BIMM-143: INTRODUCTION TO BIOINFORMATICS
The find-a-gene project assignment https://bioboot.github.io/bimm143_S20/
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Overview:

The find-a-gene project is a required assignment for BIMM-143. You should prepare a written report in PDF format that has responses to each question labeled [Q1] - [Q10] below. You may wish to consult the scoring rubric at the end of this document and the example report provided online. The objective of this assignment is for you to demonstrate your grasp of database searching, sequence analysis, structure analysis, and the R environment that we have covered in class.

Submission instructions:

Submit this preliminary report as one document with screenshots of the results inserted appropriately. See the demonstration report linked to on the course website for an example of format. I will email you my decision; proceed with subsequent questions only after we are sure you have found a novel gene.

[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.

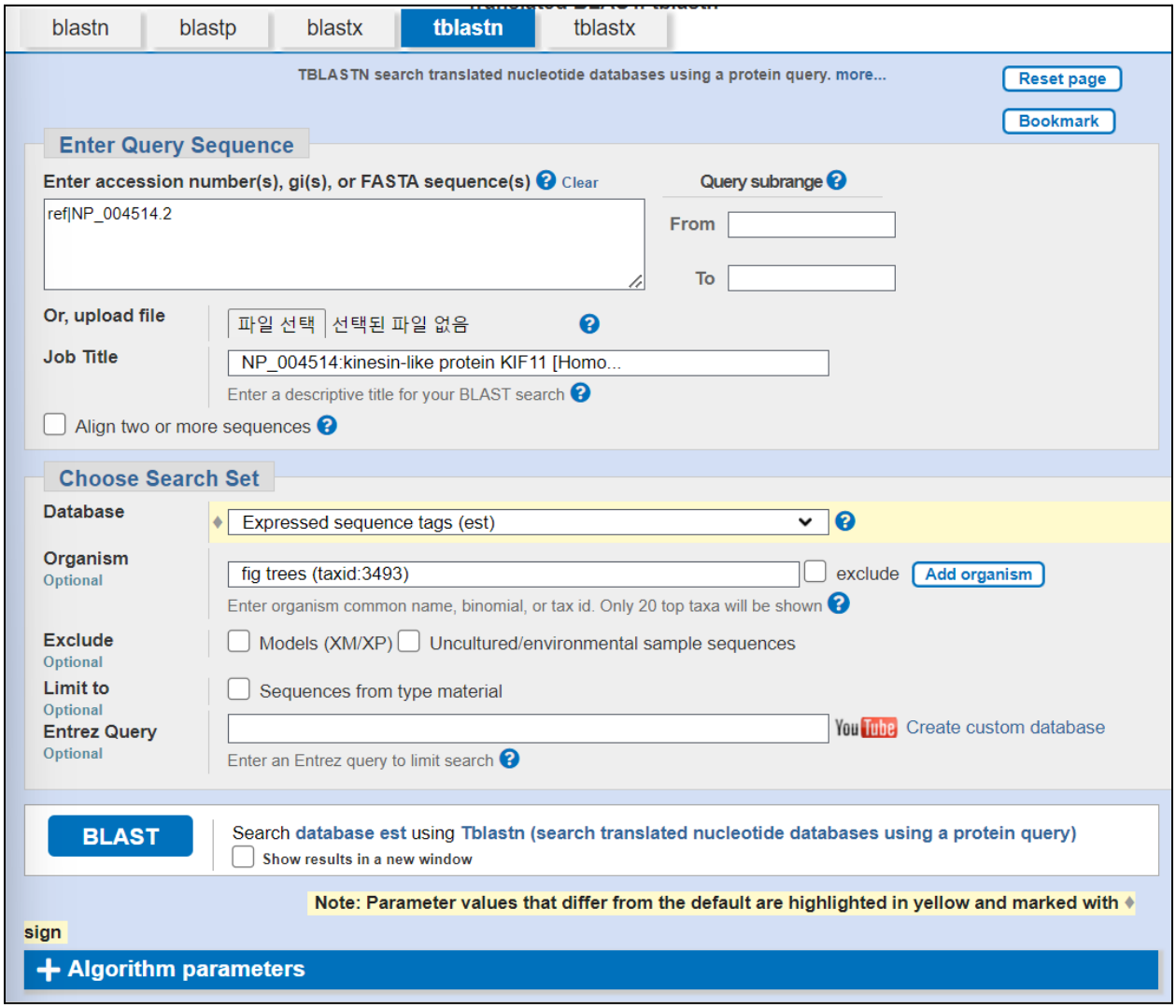
Name: KIF11
Acession: NP_004514.2
Species: Homo Sapiens

[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).

