolaimus d...	261	261	83%	2e-84	49.50%	1090	JZ673983.1
☑	Pd_3pr_63E16 Panagrolaimus davidi 4 degree Panagrolaimus davidi cDNA, mRNA sequence	Panagrolaimus d...	259	259	83%	1e-84	48.10%	916	JZ617320.1

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 JZ673983.1, a L13_f_PDT_30_053, 1090 base pair 20 degree cDNA from *Panagrolaimus davidi*

Alignment details:



L13_f_PDT_30_053 Panagrolaimus davidi 20 degree Panagrolaimus davidi cDNA, mRNA sequence

Sequence ID: JZ673983.1 Length: 1090 Number of Matches: 1

Range 1: 52 to 945 GenBank Graphics

```
Score          Expect  Method                          Identities     Positives      Gaps          Frame
261 bits(667)  2e-84   Compositional matrix adjust.    148/299(49%)   183/299(61%)   52/299(17%)   +1

Query  20    SAQNNA---KVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETKAA  76
             SA+N +   KVA+LGASGGIGQPL LLLK +P V+ L LYD+A+T GV ADLSHI+T A
Sbjct  52    SARNTSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDTHAQ  231

Query  77    VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAM  136
             V  + G       L+G D+VVIPAGVPRKPGMTRDDLFN NA IV  L  A A+ CP+A
Sbjct  232   VTAHTGXXXXHSALEGADIVVIPAGVPRKPGMTRDDLFNVNAGIVRDLAEAAAKACPKAF  411

Query  137   ICVIANP------------------------------------------GLDPARVNVPV  154
             + +I NP                                           LD ++  +PV
Sbjct  412   VAIITNPVNSTVPIAAEVYKNNGVYDPKRIFGVTTLDVVRSQAFIAELKKLDVSKTVIPV  591

Query  155   IGGHAGKTIIPLISQCTPKVDFPQDQLTALTGRIQEAGTEVVKAKAGAGSATLSMAYAGA  214
             IGGH+G TIIPL+SQC P   F   ++  LT RIQ+AGTEVVKAKAGAGSATLSMA+AGA
Sbjct  592   IGGHSGVTIIPLLSQCQPSAQFSDSEIEKLTARIQDAGTEVVKAKAGAGSATLSMAFAGA  771

Query  215   RFVFSLVDAMNGKEGVVECSFVKSQETE-CTYFSTPLLLGKKGIEK-----NLGIGKVS  267
             RFV +L+  + GK+  V+C++V+S   +   YFSTPL L   G+EK     NL   K+S
Sbjct  772   RFVDALISGLQGKK-TVQCAYVQSDVVKGVDYFSTPLELEPNGVEKFLKTVNLXFMKIS  945
```

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:

```
>52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi 20 degree
Panagrolaimus davidi cDNA, mRNA sequence
SARNTSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDTHAQ
VTAHTGXXXXHSALEGADIVVIPAGVPRKPGMTRDDLFNVNAGIVRDLAEAAAKACPKAF
VAIITNPVNSTVPIAAEVYKNNGVYDPKRIFGVTTLDVVRSQAFIAELKKLDVSKTVIPV
IGGHSGVTIIPLLSQCQPSAQFSDSEIEKLTARIQDAGTEVVKAKAGAGSATLSMAFAGA
RFVDALISGLQGKKTVQCAYVQSDVVKGVDYFSTPLELEPNGVEKFLKTVNLXFMKIS
```

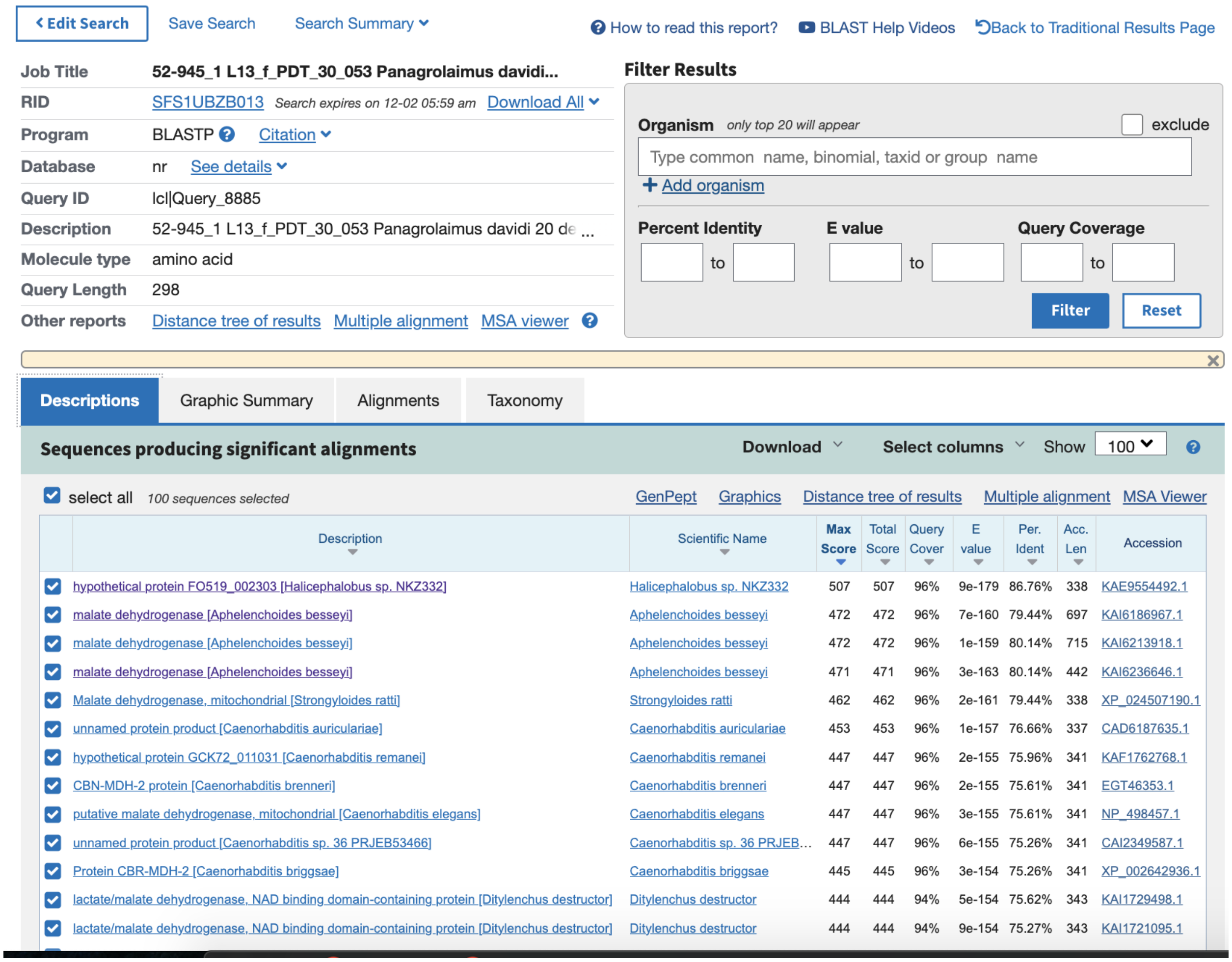
Here, tell me the name of the novel protein, and the species from which it derives. It is very unlikely (but still definitely possible) that you will find a novel gene from an organism such as S. cerevisiae, human or mouse, because those genomes have already been thoroughly annotated. It is more likely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

