## Tiantai Ma

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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 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.

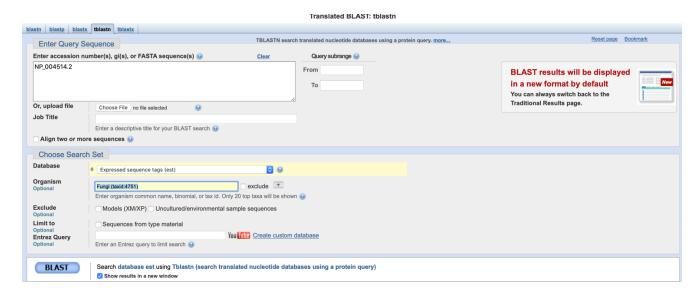
**KIF11** (kinesin family member 11) **Accession number:** NP\_004514

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 search against Fungi ESTs **Database:** Expressed sequence tag (est)

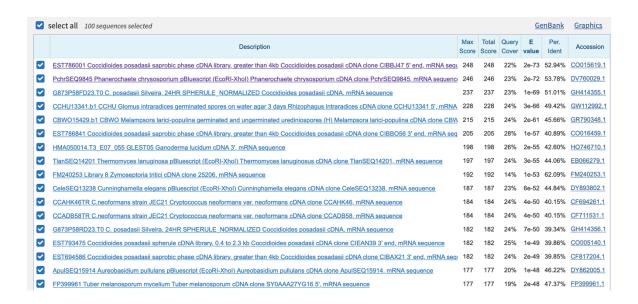
**Organism:** Fungi (taxid:4751)



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 \mathbb{H}-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.

**Chosen match:** Accession DV760029.1, 849 base pairs cDNA clone from *Phanerochaete chrysosporium*.



# PchrSEQ9845 Phanerochaete chrysosporium pBluescript (EcoRI-XhoI) Phanerochaete chrysosporium cDNA clone PchrSEQ9845, mRNA sequence

Sequence ID: DV760029.1 Length: 849 Number of Matches: 1 Range 1: 8 to 760 GenBank Graphics ▼ Next Match ▲ Previous Match Expect Method Identities Positives 246 bits(628) 2e-72 Compositional matrix adjust. 143/251(57%) 181/251(72%) 7/251(2%) +2 Query 256 VKIGKLNLVDLAGSENIGRSGAVDKRAREAGNINQSLLTLGRVITALVERTPHVPYRESK 315 +++GKLNLVDLAGSENIGRSGA DKRAREAG INQSLLTLGRVI ALV+R+ HVPYRESK
Sbjct 8 LRVGKLNLVDLAGSENIGRSGAQDKRAREAGMINQSLLTLGRVINALVDRSSHVPYRESK 
 Query
 316
 LTRILQDSLGGRTRTSIIATISPAslnleetlstleYAHRAKNILNKPEVNQKLTKKALI
 375

 Sbjct
 188
 LTRLLQDSLGGRTHT IIATISPA NHEETLSTLHYA RAKHI NKPEVNQHHTHNALL
 367
 Query 376 KEYTEEIERLKRDLAAAREKNGVYISEENFRVMSGKLTVQEEQIVELIEKIGAVEEELNR 435 KEY EIERLK D+ AAREKNG++ SEE ++ M+ + +++ + E +++ VE +L
Sbjct 368 KEYVAEIERLKADVLAAREKNGIFFSEERWQEMTAEQELKDTEMQEAKKQVEIVESOLRN 547 Query 436 ---LFMDNKNELDQCKSDLQNKTQELETTQKHLQETKLQLVKEEYITSALEST 488 V E L M EL + K LQ K EL+ T+ L+ K L +E + A + VREEFEQSMALLMRRDGELKETKERLQKKETELKATEGKLEVVKGALEEEVVVRQAYQEN 727 Sbjct 548 Query 489 EEKLHDAASKL 499 ETVLDGVATGL 760 Sbjct 728

PchrSEQ9845 Phanerochaete chrysosporium pBluescript (EcoRI-XhoI) Phanerochaete chrysosporium cDNA clone PchrSEQ9845, mRNA sequence

Sequence ID: DV760029.1 Length: 849 Number of Matches: 1 Range 1: 8 to 760GenBankGraphicsNext MatchPrevious Match

Score	Ехр	ect Method	Identities	Positives	Gaps	Frame
246 bits(628)	2e-7	'2 Compositional matrix adjust.	143/251(57%)	181/251(72%)	7/251(2%)	+2
Query	256	VKIGKLNLVDLAGSENIGRSGAVDKRAF	REAGNINQSLLTLG	RVITALVERTPHV	PYRESK 3	15
		+++GKLNLVDLAGSENIGRSGA DKRAF	REAG INQSLLTLG	RVI ALV+R+ HV	PYRESK	
Sbjct	8	LRVGKLNLVDLAGSENIGRSGAQDKRAF	REAGMINQSLLTLG	RVINALVDRSSHV	PYRESK 18	37
Query	316	LTRILQDSLGGRTRTSIIATISPAslnl	eetlstleYAHRA	KNILNKPEVNQKL	TKKALI 3	75
		LTR+LQDSLGGRT+T IIATISPA N+	EETLSTL+YA RA	K+I NKPEVNQ++	T+ AL+	
Sbjct	188	LTRLLQDSLGGRTKTCIIATISPARSNM	MEETLSTLDYAIRA	KSIKNKPEVNQRM	ITRNALL 3	67
Query	376	KEYTEEIERLKRDLAAAREKNGVYISEE	NFRVMSGKLTVQE	EQIVELIEKIGAV	EEELNR 43	35
		KEY EIERLK D+ AAREKNG++ SEE	C ++ M+ + +++	++ E +++ V	E +L	
Sbjct	368	KEYVAEIERLKADVLAAREKNGIFFSEE	CRWQEMTAEQELKD	TEMQEAKKQVEIV	ESQLRN 5	47
Query	436	VTELFMDNKNELDQCKSDLQN				88
		V E L M EL + K LQ				
Sbjct	548	VREEFEQSMALLMRRDGELKETKERLQK	KETELKATEGKLE	VVKGALEEEVVVR	QAYQEN 72	27
Query	489	EEKLHDAASKL 499				
		E L A+ L				
Sbjct	728	ETVLDGVATGL 760				

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.