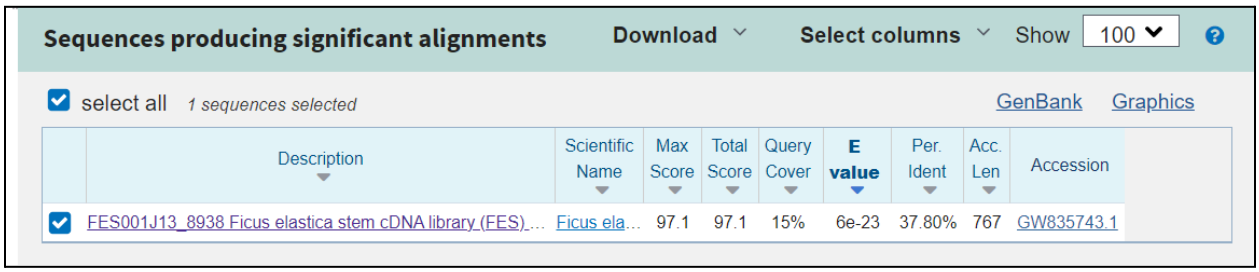
Method: TBLASTN
Database: expressed sequence tags (est)
Organism: Fig trees (taxid: 3493)

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.

Search Input: tblastn



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 number GW835743.1, acDNA sequence from Ficus elastica.

Alignment details:

FES001J13_8938 Ficus elastica stem cDNA library (FES) Ficus elastica cDNA clone FES001J13, mRNA sequence

Sequence ID: GW835743.1 Length: 767 Number of Matches: 1

```
Query  13   EEKGKNIQVVVRCRPFNLAERKASAHSIVECDPVRKEVSVRTGGLADKSSRKTYTFDMVF  72
            E KG NI+V  R RP  L +  +S    V   P   E   R   L+    + ++ FD VF
Sbjct  183  ELKG-NIRVFCRVRPL-LPDDGSSGEGKVISYPTSMETLGRGIDLSQIGQKHSFMFDKVF  356

Query  73   GASTKQIDVYRSVVCPILDEVIMGYNCTIFAYGQTGTGKTFTMEGERSPNEEYTWEEDPL  132
                 Q DV+  +   ++   + GY   IFAYGQTG+GKT+TM G+    E        L
Sbjct  357  MPDASQEDVFEEI-SQLVQSALDGYKVCIFAYGQTGSGKTYTMMGKPGQPE--------L  509

Query  133  AGIIPRTLHQIF---EKLTDNGTEFSVKVSLLEIYNEELFDLLN  173
              G+IPR+L QIF   + L   G ++ ++VS+LEIYNE + DLL+
Sbjct  510  KGLIPRSLEQIFRTRQSLLPQGWKYEMQVSMLEIYNETVRDLLS  641
```

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.

FASTA Sequence, translated from DNA sequence:

```
>GW835743.1_1 FES001J13_8938 Ficus elastica stem cDNA library (FES) Ficus
elastica cDNA clone FES001J13, mRNA sequence
RTC**MHKRNFRYPTYPSWRQKQNMKNRRKS*VNYKIAWRMPNLKLLKERCCAKSYIIRF
WN*RGTFGCSVECDHYCLMMVLLVKGRLSPIPHQWKLLDEALICHKLGKNILSCLTKFSC
LMHRKKMSLKKSHSLFKVRLTVIRSAFSPMGKRVQAKPIP*WVNQDSPS*KG*FLVP*NK
YFELDNLFCHKVGNMKCRYLCWRYITKLFGTCYLQIDHLLICCERKTVLVKHTQSNMT*M
GIHMYRI*QLWMFIVL
```

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.