Name: Malate Dehydrogenase
Species: *Panagrolaimus*

[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 the NR database produced a top hit from the Halicephalobus (Panagrolaimidae) species. Output details below:



Job Title	52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi...
RID	SFS1UBZB013 *Search expires on 12-02 05:59 am* Download All ⌄
Program	BLASTP ❓ Citation ⌄
Database	nr See details ⌄
Query ID	lcl|Query_8885
Description	52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi 20 de ...
Molecule type	amino acid
Query Length	298
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

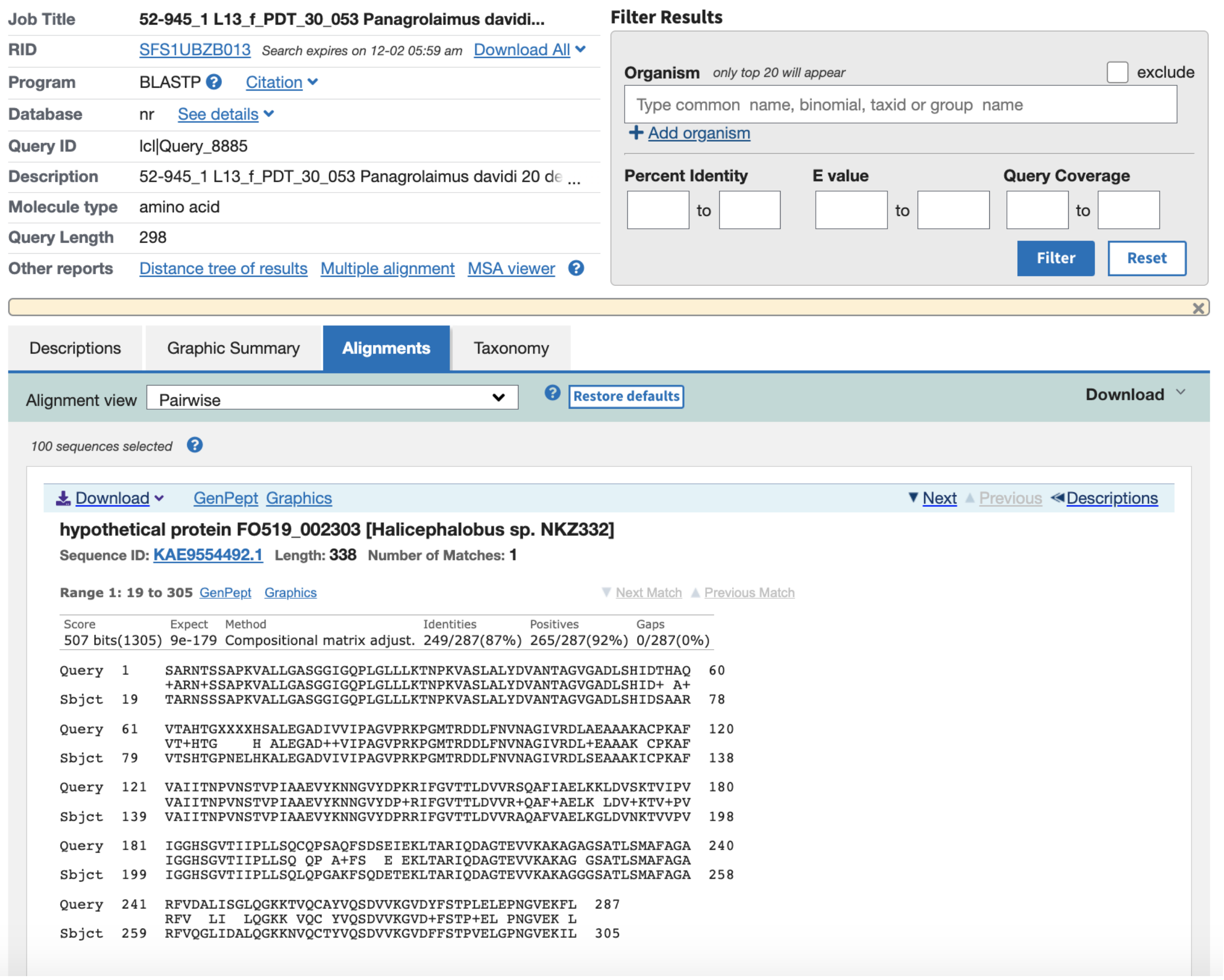
Percent Identity to **E value** to **Query Coverage** to

