BIMM-143: INTRODUCTION TO BIOINFORMATICS

Professor Barry J. Grant

Find A Gene Final Project

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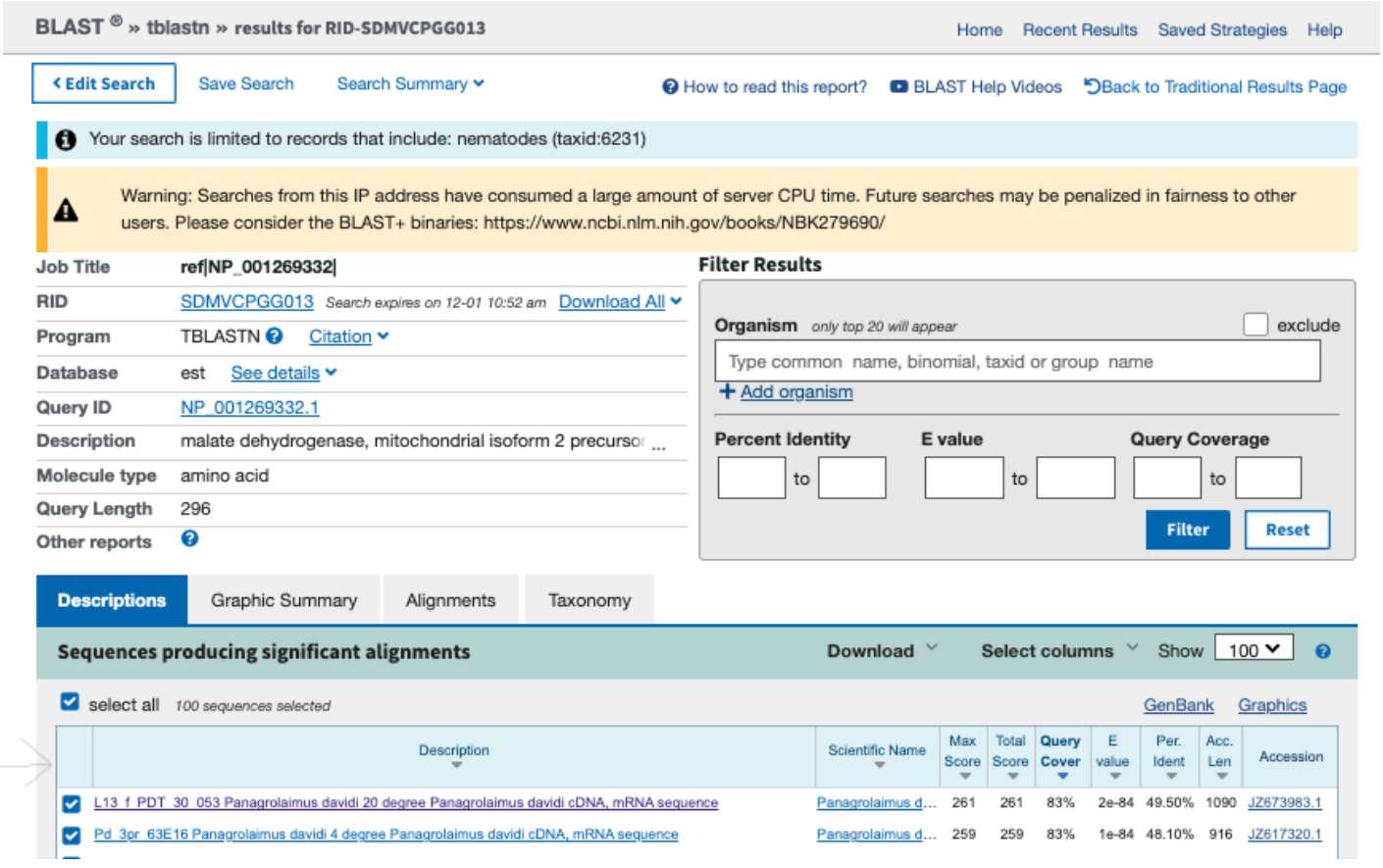
[Q1] Tell me the name of a protein you are interested in. Include the species and the accession number. This can be a human protein or a protein from any other species as long as it's function is known.