[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.

>8-760\_1 PchrSEQ9845 Phanerochaete chrysosporium pBluescript (EcoRI-XhoI) Phanerochaete chrysosporium cDNA clone PchrSEQ9845, mRNA sequence LRVGKLNLVDLAGSENIGRSGAQDKRAREAGMINQSLLTLGRVINALVDRSSHVPYRESK LTRLLQDSLGGRTKTCIIATISPARSNMEETLSTLDYAIRAKSIKNKPEVNQRMTRNALL KEYVAEIERLKADVLAAREKNGIFFSEERWQEMTAEQELKDTEMQEAKKQVEIVESQLRN VREEFEQSMALLMRRDGELKETKERLQKKETELKATEGKLEVVKGALEEEVVVRQAYQEN ETVLDGVATGL

Name: Phanerochaete chrysosporium, hypothetical protein

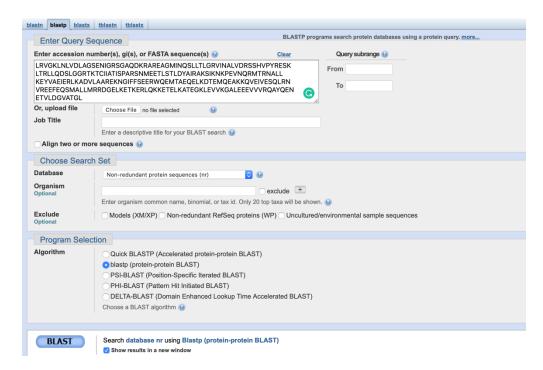
**Species:** Phanerochaete chrysosporium

Eukaryota; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Polyporales; Phanerochaetaceae; Phanerochaete

[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.

## Blastp search against nr database:



The top result is a hypothetical protein from *Phanerochaete camosa* with identity 94.42%, some results indicate that this is a kinesin-domain-cotaining protein:

	Description	Max Score		Query Cover	E value	Per. Ident	Accession
$\checkmark$	hypothetical protein PHACADRAFT_173717 [Phanerochaete carnosa HHB-10118-sp]	483	483	100%	5e-161	94.42%	XP_007395896.1
✓	hypothetical protein PHLGIDRAFT_22804 [Phlebiopsis gigantea 11061_1 CR5-6]	467	467	100%	4e-155	91.24%	KIP09572.1
✓	kinesin motor protein cin8 [Steccherinum ochraceum]	447	447	100%	1e-145	87.65%	TCD71883.1
	hypothetical protein EUX98_g250 [Antrodiella citrinella]	446	446	100%	3e-145	86.45%	THH33841.1
✓	hypothetical protein EVJ58_g2903 [Fomitopsis rosea]	442	442	100%	9e-145	86.06%	TFY64019.1
✓	hypothetical protein SERLADRAFT_451502 [Serpula lacrymans var. lacrymans S7.9]	441	441	100%	1e-144	85.66%	XP_007321258.1
$ lap{}$	hypothetical protein PHLCEN_2v10106 [Phlebia centrifuga]	444	444	100%	3e-144	85.66%	PSR74150.1
☑	hypothetical protein PAXINDRAFT_112407 [Paxillus involutus ATCC 200175]	442	442	100%	3e-144	85.26%	KIJ17421.1
✓	hypothetical protein SERLA73DRAFT_170393 [Serpula lacrymans var. lacrymans S7.3]	440	440	100%	3e-144	85.66%	EGN95950.1
✓	hypothetical protein HYDPIDRAFT_145382 [Hydnomerulius pinastri MD-312]	441	441	100%	1e-143	86.06%	KIJ69381.1
✓	kinesin-domain-containing protein [Daedalea quercina L-15889]	440	440	100%	1e-143	86.06%	KZT67731.1
✓	hypothetical protein PAXRUDRAFT_825572 [Paxillus rubicundulus Ve08.2h10]	440	440	100%	2e-143	85.26%	KIK96808.1
✓	hypothetical protein PISMIDRAFT_670188 [Pisolithus microcarpus 441]	438	438	100%	8e-143	85.26%	KIK31191.1
✓	hypothetical protein FOMPIDRAFT_1109816 [Fomitopsis pinicola FP-58527 SS1]	436	436	100%	2e-142	85.26%	EPT06162.1
✓	hypothetical protein M404DRAFT_951987.[Pisolithus tinctorius Marx 270]	436	436	100%	5e-142	85.66%	KIO13377.1
✓	predicted protein [Postia placenta Mad-698-R]	434	434	100%	5e-142	85.66%	EED84161.1
✓	hypothetical protein PLICRDRAFT_161615 [Plicaturopsis crispa FD-325 SS-3]	436	436	100%	7e-142	84.46%	KII88479.1
✓	predicted protein [Fibroporia radiculosa]	438	438	100%	1e-141	85.26%	XP_012181653.1
✓	hypothetical protein SCLCIDRAFT_113134 [Scleroderma citrinum Foug_A]	434	434	100%	2e-141	84.06%	KIM65286.1
$ lap{}$	kinesin-domain-containing protein [Rhizopogon vinicolor AM-OR11-026]	434	434	100%	2e-141	84.46%	OAX44667.1