Sequences producing significant alignments

☑ select all *100 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
☑	hypothetical protein FO519_002303 [Halicephalobus sp. NKZ332]	Halicephalobus sp. NKZ332	507	507	96%	9e-179	86.76%	338	KAE9554492.1
☑	malate dehydrogenase [Aphelenchoides besseyi]	Aphelenchoides besseyi	472	472	96%	7e-160	79.44%	697	KAI6186967.1
☑	malate dehydrogenase [Aphelenchoides besseyi]	Aphelenchoides besseyi	472	472	96%	1e-159	80.14%	715	KAI6213918.1
☑	malate dehydrogenase [Aphelenchoides besseyi]	Aphelenchoides besseyi	471	471	96%	3e-163	80.14%	442	KAI6236646.1
☑	Malate dehydrogenase, mitochondrial [Strongyloides ratti]	Strongyloides ratti	462	462	96%	2e-161	79.44%	338	XP_024507190.1
☑	unnamed protein product [Caenorhabditis auriculariae]	Caenorhabditis auriculariae	453	453	96%	1e-157	76.66%	337	CAD6187635.1
☑	hypothetical protein GCK72_011031 [Caenorhabditis remanei]	Caenorhabditis remanei	447	447	96%	2e-155	75.96%	341	KAF1762768.1
☑	CBN-MDH-2 protein [Caenorhabditis brenneri]	Caenorhabditis brenneri	447	447	96%	2e-155	75.61%	341	EGT46353.1
☑	putative malate dehydrogenase, mitochondrial [Caenorhabditis elegans]	Caenorhabditis elegans	447	447	96%	3e-155	75.61%	341	NP_498457.1
☑	unnamed protein product [Caenorhabditis sp. 36 PRJEB53466]	Caenorhabditis sp. 36 PRJEB...	447	447	96%	6e-155	75.26%	341	CAI2349587.1
☑	Protein CBR-MDH-2 [Caenorhabditis briggsae]	Caenorhabditis briggsae	445	445	96%	3e-154	75.26%	341	XP_002642936.1
☑	lactate/malate dehydrogenase, NAD binding domain-containing protein [Ditylenchus destructor]	Ditylenchus destructor	444	444	94%	5e-154	75.62%	343	KAI1729498.1
☑	lactate/malate dehydrogenase, NAD binding domain-containing protein [Ditylenchus destructor]	Ditylenchus destructor	444	444	94%	9e-154	75.27%	343	KAI1721095.1



Job Title	52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi...
RID	SFS1UBZB013 *Search expires on 12-02 05:59 am* Download All
Program	BLASTP Citation
Database	nr See details
Query ID	lcl|Query_8885
Description	52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi 20 de ...
Molecule type	amino acid
Query Length	298
Other reports	Distance tree of results Multiple alignment MSA viewer

100 sequences selected

hypothetical protein FO519_002303 [Halicephalobus sp. NKZ332]

Sequence ID: KAE9554492.1 **Length:** 338 **Number of Matches:** 1

Range 1: 19 to 305 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
507 bits(1305)	9e-179	Compositional matrix adjust.	249/287(87%)	265/287(92%)	0/287(0%)

```
Query  1    SARNTSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDTHAQ  60
            +ARN+SSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHID+ A+
Sbjct  19   TARNSSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDSAAR  78

Query  61   VTAHTGXXXXHSALEGADIVVIPAGVPRKPGMTRDDLFNVNAGIVRDLAEAAAKACPKAF  120
            VT+HTG    H ALEGAD++VIPAGVPRKPGMTRDDLFNVNAGIVRDL+EAAAK CPKAF
Sbjct  79   VTSHTGPNELHKALEGADVIVIPAGVPRKPGMTRDDLFNVNAGIVRDLSEAAAKICPKAF  138

Query  121  VAIITNPVNSTVPIAAEVYKNNGVYDPKRIFGVTTLDVVRSQAFIAELKKLDVSKTVIPV  180
            VAIITNPVNSTVPIAAEVYKNNGVYDP+RIFGVTTLDVVR+QAF+AELK LDV+KTV+PV
Sbjct  139  VAIITNPVNSTVPIAAEVYKNNGVYDPRRIFGVTTLDVVRAQAFVAELKGLDVNKTVVPV  198

Query  181  IGGHSGVTIIPLLSQCQPSAQFSDSEIEKLTARIQDAGTEVVKAKAGAGSATLSMAFAGA  240
            IGGHSGVTIIPLLSQ QP A+FS  E EKLTARIQDAGTEVVKAKAG GSATLSMAFAGA
Sbjct  199  IGGHSGVTIIPLLSQLQPGAKFSQDETEKLTARIQDAGTEVVKAKAGGGSATLSMAFAGA  258

Query  241  RFVDALISGLQGKKTVQCAYVQSDVVKGVDYFSTPLELEPNGVEKFL  287
            RFV  LI  LQGKK VQC YVQSDVVKGVD+FSTP+EL PNGVEK L
Sbjct  259  RFVQGLIDALQGKKNVQCTYVQSDVVKGVDFFSTPVELGPNGVEKIL  305
```

[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.