```
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. 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.



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

Alignment details:

Download GenBank Graphics

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 Next Match Previous Match

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
```

[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:

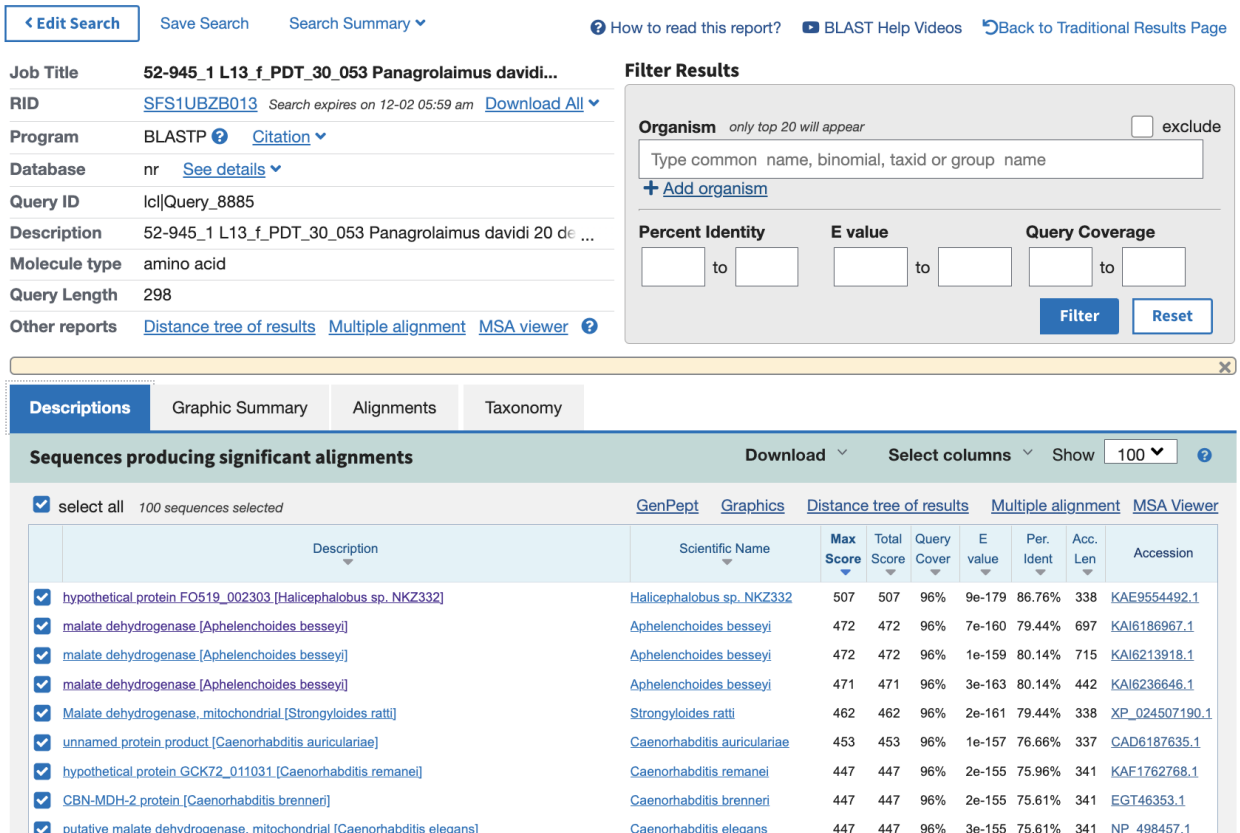
```