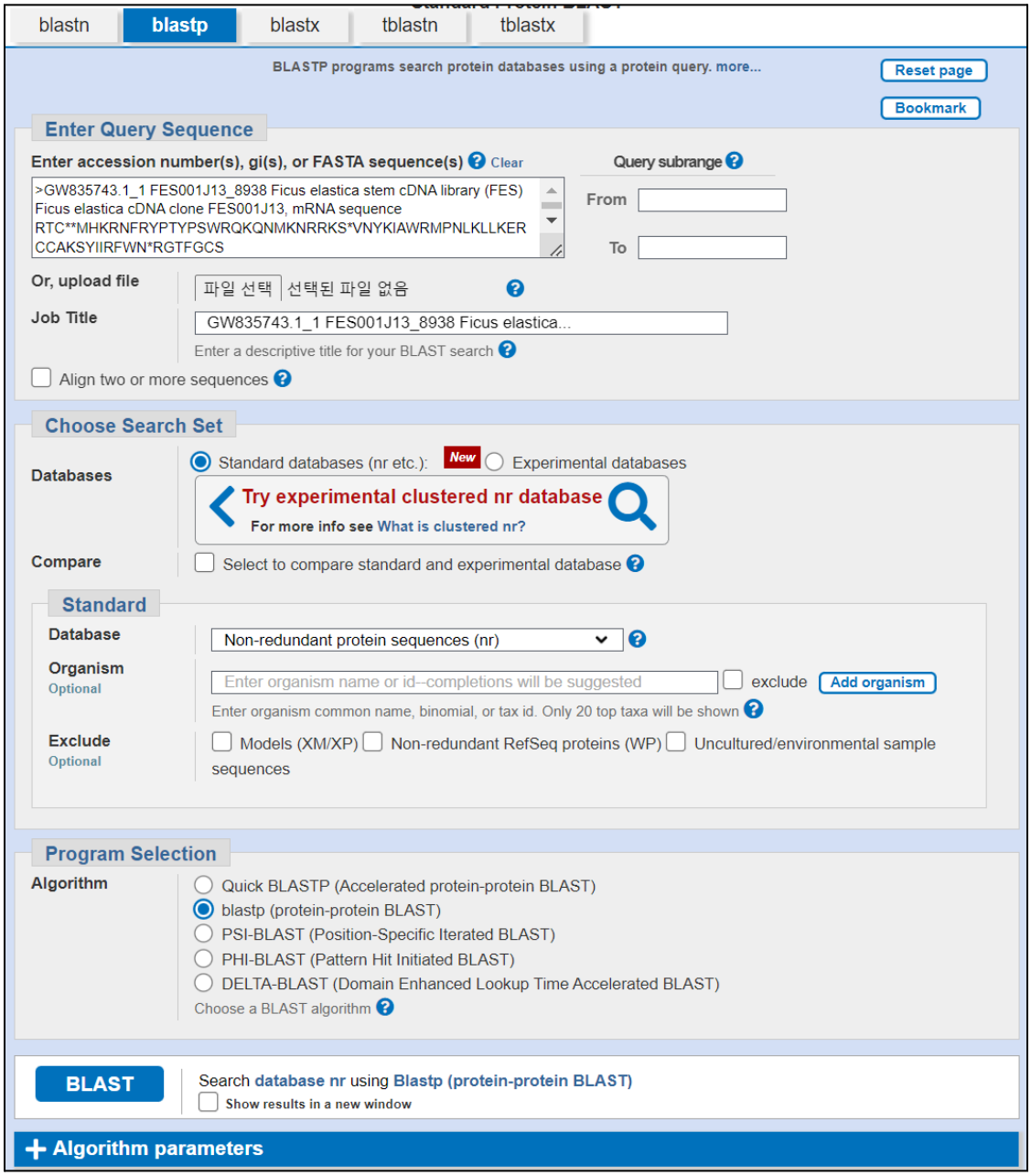
Name of novel protein: Kinesin-1 [Striga hermonthica]
Species: *Striga hermonthica*

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae; Pentapetalae; asterids; lamiids; Lamiales; Orobanchaceae; Buchnereae; Striga.

[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.