Labeled Sequences for Alignment:

```
Homo Sapiens (Humans):
> Human_MDH2 |NP_001269332.1| malate dehydrogenase, mitochondrial isoform
2 precursor [Homo sapiens]
SAQNNA---KVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETKAAVKGYLGPEQLPDCL
KGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAMICVIANP---------------------
---------------------GLDPARVNVPVIGGHAGKTIIPLISQCTPKVDFPQDQLTALTGRIQEAGTEVV
KAKAGAGSATLSMAYAGARFVFSLVDAMNGKEGVVECSFVKSQETE-CTYFSTPLLLGKKGIEK-----NLGIG
KVS

Panagrolaimus davidi (Antarctic Nematode):
> Antartic_MDH2 | 52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi 20 degree
Panagrolaimus davidi cDNA, mRNA sequence
SARNTSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDTHAQVTAHTGXXXXHSAL
EGADIVVIPAGVPRKPGMTRDDLFNVNAGIVRDLAEAAAKACPKAFVAIITNPVNSTVPIAAEVYKNNGVYDPK
RIFGVTTLDVVRSQAFIAELKKLDVSKTVIPVIGGHSGVTIIPLLSQCQPSAQFSDSEIEKLTARIQDAGTEVV
KAKAGAGSATLSMAFAGARFVDALISGLQGKKTVQCAYVQSDVVKGVDYFSTPLELEPNGVEKFLKTVNLXFMK
IS

Halicephalobus (Panagrolaimidae):
> Halicephalobus_MDH2 | KAE9554492.1:19-305 hypothetical protein
FO519_002303 [Halicephalobus sp. NKZ332]
TARNSSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDSAARVTSHTGPNELHKAL
EGADVIVIPAGVPRKPGMTRDDLFNVNAGIVRDLSEAAAKICPKAFVAIITNPVNSTVPIAAEVYKNNGVYDPR
RIFGVTTLDVVRAQAFVAELKGLDVNKTVVPVIGGHSGVTIIPLLSQLQPGAKFSQDETEKLTARIQDAGTEVV
KAKAGGGSATLSMAFAGARFVQGLIDALQGKKNVQCTYVQSDVVKGVDFFSTPVELGPNGVEKIL

Camelus Ferus (Camel):
> Camel_MDH2 | 165-1130_1 PREDICTED: Camelus ferus malate dehydrogenase 2
(MDH2), transcript variant X1, mRNA
FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRATVKGYLGPEQLPDCL
KGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAMICIISNPVNSTIPITAEVFKKHGVYNPD
KIFGVTTLDIVRANTFVAELKGLDPARVNVPVIGGHAGKTIIPVISQCTPKVDFPQDQLTTLTGRIQEAGTEVV
KAKAGAGSATLSMAYAGARFVFSLLDAMNGKEGVVECSFVKSQETDCPYFSTPLLLGKKGIEKNLGIGKISPFE
EKMIAEAIPELKASIKKGEEFVKSMK

Apodemus Sylvaticus (Wood Mouse):
> Mouse_MDH2 | 167-1132_1 PREDICTED: Apodemus sylvaticus malate
dehydrogenase 2 (LOC127672705), mRNA
FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRANVKGYLGPEQLPDCL
KGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAMICIIANPVNSTIPITAEVFKKHGVYNPN
KIFGVTTLDIVRANTFVAELKGLDPARVNVPVIGGHAGKTIIPLISQCTPKVDFPQDQLATLTGRIQEAGTEVV
KAKAGAGSATLSMAYAGARFVFSLVDAMNGKEGVVECSFVQSKETECTYFSTPLLLGKKGLEKNLGIGKITPFE
EKMIAEAIPELKASIKKGEDFVKNMK
```

Puma concolor (Cougar):

```
> Cougar_MDH2 | 138-977_1 PREDICTED: Puma concolor malate dehydrogenase 2
(MDH2), transcript variant X2, mRNA
FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAAVKGYLGPEQLPDCL
KGCDVVVIPAGVPRKPGMTRDDLFNTNASIVATLTAACAQHCPEAMICIISNPGLDPARVNVPVIGGHAGKTII
PLISQCTPKVDLPQDQLTAVTGRIQEAGTEVVKAKAGAGSATLSMAYAGARFVFSLVDAINGKEGVVECSFVKS
QETDCPYFSTPLLLGKKGIEKNLGIGKISPFEEKMIAEALPELKASIKKGEEFVKNMK
```

Erinaceus Eeuropaeus (European Hedgehog):

```
> HedgeHog_MDH2 | 172-1011_1 PREDICTED: Erinaceus europaeus malate
dehydrogenase 2 (MDH2), transcript variant X2, mRNA
FSTSTQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRANVKGYLGPEQLPDCL
KGCDVVVVPAGVPRKPGMTRDDLFNTNATIVATLAAACAQHCPEAMICIIANPGLDPARVNVPVIGGHAGKTII
PLISQCTPKVDLPQDKLTALTGRIQEAGTEVVQAKAGAGSATLSMAYAGARFVFSLVDAMNGKEGVVECSFVKS
QETDCTYFSTPLLLGRKGLEKNLGIGKVTPFEEKMISEAIPELKASIKKGEEFVKNMK
```