>52-945_1 L13_f_PDT_30_053 Panagrolaimus davidi 20 degree
Panagrolaimus davidi cDNA, mRNA sequence
SARNTSSAPKVALLGASGGIGQPLGLLLKTNPKVASLALYDVANTAGVGADLSHIDTHAQ
VTAHTGXXXXHSALEGADIVVIPAGVPRKPGMTRDDLFNVNAGIVRDLAEAAAKACPKAF
VAIITNPVNSTVPIAAEVYKNNGVYDPKRIFGVTTLDVVRSQAFIAELKKLDVSKTVIPV
IGGHSGVTIIPLLSQCQPSAQFSDSEIEKLTARIQDAGTEVVKAKAGAGSATLSMAFAGA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

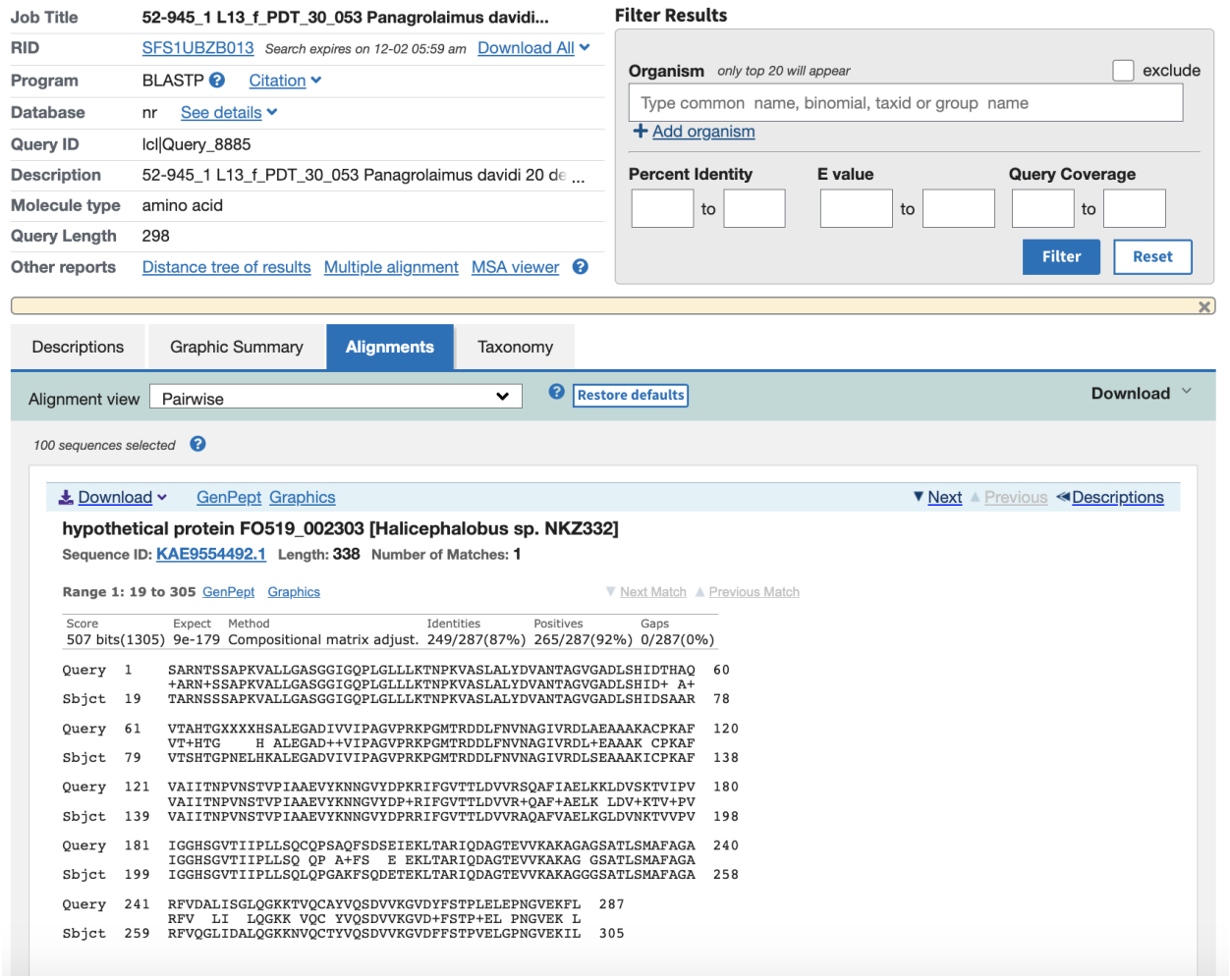