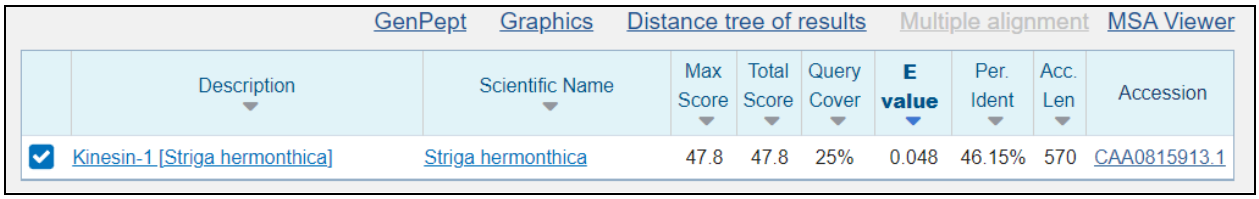
- If there is a match with 100% amino acid identity to a protein in the database, from the same species, then your protein is NOT novel (even if the match is to a protein with a name such as “unknown”). Someone has already found and annotated this sequence, and assigned it an accession number.
- If the 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: Using FASTA sequence from Q3



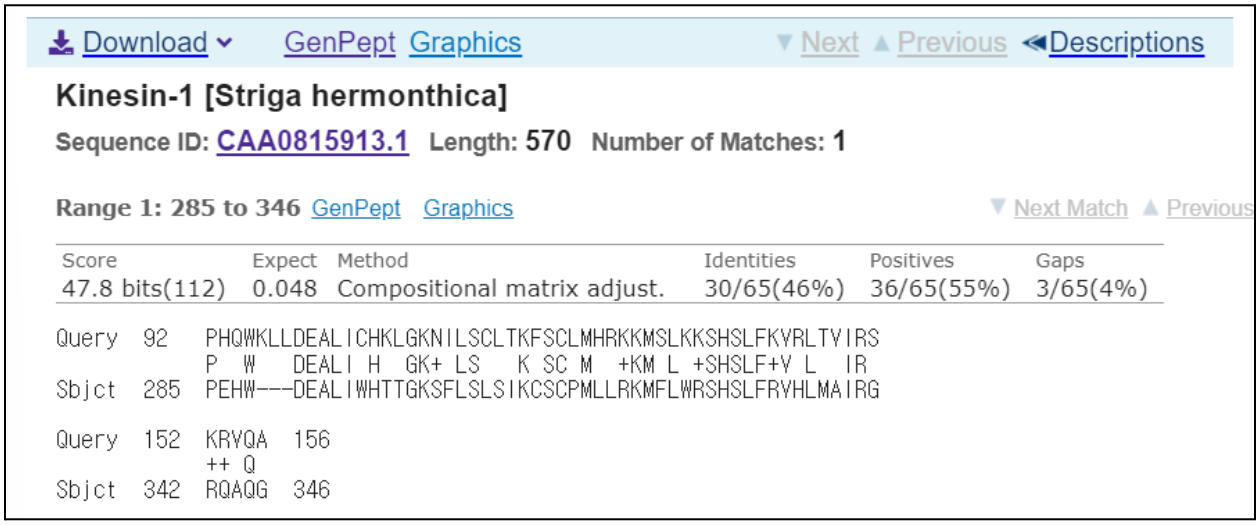
The chosen protein is:
Kinesin-1 [Striga hermonthica], the search query loads a match with a low e- value, and low percent identity, indicating that this is likely a novel protein.

Sequence ID: CAA0815913.1



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
☑	Kinesin-1 [Striga hermonthica]	Striga hermonthica	47.8	47.8	25%	0.048	46.15%	570	CAA0815913.1



Download GenPept Graphics ▼Next ▲Previous ◀◀Descriptions

Kinesin-1 [Striga hermonthica]

Sequence ID: CAA0815913.1 Length: 570 Number of Matches: 1

Range 1: 285 to 346 GenPept Graphics ▼Next Match ▲Previous

Score	Expect	Method	Identities	Positives	Gaps
47.8 bits(112)	0.048	Compositional matrix adjust.	30/65(46%)	36/65(55%)	3/65(4%)

```
Query  92   PHQWKLLDEALICHKLGKNILSCLTKFSCLMHRKKMSLKKSHSLFKVRLTVIRS
            P  W   DEALI H  GK+ LS   K SC M  +KM L +SHSLF+V L  IR
Sbjct  285  PEHW---DEALIWHTTGKSFLSLSIKCSCPMLLRKMFLWRSHSLFRVHLMAIRG

Query  152  KRVQA  156
            ++ Q
Sbjct  342  RQAQG  346
```