Lipotes vexillifer (Yangtze River Dolphin):

```
> Dolphin_MDH2 | 49-888_1 PREDICTED: Lipotes vexillifer malate
dehydrogenase 2, NAD (mitochondrial) (MDH2), transcript variant X2, mRNA
FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRATVKGYLGPEQLPDCL
KGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAMICIISNPGLDPARVSVPVIGGHAGKTII
PLASQCTPKVDFPQDQLTTLIGRIQEAGTEVVKAKAGAGSATLSMAYAGARFVFSLVDAMNGKEGVVECSFVKS
QETDCPFFSTPLLLGKKGIEKNLGIGKISPFEEKMIAEAIPELKASIKKGEEFVKNMK
```

Myotis lucifugus (Little Brown Bat):

```
> Bat_MDH2 | 171-1136_1 PREDICTED: Myotis lucifugus malate dehydrogenase 2
(MDH2), transcript variant X1, mRNA
FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRASVKGYLGPEQLPDCL
KGCDLVVIPAGVPRKPGMTRDDLFNTNATIVANLTAACAQNCPEAMICVIANPVNSTIPITSEVFKKHGVYNPN
KIFGVTTLDVVRANAFVAELKGLDPARVNVPVIGGHAGKTIIPLISQCTPKVEFPQDQLTTLTGRIQEAGTEVV
KAKAGAGSATLSMAYAGARFVFSLLDAINGKEGVVECSFVKSQETDCSYFSTPLLLGKKGIEKNLGIGKISSFE
EKMIAEAIPELKASIKKGEDFVKNMK
```

Carlito syrichta (Philippine tarsier):

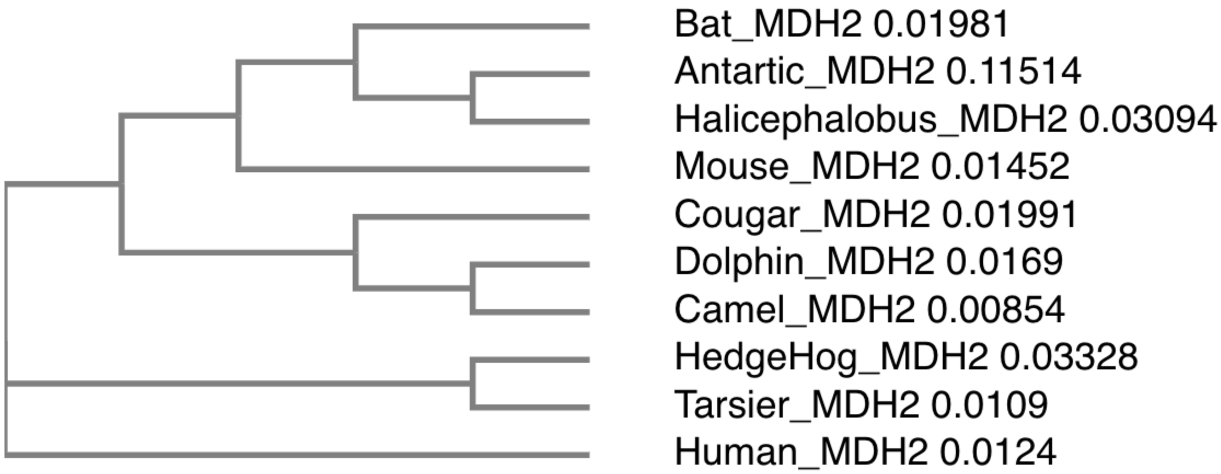
```
> Tarsier_MDH2 | 194-1159_1 PREDICTED: Carlito syrichta malate
dehydrogenase 2 (MDH2), transcript variant X1, mRNA
FGTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRATVKGYLGPEQLPDCL
KGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLAAACAQHCPEAMICIIANPVNSTIPITAEVFKKHGVYNPN
KVFGVTTLDIVRANTFVAELKGLDPARVNVPVIGGHAGKTIIPLISQCTPKVDFPQDQLTALTGRIQEAGTEVV
KAKAGAGSATLSMAYAGARFVFSLVDAMNGKEGVVECSFVKSQETDCTYFSTPLLLGKKGLEKNLGIGKVSSFE
EKMITEAMPELKASIKKGEEFVKNMK
```

Alignment (Obtained using MUSCLE via EBI):