Here, tell me the name of the novel protein, and the species from which it derives. It is very unlikely (but still definitely possible) that you will find a novel gene from an organism such as S. cerevisiae, human or mouse, because those genomes have already been thoroughly annotated. It is more likely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





[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
                        ****:* ****: **  * ..:.:.:   : .***:******:****.********:***
```

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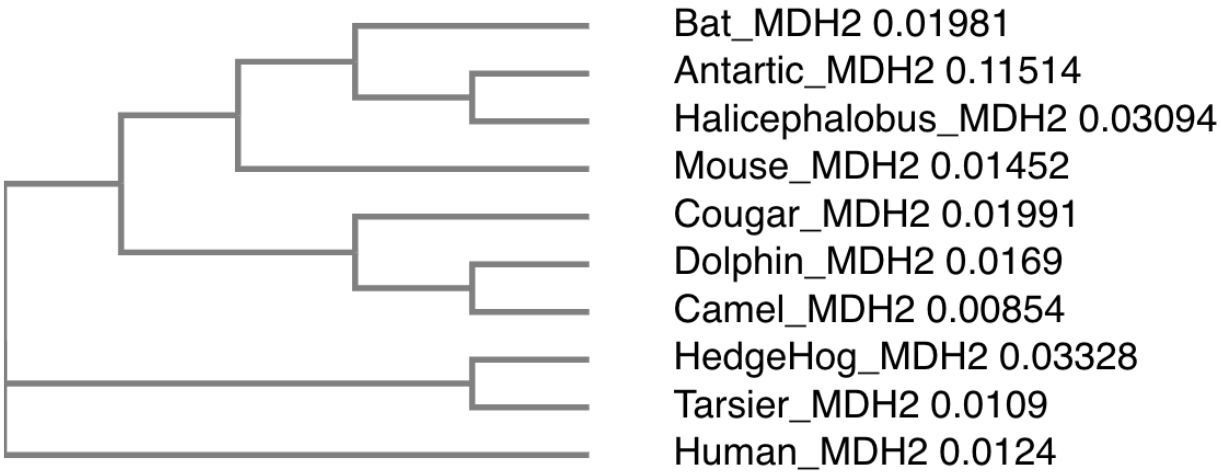
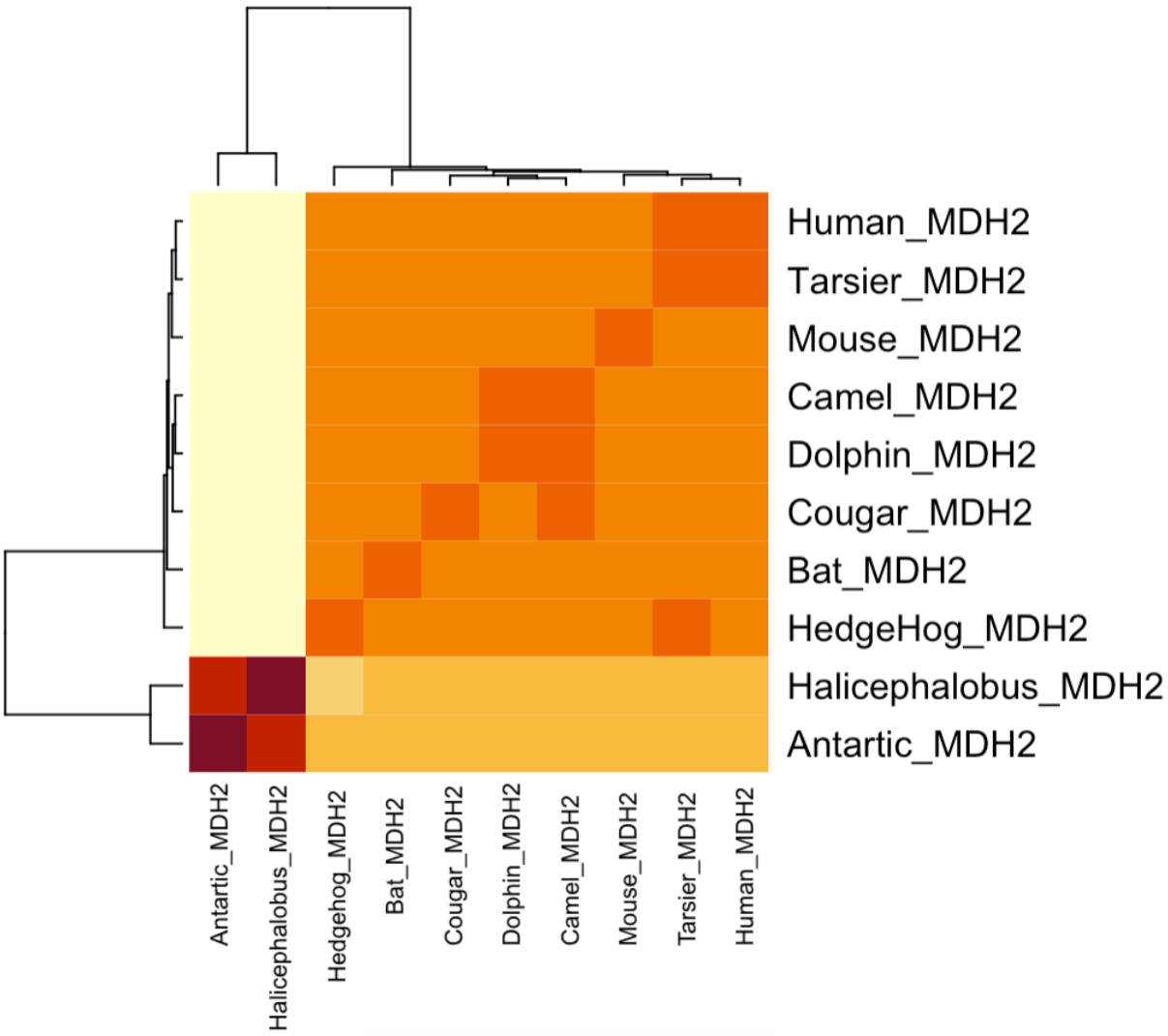
Bat_MDH2                RFVFSLLDAINGKEGVVECSFVKSQETDCS-YFSTPLLLGKKGIEKNLGIGKISSFEEKM
HedgeHog_MDH2           RFVFSLVDAMNGKEGVVECSFVKSQETDCT-YFSTPLLLGRKGLEKNLGIGKVTPFEEKM
Cougar_MDH2             RFVFSLVDAINGKEGVVECSFVKSQETDCP-YFSTPLLLGKKGIEKNLGIGKISPFEEKM
Dolphin_MDH2            RFVFSLVDAMNGKEGVVECSFVKSQETDCP-FFSTPLLLGKKGIEKNLGIGKISPFEEKM