## **L** Download **→** GenPept Graphics

## hypothetical protein PHACADRAFT\_173717 [Phanerochaete carnosa HHB-10118-sp]

Sequence ID: XP 007395896.1 Length: 1058 Number of Matches: 1

See 1 more title(s) ❤

#### Range 1: 320 to 570 GenPept Graphics

▼ Next Match ▲ Previous Match

Score		Expect	Method				Identities	5	Positiv	es	Gaps	
483 bits	s(1243)	5e-161	Compo	ositional	matrix	adjust.	237/25	L(94%)	245/2	251(97%)	0/251(	0%)
Query										DRSSHVP		60
Sbjct										DRSAHVP		379
Query										EVNORMTE EVNORMTE		120
Sbjct										PEVNORMT		439
Query										KQVEIVES		180
Sbjct										KÖVETVE		499
Query										EEVVVRQ/		240
Sbjct										EEVVVRQ		559
Query		ETVLDGV E LDGV	ATGL ATGL	251								
Sbjct	560	EAALDGV		570								

[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.

## Sequence selected for comparing (re-labeled):

>Cricetulus\_griseus RLQ59498.1 KIF11 [Cricetulus griseus]
MNGKLTVQEEQIVESAEKIGALEEELSKVTGLFVDSKNELDQCKSDLQTKTQELETTQKHLQETKLQLVK
EEYVSSALERTEEKLHDAASKLLSTVKETTRDVSGLHSKLDRKRAIDEHNAEAQDIFGNLMSSSVSALDT
VTTTALGTLMSIPQNVSARVTQISDMILKEQSLAAQSKTVLRGLINGLETDLLGSLKTILAPGVVSILNI
NRQLQHIFRTSLTMAEKELSQLVSSWTERFCALEKKCENIQKSLNKIQENTEHLFSEQWASCLSKSKEEL
QNLMEFINECCEATGSEITAKQSVHAAAIENQHTSFIVQITSDDEKLKAGNLKLDETIKTGLTKLNCFLQ
EDLKLDIPTGMTPERKNYLYPSTLVRTQPREQLLDQLQKKQHNQLNCSENDKEAFQHKKPHGKDKENRGI
NPVEKSKVEETSEYSVTKSRLPLRASNL

>Leptonychotes weddellii XP 006733941.1 kinesin-like protein KIF11 [Leptonychotes weddellii] MSGRPFNLAERKANAHSVVECDHVRKEVSVRTGGLADKSSRKTYTFDMVFGASTKQIDVYRSVVCPILDE VIMGYNCTIFAYGQTGTGKTFTMEGERSPNEEYTWEEDPLAGIIPRTLHQIFEKLTDNGTEFSVKVSLLE IYNEELFDLLNPSSDVSERLOMFDDPRNKRGVIIKGLEEITVHNKDEVYQILEKGAAKRTTAATLMNAYS SRSHSVFSVTIHMKETTIDGEELVKIGKLNLVDLAGSENIGRSGAVDKRAREAGNINQSLLTLGRVITAL VERTPHVPYRESKLTRILQDSLGGRTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQKLTKR ALIKEYTEEIERLKRDLAAAREKNGVYISEENFRAMSGKLTVOEEOIVELIEKIGAIEEELNRVTELFMD NKNELDQCKSDLQNKTQELETTQKHLQETKLQLVEEEYITSALESTEEKLHDAASRLLTTVEETTKDVSG LHSKLDRKKAIDQHNAEAQDIFGKNLNSLFSNMEELIKDSSSKQKAMLETHKTLFGNLLSSSVSALDTIT TTALGSLTSVPENVSIRVSQISNRILKEQSLAAESKTVLQTLIVSKSLKVFFKCYFAKPSKTMTSKDIIS KTASHSTQFCADCDGLSQELRCCNQEGTKLVEESVKHCDKLSSNLEVVSQETEQRCEALDASTLAFSEQW VSCLNKREEELONLLEVINOGCKAASSEITEKLNGHKAANENOHNTFFGOITTDEEKLMAOSLELNETIK MGLTKLNCFLQQDLKLDIPTGTTPQRKNYLYPSTLVRTEPREQLLDQLKRKQPELLMMLDCSENNKEEIS QDLDEEKSVLGHSVEELPGQEPSIDASVDCSSSGGIPFFQHKKSHGKDKENRGINSVERSKVEETTEHSV TRSRLPLRAQINL

>Symbiodinium\_microadriaticum OLP75816.1 Kinesin-like protein KIF11 [Symbiodinium microadriaticum]
MHEPDGCPDNRGTACRRQARSRPATPTGASNAEIPVTNVRVVCRIRPMNDREKKADPQCIEYAGATPAAT
ASTERREVAVVRSTFHFDDVLTSFSSQEDVFRATLQPLVGQVHVARAMLVLAVVSLPGWRANWTQRKAHP
EFTESTVTVSYLEIYNEELSDLLATAERHPKLDLKDIGSGRGVCCQGLSEALPHKDASKAVHGETLTPLE
RPRSLCAMPDGASPAPTVELRLLSTAAAPSPEAEHHGFLEVPSARRGHRRSSLARLTELEHQLHRDKGHL
AEKKLAKALALVLYTLARISANLVSIDYENHIRIVNALVRPLVTGLGVGMFLIREERFSTKSRLLIWSRR
TVFVLAIFVFDESMSTLRAALPIFSVLPWEITCNVMQVHMTNLRLQALGWRLTAKFCHIMAAIGCSFLLG