Here is a side by side alignment of the search query and the top search hit (Kinesin-1):

```
Query  92   PHQWKLLDEALICHKLGKNILSCLTKFSCLMHRKKMSLKKSHSLFKVRLTVIRSAFSPMG  151
            P  W   DEALI H  GK+ LS   K SC M  +KM L +SHSLF+V L  IR     M
Sbjct  285  PEHW---DEALIWHTTGKSFLSLSIKCSCPMLLRKMFLWRSHSLFRVHLMAIRGLHGYMV  341

Query  152  KRVQA  156
            ++ Q
Sbjct  342  RQAQG  346
```

Here is a full length sequence of the isolated protein (top search hit) in FASTA format:

```
>CAA0815913.1 Kinesin-1 [Striga hermonthica]
MRSAGRIYTRLSKFSVLPPVVLDFSQSEKLEIVANVKKYLQFFHLEYYPKLELEAYTNLTFEFYTTFKFV
KNGTDVVCRLGDKRKTIDTALMHQIFGFVSTGAEAPTNGLIVASIQTSDRFSPSFGMLVAALTRHFKSPM
REEDVVEAQRLVIKYFCAERNGSTEAEDTDLRGMVKEIVARMEFLMEALGGEVATLRLDLQQVQDEVATY
KEWIGKSIPELHSWQTKATESTCLSQSEQIRRLQKQLAVSKELKGNIRVFCRVRPFLSDDGVGNNAKVVS
FPTSPEHWDEALIWHTTGKSFLSLSIKCSCPMLLRKMFLWRSHSLFRVHLMAIRGLHGYMVRQAQGWKYD
MRISMLEIYNETIRDLLAPNRTCSDASRAENAGKQYAIKHDANGNTQVFDLTVVDVQSSKEVSYLLERAA
QSRSVGKTQMNEQSSRSHFVFTLRIMGFNENTDQQVCVLNLIDLAGSERLSKSGSTGNQLKETQAINKSL
SSLSDVIFALAKKEEHVPYRNSKLTYLLQPCLGGDSKTLMFVNVSPDHSLEGESLCSLRFAARVNACEIG
VPRRQTNLRS
```

[Q5] Generate a multiple sequence alignment with your novel protein, your original query protein, and a group of other members of this family from different species. A typical number of proteins to use in a multiple sequence alignment for this assignment purpose is a minimum of 5 and a maximum of 20 - although the exact number is up to you. Include the multiple sequence

alignment in your report. Use Courier font with a size appropriate to fit page width. Side-note: Indicate your sequence in the alignment by choosing an appropriate name for each sequence in the input unaligned sequence file (i.e. edit the sequence file so that the species, or short common, names (rather than accession numbers) display in the output alignment and in the subsequent answers below). The goal in this step is to create an interesting alignment for building a phylogenetic tree that illustrates species divergence.