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

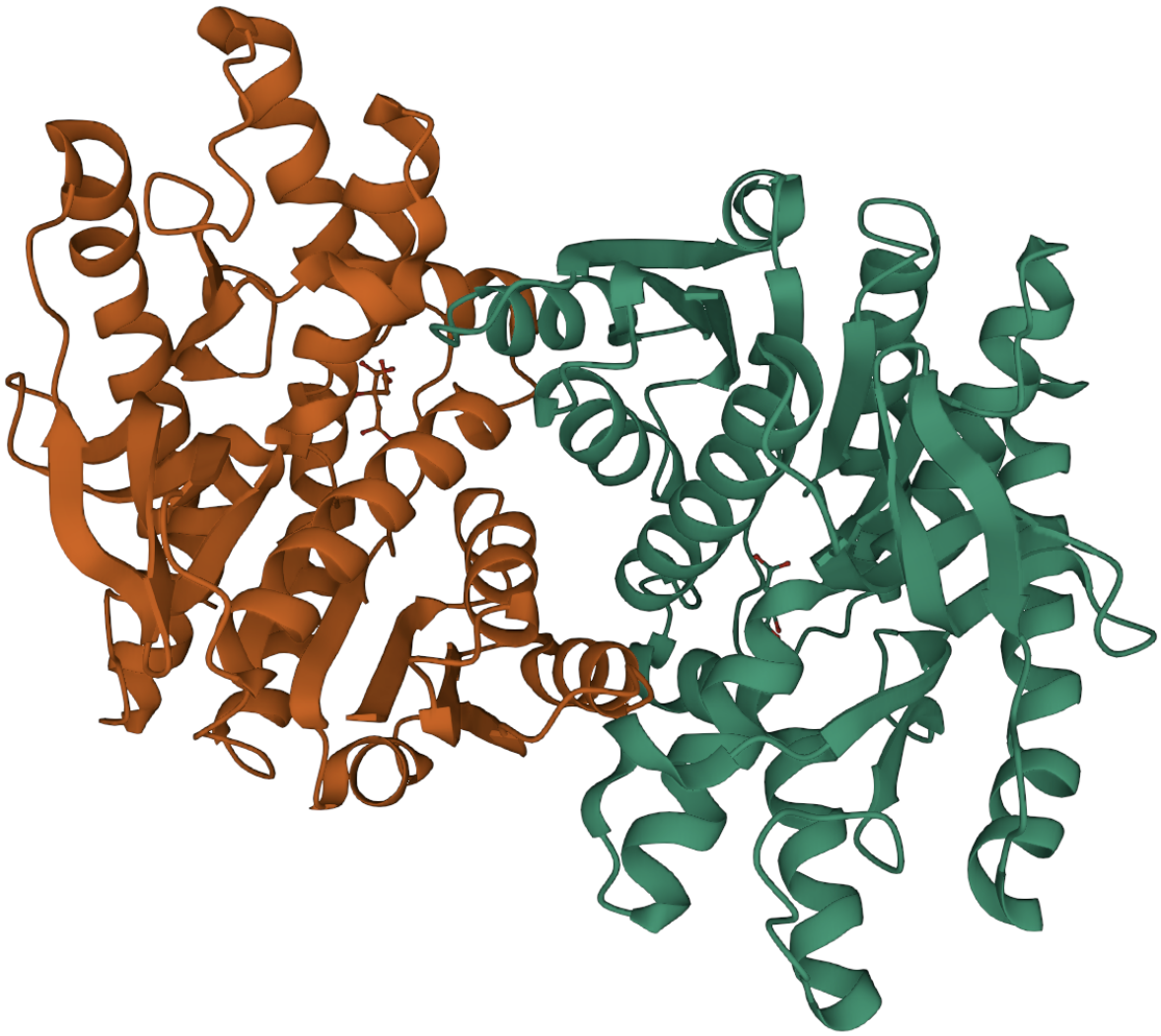
for completing this task. Note that the results of blast.pdb() contain the hits PDB identifier (or pdb.id) as well as Evalue and identity. The results of pdb.annotate() contain the other annotation terms noted above.

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	4	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%**