GLVILTSRISISEFGKEGNLTVERVGAPASFLLFFSFFCCWTTLFTQCGALCQAGSKAMVAGRADLDLDL KLTACFLYLNALLVLLGPALGFWSWAQFVRRIEMTWLTIDICVQIFTALALSGMMGPRNPMDAFRKLADL SGYGFASKRITFPGHINERAAKCIVSFPGVYNKLWDKAVKAVRDDFVTEEDLCSLACVFLTDSASGLGQH SMNPDTPGKCWCHAIYGQIRASAYLRVVEVDPCEADSIESREKLRHEVADADAMNQVLLIKSEQNDLEWQ KQYARALRTARDLGQENGGRAPWGCQWFEVWKKNVDRAVELKQELHVFYFQGRKGQGKLSWEDLSSDAAK NRVRPESGLGASQTAEVAYLLKMGIPFKEHDVQDFLSFLSSESS

>Homo sapiens NP 004514.2 kinesin-like protein KIF11 [Homo sapiens] MASQPNSSAKKKEEKGKNIQVVVRCRPFNLAERKASAHSIVECDPVRKEVSVRTGGLADKSSRKTYTFDM VFGASTKOIDVYRSVVCPILDEVIMGYNCTIFAYGOTGTGKTFTMEGERSPNEEYTWEEDPLAGIIPRTL HOIFEKLTDNGTEFSVKVSLLEIYNEELFDLLNPSSDVSERLOMFDDPRNKRGVIIKGLEEITVHNKDEV YOILEKGAAKRTTAATLMNAYSSRSHSVFSVTIHMKETTIDGEELVKIGKLNLVDLAGSENIGRSGAVDK RAREAGNINOSLLTLGRVITALVERTPHVPYRESKLTRILQDSLGGRTRTSIIATISPASLNLEETLSTL EYAHRAKNILNKPEVNQKLTKKALIKEYTEEIERLKRDLAAAREKNGVYISEENFRVMSGKLTVQEEQIV ELIEKIGAVEEELNRVTELFMDNKNELDOCKSDLONKTOELETTOKHLOETKLOLVKEEYITSALESTEE KLHDAASKLLNTVEETTKDVSGLHSKLDRKKAVDOHNAEAODIFGKNLNSLFNNMEELIKDGSSKOKAML EVHKTLFGNLLSSSVSALDTITTVALGSLTSIPENVSTHVSOIFNMILKEOSLAAESKTVLOELINVLKT DLLSSLEMILSPTVVSILKINSQLKHIFKTSLTVADKIEDQKKELDGFLSILCNNLHELQENTICSLVES QKQCGNLTEDLKTIKQTHSQELCKLMNLWTERFCALEEKCENIQKPLSSVQENIQQKSKDIVNKMTFHSQ KFCADSDGFSQELRNFNQEGTKLVEESVKHSDKLNGNLEKISQETEQRCESLNTRTVYFSEQWVSSLNER EOELHNLLEVVSOCCEASSSDITEKSDGRKAAHEKOHNIFLDOMTIDEDKLIAONLELNETIKIGLTKLN CFLEODLKLDIPTGTTPORKSYLYPSTLVRTEPREHLLDOLKRKOPELLMMLNCSENNKEETIPDVDVEE AVLGOYTEEPLSQEPSVDAGVDCSSIGGVPFFQHKKSHGKDKENRGINTLERSKVEETTEHLVTKSRLPL RAQINL

>Phanerochaete\_chrysosporium 8-760\_1 PchrSEQ9845 Phanerochaete chrysosporium pBluescript (EcoRI-XhoI) Phanerochaete chrysosporium cDNA clone PchrSEQ9845, mRNA sequence

LRVGKLNLVDLAGSENIGRSGAQDKRAREAGMINQSLLTLGRVINALVDRSSHVPYRESKLTRLLQDSLGGRTKTC IIATISPARSNMEETLSTLDYAIRAKSIKNKPEVNQRMTRNALLKEYVAEIERLKADVLAAREKNGIFFSEERWQE MTAEQELKDTEMQEAKKQVEIVESQLRNVREEFEQSMALLMRRDGELKETKERLQKKETELKATEGKLEVVKGALE EEVVVRQAYQENETVLDGVATGL

>Nothobranchius\_rachovii SBS17602.1 kinesin family member 11 [Nothobranchius rachovii]