BLASTP Search: Using FASTA sequence from Q3

```
>NP_004514.2 kinesin-like protein KIF11 [Homo sapiens]
MASQPNSSAKKKEEKGKNIQVVVRCRPFNLAERKASAHSIVECDPVRKEVSVRTGGLADKSSRKTYTFDMVFGASTKQI
DVYRSVVCPILDEVIMGYNCTIFAYGQTGTGKTFTMEGERSPNEEYTWEEDPLAGIIPRTLHQIFEKLTDNGTEFSVKV
SLLEIYNEELFDLLNPSSDVSERLQMFDDPRNKRGVIIKGLEEITVHNKDEVYQILEKGAAKRTTAATLMNAYSSRSHS
VFSVTIHMKETTIDGEELVKIGKLNLVDLAGSENIGRSGAVDKRAREAGNINQSLLTLGRVITALVERTPHVPYRESKL
TRILQDSLGGRTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQKLTKKALIKEYTEEIERLKRDLAAAREK
NGVYISEENFRVMSGKLTVQEEQIVELIEKIGAVEEELNRVTELFMDNKNELDQCKSDLQNKTQELETTQKHLQETKLQ
LVKEEYITSALESTEEKLHDAASKLLNTVEETTKDVSGLHSKLDRKKAVDQHNAEAQDIFGKNLNSLFNNMEELIKDGS
SKQKAMLEVHKTLFGNLLSSSVSALDTITTVALGSLTSIPENVSTHVSQIFNMILKEQSLAAESKTVLQELINVLKTDL
LSSLEMILSPTVVSILKINSQLKHIFKTSLTVADKIEDQKKELDGFLSILCNNLHELQENTICSLVESQKQCGNLTEDL
KTIKQTHSQELCKLMNLWTERFCALEEKCENIQKPLSSVQENIQQKSKDIVNKMTFHSQKFCADSDGFSQELRNFNQEG
TKLVEESVKHSDKLNGNLEKISQETEQRCESLNTRTVYFSEQWVSSLNEREQELHNLLEVVSQCCEASSSDITEKSDGR
KAAHEKQHNIFLDQMTIDEDKLIAQNLELNETIKIGLTKLNCFLEQDLKLDIPTGTTPQRKSYLYPSTLVRTEPREHLL
DQLKRKQPELLMMLNCSENNKEETIPDVDVEEAVLGQYTEEPLSQEPSVDAGVDCSSIGGVPFFQHKKSHGKDKENRGI
NTLERSKVEETTEHLVTKSRLPLRAQINL

>CAA0815913.1 Kinesin-1 [Striga hermonthica]
MRSAGRIYTRLSKFSVLPPVVLDFSQSEKLEIVANVKKYLQFFHLEYYPKLELEAYTNLTFEFYTTFKFVKNGTDVVCR
LGDKRKTIDTALMHQIFGFVSTGAEAPTNGLIVASIQTSDRFSPSFGMLVAALTRHFKSPMREEDVVEAQRLVIKYFCA
ERNGSTEAEDTDLRGMVKEIVARMEFLMEALGGEVATLRLDLQQVQDEVATYKEWIGKSIPELHSWQTKATESTCLSQS
EQIRRLQKQLAVSKELKGNIRVFCRVRPFLSDDGVGNNAKVVSFPTSPEHWDEALIWHTTGKSFLSLSIKCSCPMLLRK
MFLWRSHSLFRVHLMAIRGLHGYMVRQAQGWKYDMRISMLEIYNETIRDLLAPNRTCSDASRAENAGKQYAIKHDANGN
TQVFDLTVVDVQSSKEVSYLLERAAQSRSVGKTQMNEQSSRSHFVFTLRIMGFNENTDQQVCVLNLIDLAGSERLSKSG
STGNQLKETQAINKSLSSLSDVIFALAKKEEHVPYRNSKLTYLLQPCLGGDSKTLMFVNVSPDHSLEGESLCSLRFAAR
VNACEIGVPRRQTNLRS

>KAI3457004.1 hypothetical protein Pfo_013667 [Paulownia fortunei]
MASRNQNKPPSSPSHSKYSVDEVSVDKRRRIGNTKMPPNTGTRMQTRQAFSVVNGGQDLPPISGPPSNSGSDSGVIEFT
KEDVEALLNEKLRIKNKFNYKEKSEQMAECIKRLKQCIKWFQQLEGNYVTEQEKLKNLLELAEKRSNDMKLLMKAKEDE
LNSIIMELRKNLEALQEKFAKEELDKLEALDSLAREKDSRLAAERVQASISEELKRTQQDNASGIQKIQSLNDMYKRLQ
EYNTSLQQYNSRLQSELHATNETLKRVEKEKAAVVENLSTLRGHYTSLQEQLTSSRALQDEAMKQKEALGSEVTCLRGD
LQQVRDDRDRQLLQVQALSAEVVKYKECTGKSIAELDSLTTKTNELESTCLSQSEQIRRLQEQLAFAEKKLKLSDMSAM
ETRSEFEEQKTFILELQNRLADAELKIVEGEKLRKKLHNTILELKGNIRVFCRVRPLLSDDGVGIDAKVVSFPTSMEAL
GRGIDLTQNGQKLSFTFDKVFLPDASQEDVFVEISQLVQSALDGYKVCIFAYGQTGSGKTYTMMGKPGPPDQKGLIPRS
LEQVFETRQILQAQGWKYEMQVSMLEIYNETIRDLLAPNRSGFDASRAETGGKQYAIKHDANGNTHVSDLTIVDVRSSK
EVSYLLDRAAQSRSVGKTQMNEQSSRSHFVFTMRIMGFNESTDQQVQGVLNLIDLAGSERLSKSGSTGDRLKETQAINK
SLSSLSDVIFALAKKEEHVPYRNSKLTYLLQPCLGGDSKTLMFVNVSPDPSSVGESLCSLRFAARVNACEIGVPRRQTN
LRSSDSRLSIG
```