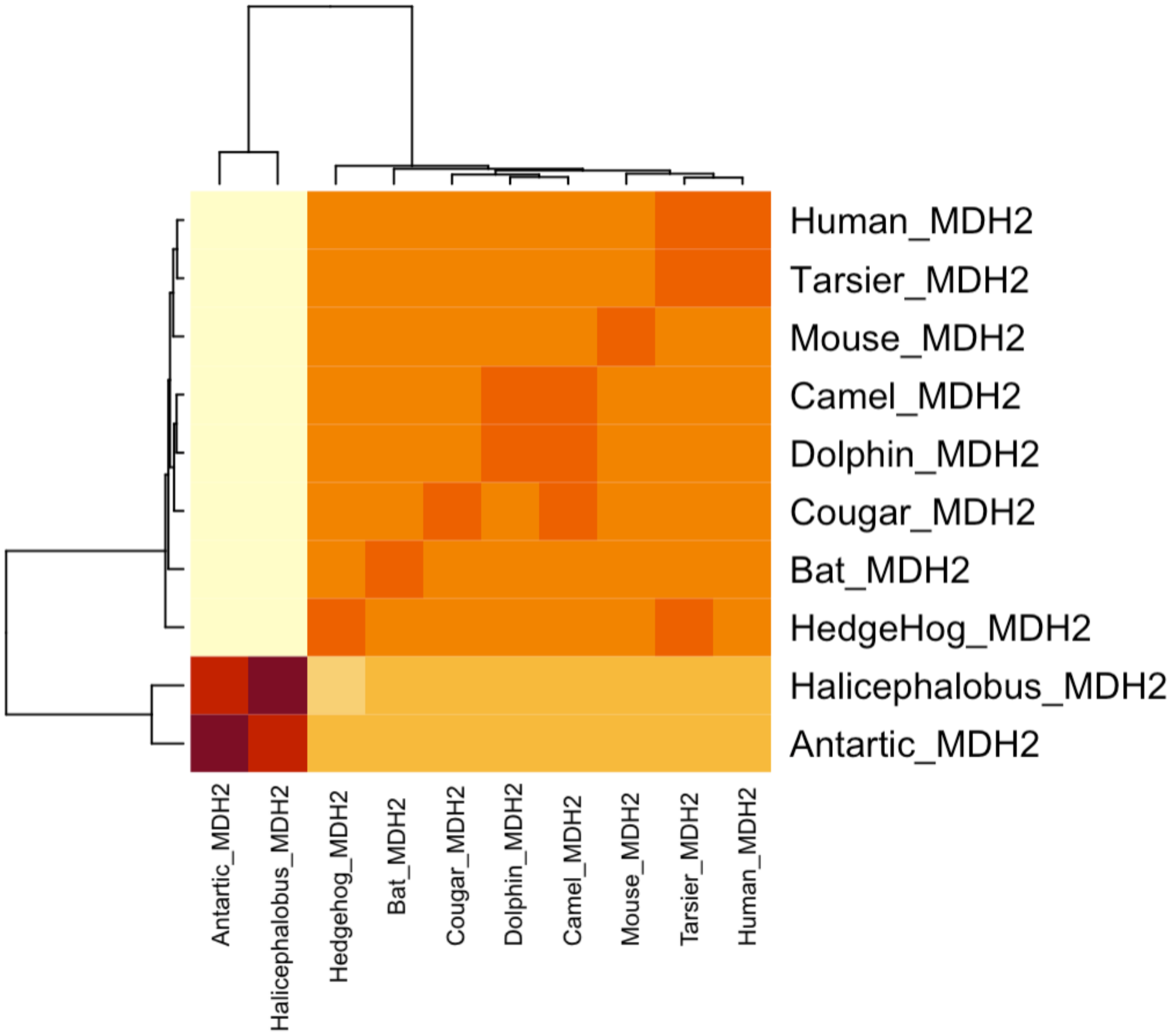
```
CLUSTAL multiple sequence alignment by MUSCLE (3.8)

Bat_MDH2                 FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAS
HedgeHog_MDH2            FSTSTQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAN
Cougar_MDH2              FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAA
Dolphin_MDH2             FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAT
Camel_MDH2               FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAT
Mouse_MDH2               FSTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAN
Human_MDH2               ---SAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETKAA
Tarsier_MDH2             FGTSAQNNAKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETRAT
Antartic_MDH2            SARNTSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDTHAQ
Halicephalobus_MDH2      TARNSSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDSAAR
                            .:.. .***:***********.****..* *: *:***:*:*.**.******:: *

Bat_MDH2                 VKGYLGPEQLPDCLKGCDLVVIPAGVPRKPGMTRDDLFNTNATIVANLTAACAQNCPEAM
HedgeHog_MDH2            VKGYLGPEQLPDCLKGCDVVVVPAGVPRKPGMTRDDLFNTNATIVATLAAACAQHCPEAM
Cougar_MDH2              VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNASIVATLTAACAQHCPEAM
Dolphin_MDH2             VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAM
Camel_MDH2               VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAM
Mouse_MDH2               VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAM
Human_MDH2               VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLTAACAQHCPEAM
Tarsier_MDH2             VKGYLGPEQLPDCLKGCDVVVIPAGVPRKPGMTRDDLFNTNATIVATLAAACAQHCPEAM
Antartic_MDH2            VTAHTGXXXXHSALEGADIVVIPAGVPRKPGMTRDDLFNVNAGIVRDLAEAAAKACPKAF
Halicephalobus_MDH2      VTSHTGPNELHKALEGADVIVIPAGVPRKPGMTRDDLFNVNAGIVRDLSEAAAKICPKAF
                         *..: *     ..*:*.*::*:*****************.** **  *: *.*: **:*:

Bat_MDH2                 ICVIANPVNSTIPITSEVFKKHGVYNPNKIFGVTTLDVVRANAFVAELKGLDPARVNVPV
HedgeHog_MDH2            ICIIANP-------------------------------------------GLDPARVNVPV
Cougar_MDH2              ICIISNP-------------------------------------------GLDPARVNVPV
Dolphin_MDH2             ICIISNP-------------------------------------------GLDPARVSVPV
Camel_MDH2               ICIISNPVNSTIPITAEVFKKHGVYNPDKIFGVTTLDIVRANTFVAELKGLDPARVNVPV
Mouse_MDH2               ICIIANPVNSTIPITAEVFKKHGVYNPNKIFGVTTLDIVRANTFVAELKGLDPARVNVPV
Human_MDH2               ICVIANP-------------------------------------------GLDPARVNVPV
Tarsier_MDH2             ICIIANPVNSTIPITAEVFKKHGVYNPNKVFGVTTLDIVRANTFVAELKGLDPARVNVPV