MAGASVKVAVRVRPFNSRETEKESKCIIQMSGNTTTIINPKQAKDNKSFNFDFSYWSHTSPEDVNYASQM RVYKDIGEEMLLHAFEGYNVCIFAYGQTGAGKSYTMMGKQDVKDQQGIIPLLCEDLFTKINDNTNNSMSY SVEVSYMEIYCERVRDLLNPKNKGNLRVREHPLMGPYVEDLSKLAVTSYNDIQDLMDSGNKARTVAATNM NETSSRSHAVFNIIFTQKRYDAETDNTSEKVSKISLVDLAGSENIGRSGAVDKRAREAGNINQSLLTLGR VITALVEKRPHIPYRESKLTRILQDSLGGRTKTSIIATVSPSSSNMEETMSTLEYACRAKNIMNKPEVNQ KLTKRTLIKEYTEEIERLKRDLIAVREKNGVYLSSENYESMMTQIMAHEEQIAEYVDKIIIMEEELRKVT ELFEDSQTKLEQCTADLDEKHKRLEETSKDLQQTKEKLVEEEFICSEVTSVHESLYNTAGQLLSTADAST RDVSGLHDKLDRKKKVESHNKQIQQSFAERMDGALSSMQRCVHRHGAKHNELLSNCSQAVDGLLMMNEVA LKGTVTTMESFVGGVRSVVAEGVARCREKLQQHESVCLQDKESLLQLLEEHQQDMEDVLAARTLMGLSAV NELSDVLRSSVEKQRALADKVEESVGLFFNSLCRDLDSLREEAVHRLSSLQDEQDKLEDRISQAQERQRL GMQQTIQCLQDQLNLLSMQSQQDYADLRSASEALKAPALSLQESICSGCSSMKDQATTQADLHLTTSSNL TSSLHQTQQHVEEMNGYCADLHSSISGLVDRDLRWNLGVRDHTERQTREHLSVMEKLSAEAASLDQDVDT CCTNQLQTAEKELSGHREEVKQALLAIQNQTSMDQTILEQQQAELEDHVEVNQQLVHKFLQSELQQDIPT GTTPQQETSKSNESLATELSFDENLVFNESKRVPFFKQKKGKMTKIPTRSKVSENNDSLQTTPQKTRAPL HCVN

>Hydra\_vulgaris CDG70760.1 Hydra vulgaris Kinesin-like protein KIF11, partial [Hydra vulgaris]

MGKPILKLDKNQNIQVAVRCRPRNSQEIKAGSADVVDINNQTKEITVRQDMNYMDKGNNKTFSFDKVFGP KSKOIDIYOSMVCPVIDEVLOGYNCTIFAYGOTGTGKTFTMEGDRLDSEEFSWODDPLAGIIPRAMHOLF ELLNIMEECAEFSVRVSFLEIYNEELFDLLGTSLDSQKLRLFEDTTKKGSVVIQGLEEVIVHSRNEVYHI LERGAARRQTAATLLNAQSSRSHSLFMVTIHMKENNINGEEFLKTGKLNLVDLAGSENIGRSGAVEKRAR EAGTINOSLLTLGRVITALVENAPHVPYRESKLTRLLKDSLGGRTKTSIIATISPAACNLEETLSTLDYA ORAKNITNKPEINOKLTKKALIKEYTEEIEKLKKDLFAAREKNGIFIAEDNYILMONOLSSOKSSLREYV EKIQFMEEEMKKTEQLFASMOOKLEKTTQHLOWIKQERDETKVLVSKHVETEQQLFNQASELLDTVEETI NHKNILHSSLDRNKNLHVVNLLSSQKFKENAHAKVSSLNESIINGRHSSFAYHQDIVTELNNFKSEKASS YNOLSKIINISVEEIYKCLEIARTKQNEIKSECTKKDLLFSHNVETCKNETLQAINNYHKEIFTNALKEF HECILRMQECDERLVNSVNAMTEHFCETTKGFIEKQTVELRDLGQTVDEHICCQKEAIEKVNSYVEKTVQ NQDKMQKELKDGFIKSLQASILDVVEKNFSNLFQTQQTQLQEDTQMICSELSCQTENESNFKNSISSKLK DMNLTAISTVDGITTLAYQNLQYATENIDALKTCANSLKTKLDDLEKNETSFENVLIDNSNKYHDMTLKS YKELVTLSDNLISENHKNINELQTLHNSLCDQCLSNINTQVLSCDSVIDGLTEKMKQNQADSMNHSLSLE NGLHLLENDIIEFVEEELKQDSPTGLTPSQKEYNYPRSLQRTQEHEQIINEYRQRHDIPPLPSLLEYESD ENHDGSFLOOSEDKOKESEFLIPRSPDETGNEVLSIDTASDKENRNALLNNYYSSASKIGNNRPRIPLRS SNTLS

>Zonotrichia albicollis XP 014131087.1 kinesin-like protein KIF11 isoform X2 [Zonotrichia albicollis]

MVFGAQAKQIDVYRSVVCPILDEVIMGYNCTVFAYGQTGTGKTFTMEGERSPNEEYTWEEDPLAGIIPRT LHOIFEKLTENGTEFSVKVSLLEIYNEELFDLLNPTPDVGERLOMFDDPRNKRGVIIKGLEEVTVHNKNO VYOILERGAAKRTTAATYMNAYSSRSHSVFSITIHMKETTVDGEELVKIGKLNLVDLAGSENIGRSGAVD KRAREAGNINOSLLTLGRVITALVERAPHIPYRESKLTRILODSLGGRTKTSIIATISPASVNLEETLST LEYAHRAKNIMNKPEVNQKLTKKALIKEYTEEIERLKRDLAAAREKNGVYISAENYEALNGKLTVQEEQI TEYIDKISVMEEEVKRVTELFRVSKNELEOCKTDLOIKEKELEETOKDLOETKVOLAEEEYVVSVLESTE OELHDTASOLLTTVEETTRDVSGLHAKLERKRAVDOHNAAVONTFAGOMNASFSKIODSITENSLKOOOM LTYYTNCIGDLLSTSSSTADMFASVVSASFACLKELVSTEVSHISEKITQHENLSLDCKAELLRLIEEHQ TGLGRAVNSLTPMVEFVLGLNCQFQSNMKKYSAVADQMEDHKKEMDTFFADLSLTLKKIQEQTAGGFAQL QHNCDSLKEEVEMMRLAHRKSAAELMSSLQSQLDLFAQETQKSLTDVLTRNGSLKTTITAMQENVHLKTT DLVSSTNSNHNKFAASLDNFSQELRSINAENKAMLEESNDHCQHLLTNLKNVAQHTNTWGEFTTAQMVNF TNQHLLSFKDEKQQFQYLQKKNEENCDQAIAEIADHIGSQKAAEEKVLNGLLDQIKVDQEILVEQKLALR EOVOHGLTOVNGFLOEDLKVDVPTGTTPORKDYSYPVTLVRTEPROLLLEOLROKOPNLDAMLSSVGKEM EDSAGODLLEEGVLOEPSESLACDKYSMDTNVYCHTNGGIPFFQHKRSLKKGKENKSAAPLENKMEDMTE ELLQKSKHPLRLLN

### Alignment using MUSCLE at EBI:

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

Symbiodinium microadriaticum Nothobranchius rachovii Hydra\_vulgaris Phanerochaete chrysosporium Zonotrichia\_albicollis Cricetulus\_griseus Leptonychotes\_weddellii Homo\_sapiens

MHEPDGCPDNRGTACRRQARSRPATPTGASNAEIPVTNVRVVCRIRPMNDREKKADPQCI ----MAGASVKVAVRVRPFNSRETEKESKCI ----MGKPILKLDKNQNIQVAVRCRPRNSQEIKAGSADV ----MSGRPFNLAERKANAHSV -----MASQPNSSAKKKEEKGKNIQVVVRCRPFNLAERKASAHSI

Nothobranchius rachovii

Symbiodinium\_microadriaticum -EYAGATPAATASTE-RREVAVVRSTFHFDDVLTSFSSQED-------VFRATLQPLVG
Nothobranchius\_rachovii IQMSGNTTTII----NPKQAKDNKSFNFDFSYWSHTSPEDVNYASQMRVYKDIGEEMLL

Hydra\_vulgaris
Phanerochaete\_chrysosporium
Zonotrichia\_albicollis
Cricetulus\_griseus
Leptonychotes\_weddellii
Homo\_sapiens

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Nothobranchius\_rachovii
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-----KDASKAVHGETLTPLERPRSLCAMP------DGASPA-PTVELRLLSTA
DIQDLMDSGNKARTVAATNMNETSSRSHAVFNIIFTQKRYDAETDNTSEKVSKISLVDLA
EVYHILERGAARRQTAATLLNAQSSRSHSLFMVTIHMKENNINGEEFL-KTGKLNLVDLA
QVYQILERGAAKRTTAATYMNAYSSRSHSVFSITIHMKETTVDGEELV-KIGKLNLVDLA
EVYQILEKGAAKRTTAATLMNAYSSRSHSVFSVTIHMKETTIDGEELV-KIGKLNLVDLA
EVYQILEKGAAKRTTAATLMNAYSSRSHSVFSVTIHMKETTIDGEELV-KIGKLNLVDLA

AAPSPEAEHHGFLEVPSARRGHRRSSLARLTELEHQLHRDKGHLA--EKKLAKAL---G--SENIGRSGAVDKRAREAGNINQSLLTLGRVITALVEKRPHIPYRESKLTRILQDSLG
G--SENIGRSGAVEKRAREAGTINQSLLTLGRVITALVENAPHVPYRESKLTRLLKDSLG
G--SENIGRSGAQDKRAREAGMINQSLLTLGRVINALVDRSSHVPYRESKLTRLLQDSLG
G--SENIGRSGAVDKRAREAGMINQSLLTLGRVITALVERAPHIPYRESKLTRILQDSLG
G--SENIGRSGAVDKRAREAGMINQSLLTLGRVITALVERTPHVPYRESKLTRILQDSLG
G--SENIGRSGAVDKRAREAGMINQSLLTLGRVITALVERTPHVPYRESKLTRILQDSLG

---ALVLYTLARISAN----LVSIDYENHIRIV------NALVRPLVTGLGV-GRTKTSIIATVSPSSSNMEETMSTLEYACRAKNIMNKPEVNQKLTKRTLIKEYTEEIERL GRTKTSIIATISPAACNLEETLSTLDYAQRAKNITNKPEINQKLTKKALIKEYTEEIEKL GRTKTCIIATISPARSNMEETLSTLDYAIRAKSIKNKPEVNQRMTRNALLKEYVAEIERL GRTKTSIIATISPASVNLEETLSTLEYAHRAKNIMNKPEVNQKLTKKALIKEYTEEIERL GRTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQKLTKRALIKEYTEEIERL GRTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQKLTKKALIKEYTEEIERL GRTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQKLTKKALIKEYTEEIERL

-----GMFLIREERFSTKSRLLIWSRRTV-FVLAIFVFDESM-----S
KRDLIAVREKNGVYLSSENYESMMTQIMAHEEQIAEYVDKIIIMEEELRKVTELFEDSQT
KKDLFAAREKNGIFIAEDNYILMQNQLSSQKSSLREYVEKIQFMEEEMKKTEQLFASMQQ
KADVLAAREKNGIFFSEERWQEMTAEQELKDTEMQEAKKQVEIVESQLRNV-----RE
KRDLAAAREKNGVYISAENYEALNGKLTVQEEQITEYIDKISVMEEEVKRVTELFRVSKN
------MNGKLTVQEEQIVESAEKIGALEEELSKVTGLFVDSKN
KRDLAAAREKNGVYISEENFRAMSGKLTVQEEQIVELIEKIGAIEEELNRVTELFMDNKN
KRDLAAAREKNGVYISEENFRVMSGKLTVQEEQIVELIEKIGAVEEELNRVTELFMDNKN