Antartic_MDH2            VAIITNPVNSTVPIAAEVYKNNGVYDPKRIFGVTTLDVVRSQAFIAELKKLDVSKTVIPV
Halicephalobus_MDH2      VAIITNPVNSTVPIAAEVYKNNGVYDPRRIFGVTTLDVVRAQAFVAELKGLDVNKTVVPV
                         :.:*:**                                           **  .. :**

Bat_MDH2                 IGGHAGKTIIPLISQCTPKVEFPQDQLTTLTGRIQEAGTEVVKAKAGAGSATLSMAYAGA
HedgeHog_MDH2            IGGHAGKTIIPLISQCTPKVDLPQDKLTALTGRIQEAGTEVVQAKAGAGSATLSMAYAGA
Cougar_MDH2              IGGHAGKTIIPLISQCTPKVDLPQDQLTAVTGRIQEAGTEVVKAKAGAGSATLSMAYAGA
Dolphin_MDH2             IGGHAGKTIIPLASQCTPKVDFPQDQLTTLIGRIQEAGTEVVKAKAGAGSATLSMAYAGA
Camel_MDH2               IGGHAGKTIIPVISQCTPKVDFPQDQLTTLTGRIQEAGTEVVKAKAGAGSATLSMAYAGA
Mouse_MDH2               IGGHAGKTIIPLISQCTPKVDFPQDQLATLTGRIQEAGTEVVKAKAGAGSATLSMAYAGA
Human_MDH2               IGGHAGKTIIPLISQCTPKVDFPQDQLTALTGRIQEAGTEVVKAKAGAGSATLSMAYAGA
Tarsier_MDH2             IGGHAGKTIIPLISQCTPKVDFPQDQLTALTGRIQEAGTEVVKAKAGAGSATLSMAYAGA
Antartic_MDH2            IGGHSGVTIIPLLSQCQPSAQFSDSEIEKLTARIQDAGTEVVKAKAGAGSATLSMAFAGA
Halicephalobus_MDH2      IGGHSGVTIIPLLSQLQPGAKFSQDETEKLTARIQDAGTEVVKAKAGGGSATLSMAFAGA
                         ****:* ****: **  * ..:.:.:   : .***:******:****.********:***
```

```
Bat_MDH2                 RFVFSLLDAINGKEGVVECSFVKSQETDCS-YFSTPLLLGKKGIEKNLGIGKISSFEEKM
HedgeHog_MDH2            RFVFSLVDAMNGKEGVVECSFVKSQETDCT-YFSTPLLLGRKGLEKNLGIGKVTPFEEKM
Cougar_MDH2              RFVFSLVDAINGKEGVVECSFVKSQETDCP-YFSTPLLLGKKGIEKNLGIGKISPFEEKM
Dolphin_MDH2             RFVFSLVDAMNGKEGVVECSFVKSQETDCP-FFSTPLLLGKKGIEKNLGIGKISPFEEKM
Camel_MDH2               RFVFSLLDAMNGKEGVVECSFVKSQETDCP-YFSTPLLLGKKGIEKNLGIGKISPFEEKM
Mouse_MDH2               RFVFSLVDAMNGKEGVVECSFVQSKETECT-YFSTPLLLGKKGLEKNLGIGKITPFEEKM
Human_MDH2               RFVFSLVDAMNGKEGVVECSFVKSQETECT-YFSTPLLLGKKGIEKNLGIGKVS------
Tarsier_MDH2             RFVFSLVDAMNGKEGVVECSFVKSQETDCT-YFSTPLLLGKKGLEKNLGIGKVSSFEEKM
Antartic_MDH2            RFVDALISGLQGKKT-VQCAYVQSDVVKGVDYFSTPLELEPNGVEKFLKTVNLXFMKIS-
Halicephalobus_MDH2      RFVQGLIDALQGKKN-VQCTYVQSDVVKGVDFFSTPVELGPNGVEKIL------------
                         *** .*:..::**:  *:*::*:*. ..   :****: *  :*:** *

Bat_MDH2                 IAEAIPELKASIKKGEDFVKNMK
HedgeHog_MDH2            ISEAIPELKASIKKGEEFVKNMK
Cougar_MDH2              IAEALPELKASIKKGEEFVKNMK
Dolphin_MDH2             IAEAIPELKASIKKGEEFVKNMK
Camel_MDH2               IAEAIPELKASIKKGEEFVKSMK
Mouse_MDH2               IAEAIPELKASIKKGEDFVKNMK
Human_MDH2               -----------------------
Tarsier_MDH2             ITEAMPELKASIKKGEEFVKNMK
Antartic_MDH2            -----------------------
Halicephalobus_MDH2      -----------------------
```