TLRAALPIFSVLPWEITCNVMQVHMTNLR------LQALGWRLTAKFCHIMAAIGC KLEQCTADLDEKHKRLEETSKDLQQTKEKLVEEEFICSEVTSVHESLYNTAGQLLSTADA KLEKTTQHLQWIKQERDETKVLVSKH------VETEQQLFNQASELLDTVEE EFEQSMALLMRRDGELKETKERLQKKETE----LKATEGKLE----ELEQCKTDLQIKEKELEETQKDLQETKVQLAEEEYVVSVLESTEQELHDTASQLLTTVEE

Cricetulus\_griseus Leptonychotes\_weddellii Homo\_sapiens

Symbiodinium\_microadriaticum
Nothobranchius\_rachovii
Hydra\_vulgaris
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Homo sapiens

ELDQCKSDLQTKTQELETTQKHLQETKLQLVKEEYVSSALERTEEKLHDAASKLLSTVKE
ELDQCKSDLQNKTQELETTQKHLQETKLQLVEEEYITSALESTEEKLHDAASRLLTTVEE
ELDQCKSDLQNKTQELETTQKHLQETKLQLVKEEYITSALESTEEKLHDAASKLLNTVEE
: \*

AVRDDFVTEEDL-----CSLACVFLTDSASGLGQHSMN------PDTPGKCWCHANLTSSLHQTQQH----VEEMNGYCADLHSSISGLVDRDLRWNLGVRDHTERQTREHLSVGITTLAYQNLQYATENIDALKTCANSLKTKLDDLEKNETSFENVLIDNSNKYHDMTLKSY

NFSQELRSINAENKAMLEESNDHCQHLLTNLKNVAQHTNTWGEFTTAQMVNFTNQHLLS-----LEKKCENIQKSLNKIQENTEH----LFSEQWASCGLSQELRCCNQEGTKLVEESVKHCDKLSSNLEVVSQETEQRCEALDASTLAFSEQWVSCGFSQELRNFNQEGTKLVEESVKHSDKLNGNLEKISQETEQRCESLNTRTVYFSEQWVSS-

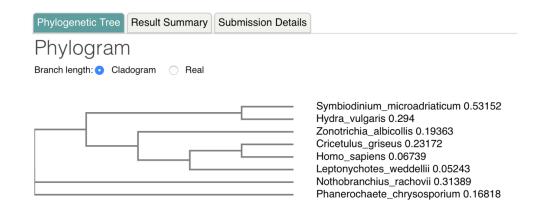
-----IYGQIRASAYLRVVEVDPCEADSIESREKLRHEVADADAMNQVLLIKSEQ
-----MEKLSAEAASLDQDVDTCCTNQLQTAEKELSGHREEVKQALLAIQNQTSM
KELVTLSDNLISENHKNINELQTLHNSLCDQCLSNI---NTQVLSCDSVIDGLTEKMKQ
-----FKDEKQQFQYLQKKNEENCDQAIAEIADHIGSQKAAEEKVLNGLLDQIKV
----LSKSKEELQNLMEFINECCEATGSEITAKQSVHAAAIENQHTSFIVQITS
-----LNKREEELQNLLEVINQGCKAASSEITEKLNGHKAANENQHNTFFGQITT
-----LNREEQELHNLLEVVSQCCEASSSDITEKSDGRKAAHEKQHNIFLDQMTI