[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.



[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).

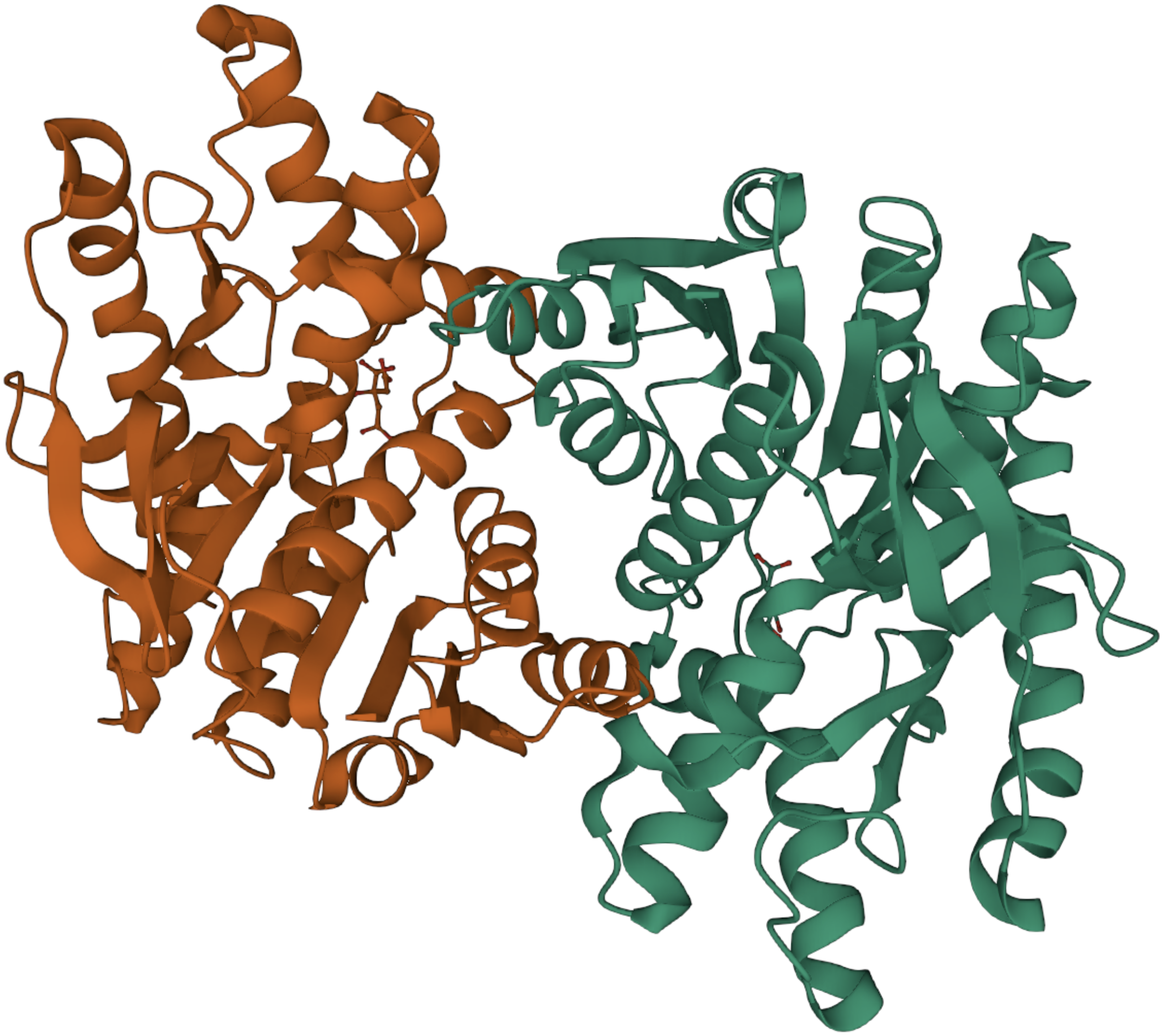
HINT: You can use a single sequence from your alignment or generate a consensus sequence from your alignment using the Bio3D function consensus(). The Bio3D functions blast.pdb(), plot.blast() and pdb.annotate() are likely to be of most relevance for completing this task. Note that the results of blast.pdb() 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 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	Evalue	Identity
1MLD	X-RAY DIFFRACTION	1.83 Å	Sus scrofa (Wild Boar)	0	63%
4E0B	X-RAY DIFFRACTION	2.17 Å	Vibrio vulnificus (Bacteria)	0	60%
1SMK	X-RAY DIFFRACTION	2.50 Å	Citrullus lanatus (Watermelon)	0	56%

[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?

Based on the sequence similarity, I believe the structure to be somewhat similar to my "novel" protein since the sequence similarity is greater than 60%.



[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?

```
The "Target" search via ChEMBEL outputted 2 Functional Assay
(CHEMBL614281, CHEMBL2366649)and 5 Binding Assay (CHEMBL2095180,
CHEMBL2189156, CHEMBL2242736, CHEMBL2326, CHEMBL2216)

Only three Binding Assay had ligand efficiency data, which is
reported as follows:
CHEMBL2095180 | BEI: 27 | SEI: 6.57
CHEMBL2326 | BEI: 27.69 | SEI: 9.20
CHEMBL2216 | BEI: 35.08 | SEI: 6.78
```

Scoring Rubric:
[45 total points available]

Q1 (4 points)

Protein name	1	1
Species	1	1
Accession number	1	1
Function known	0.5	1

Q2 (6 points)

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

Q3 (3 points)

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

Q4 (3 point)

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

Q5 (3 points)

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

Q6 (1 point)

Figure illustrates sequence clustering pattern	1	1

Q7 (10 points)

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

Q8 (10 points)

PDB identifiers from multiple species reported	5	5
Annotation of PDB source, resolution and technique	5	4

Annotation of Evalue and Sequence Identity	1		1		
Q9 (4 points)					
Structure figure provided	2		2		
Uses white background for molecular figure	1		1		
Figure of high resolution (i.e. not just snapshot)	1		1		
Q10 (1 point)					
Evidence of ChEMBEL searches	1		1		
Final Score:	**44.5**	**/**	**45**	**=**	**98.8%**