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NDLEWQKQYARALRTARDLGQENGGRAPWGCQWFEVWKKNVDRAVELKQELHVFYFQGRK
Symbiodinium_microadriaticum
Nothobranchius rachovii
                                DQTILEQQQAELEDHVEV-----NQQLVHKFLQSELQQDIPTGTTPQQET---
                                NQADSMNHSLSLENGLHL----LENDIIEFVEEELKQDSPTGLTPSQKEYNYPRSLQR
Hydra vulgaris
Phanerochaete_chrysosporium
                                EEEVVVRQAYQENETVLD-----G-------VATGL------
                                DQEILVEQKLALREQVQH-----GLTQVNGFLQEDLKVDVPTGTTPQRKDYSYPVTLVR
Zonotrichia albicollis
Cricetulus_griseus
                                DDEKLKAGNLKLDETIKT----GLTKLNCFLQEDLKLDIPTGMTPERKNYLYPSTLVR
Leptonychotes_weddellii
                                DEEKLMAQSLELNETIKM----GLTKLNCFLQQDLKLDIPTGTTPQRKNYLYPSTLVR
Homo sapiens
                                DEDKLIAQNLELNETIKI-----GLTKLNCFLEQDLKLDIPTGTTPQRKSYLYPSTLVR
                                GQGKLSWEDLSSDAAKNRVRPE--SGLGASQTAEVA-----
Symbiodinium microadriaticum
Nothobranchius_rachovii
                                SKSN---ESTATELSEDE-----NLVENESKRVP------
Hydra_vulgaris
                                TQEH---EQIINEYRQRHDIPPLPSLLEYESDENHD-----GSFLQQSEDKQKESEF
Phanerochaete chrysosporium
Zonotrichia_albicollis
                                TEPR---QLLLEQLRQKQ--PNLDAMLSSVGKEMEDSAGQDLLEEGVLQEPSESLACDKY
                                TQPR---EQLLDQLQKKQ--HN---QLNCSENDKEA-----
Cricetulus griseus
Leptonychotes weddellii
                                TEPR---EQLLDQLKRKQ--PELLMMLDCSENNKEEISQDLDEEKSVLGHSVEELPGQEP
Homo_sapiens
                                TEPR---EHLLDOLKRKO--PELLMMLNCSENNKEETIPDVDVEEAVLGOYTEEPLSOEP
Symbiodinium_microadriaticum
                                -----PFKEHDVQDFLSFLSSESS--
Nothobranchius_rachovii
                                ----FFKQKKGKMTKIPTRS---KVSENNDSLQTTPQKTRAPLHCVN-
Hydra vulgaris
                               LIPRSPDETGN----EVLSIDTASDKENR--NALLNNYYSSASKIGNNRPRIPLRSSNT
Phanerochaete_chrysosporium
Zonotrichia albicollis
                                SMDTNVYCHTNGGIPFFQHKRSLKKGKENKSAAPLE-NKMEDMTEELLQKSKHPLRLL-N
                                -----FQHKKPHGKDKENRGINPVEKSKVEETSEYSVTKSRLPLRAS-N
Cricetulus griseus
Leptonychotes_weddellii
                                {\tt SIDASVDCSSSGGIPFFQHKKSHGKDKENRGINSVERSKVEETTEHSVTRSRLPLRAQIN}
Homo sapiens
                                SVDAGVDCSSIGGVPFFQHKKSHGKDKENRGINTLERSKVEETTEHLVTKSRLPLRAQIN
Symbiodinium_microadriaticum
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Zonotrichia_albicollis
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Homo_sapiens
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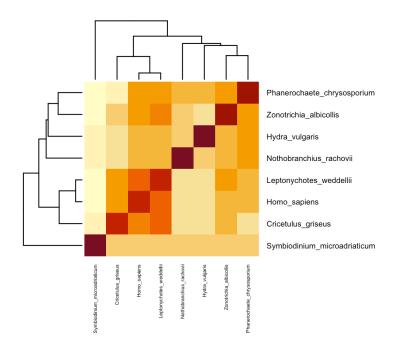
[Q6] Create a phylogenetic tree, using either a parsimony or distance-based approach. Bootstrapping and tree rooting are optional. Use "simple phylogeny" online from the EBI or any respected phylogeny program (such as MEGA, PAUP, or Phylip). Paste an image of your Cladogram or tree output in your report.

Using "simple phylogeny" from EBI, the phylogeny options are shown below:





[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 (structureld), 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.

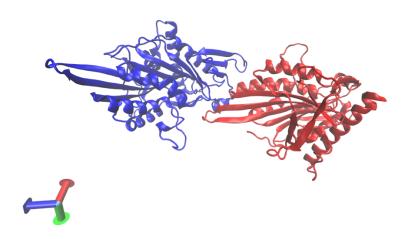
Since the consensus sequence from my alignment has lots of gaps. I chose to use single sequence for blast search. Based on my sequence identity matrix, the highest identity to all other sequence is from *Leptonychotes weddellii*. So, I proceed blast search using Bio3D with *Leptonychotes weddellii* sequence.

ID	Technique	Resolution	Source	Evalue	Identity
3HQD_A	X-RAY DIFFRACTION	2.19	Homo sapiens	0.00E+00	99.128
1116_A	X-RAY DIFFRACTION	2.1	Homo sapiens	0.00E+00	99.125
3WPN_A	X-RAY DIFFRACTION	2.8	Homo sapiens	0.00E+00	99.128

[Q9] Generate a molecular figure of one of your identified PDB structures using VMD. 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?

The structure of 3HQD (Human kinesin Eg5 motor domain in complex with AMPPNP and Mg2+) is shown here.

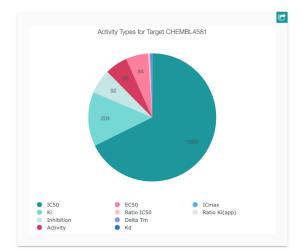


The similarity between my novel protein sequence and chain A sequence from PDBid: 3HQD is 20.8%. So, it is highly unlikely that the structure presented here is similar to the real structure of my novel protein.

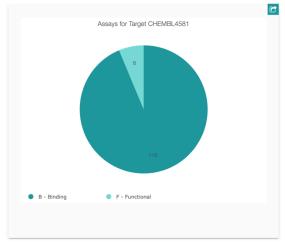
[Q10] Perform a "Target" search of ChEMBEL (https://www.ebi.ac.uk/chembl/) with your novel sequence. Are there any **Target Associated Assays** and **ligand efficiency data** reported that may be useful starting points for exploring potential inhibition of your novel protein?

After searching with my novel protein sequence, ChEMBEL feathered 119 binding assays and 8 functional assays. Most of them target kinesin-mediated microtubule function. (https://www.ebi.ac.uk/chembl/target\_report\_card/CHEMBL4581/)

#### Associated Bioactivities



#### Associated Assays



For example, in the review paper reported by *Visanath et al.* (DOI: 10.1021/jm401071u), a allosteric human KIF11 inhibitor SB743921 along with inhibitors AZD4877, MK0731, ARQ621, and ARRY520 are making huge progress towards clinical trials. Considering the similarity between by novel protein and human KIF11, it is highly likely that those inhibitors might work on this novel protein from *Phanerochaete chrysosporium* as well.