>GER43185.1 kinesin [Striga asiatica]
MSSSKQLDIQFEDHRQNQNKWLCPLREDVFAAFISKDNPTVHNIFGAASSLFSPFLFGKFFDPSDAFPLWEFDPQALLP
NNFNSSEHETVDWFRMENGYVLRAQLPNGTSQNTIQVCIANGKILEIYGQWKQQRESKTKDWKSSHWWEHGFVRRLELP
EQADWRKLEAHVKNELVLEIKVPDITTEGDVAQMVERSLSMRENKDSVDKVSVDRRRKMPLNTGIRVRKAFSVVNGGQN
LPQVSGPPSSSGSECGVSEFTKEEVEALLNGKLQIKNKFNYKEKSEQMAECIKKLKQCIKWFQQLEGNYVTEQGKLKDL
LGVAEKRSNDMELLMKAKEDELNSIIMDLRQKLEDLQEKFVGEEREKLDALDSLEKEKFYRLAAEKLQFSISEELKRVQ
EDNAAGIQKIQTLNDMYKRLQEYNTSLQQYNSRLQSELQATNETLKRVEKEKAAVVENLSTLRGHYTSLQEQLTSSRAL
QDEAMKQKEALGSEVTSLRGDLQQVRDDRDRQLLQVQALSAEVVKYKECTGKSIAELDSLSTKTTELESTCLSQSEQIR
RLQEQLAFAEKKLKLSDISAMETRSEFEEQKTTILELQNCLADAESKIVEGEKLRKKLHNTILELKGNIRVFCRVRPLL
SDDGVGNDAKVVSFPISTETLGRGIDLAQNGQKHSFTFDKVFMPDASQEDVFVEISQLVQSALDGYKVCIFAYGQTGSG
KTHTMMGKPGLPDQKGLIPRSLEQVFETRQILQAQGWKYDMQVSMLEIYNETIRDLLAPNRTGLDASRAENAGKQYAIK
HDANGNTHVSELTVVDVRSSKEVSYLLDRAAQSRSVGKTQMNEQSSRSHFVFTLRIMGFNESTDQQVQGVLNLIDLAGS
ERLSKSGSTGDRLRETQAINKSLSSLSDVIFALAKKEEHVPYRNSKLTYLLQPCLGGDSKTLMFVNVSPDPSSVGESLC
SLRFAARVNACEIGVPRRQTNLRSSSSSSSDSRLSIG

>XP_011095312.1 kinesin-like protein KIN-14N [Sesamum indicum]
MASKNQNKPPSSPSHSKYSVDDVSVDKRRRIGNTKMPPNSGTRVQTRQAFSVVNGGQDPPPTSGPPSNSG
SDSGVTEFTREDVEALLIEKLRIKNKFNYKEKSEQMAEYIKRLKQCIKWFQQCEGNYVTEQEKLKNLLELAEKKCNDME
LLMKAKEDELNSIIMELRNNLEALQEKFSKEELDKLEALDSLAKEKDSRLAAERLNASLSEELKRSQEDNASNVQKIQS
LNDMYKRLHEYNTSLQQYNSKLQSEIHAIKETLKHVEQEKSAIVENLSTLRGHSTSLQEQLASSRASQDEALKQKEALG
SEVTCLRGELQQVRDDRDRQLVQVQALSAEVVKYKECTGKSIAELDSLTTKTNELESTCLSQSEQIRRLHEQLAFAEKK
LKLSDMSAMETRSEFEEQKTIISQLQNRLADAESKIVEGEQLRKKLHNTILELKGNIRVFCRVRPLLSDDGVGADTKVV
SFPTSMEAQGRGIDLTQNGQKLSFTFDKVFVPDASQEDVFVEISQLVQSALDGYKVCIFAYGQTGSGKTYTMMGKPAPI
DQKGLIPRSLEQVFETRQILQAQGWKYGMQVSMLEIYNETIRDLLAPNRSGFDASRAENAGKQYSIKHDANGNTHVSDL
TIVDVHSSKEVSYLLDRAAQSRSVGKTQMNEQSSRSHFVFTLRITGFNESTDQQVQGVLNLIDLAGSERLSKSGSTGDR
LKETQAINKSLSSLSDVIFALAKKEEHVPYRNSKLTYLLQPCLGGDSKTLMFVNVSPDPSSVGESLCSLRFAARVNACE
IGVPRRQTNLRSLDSRLSIG

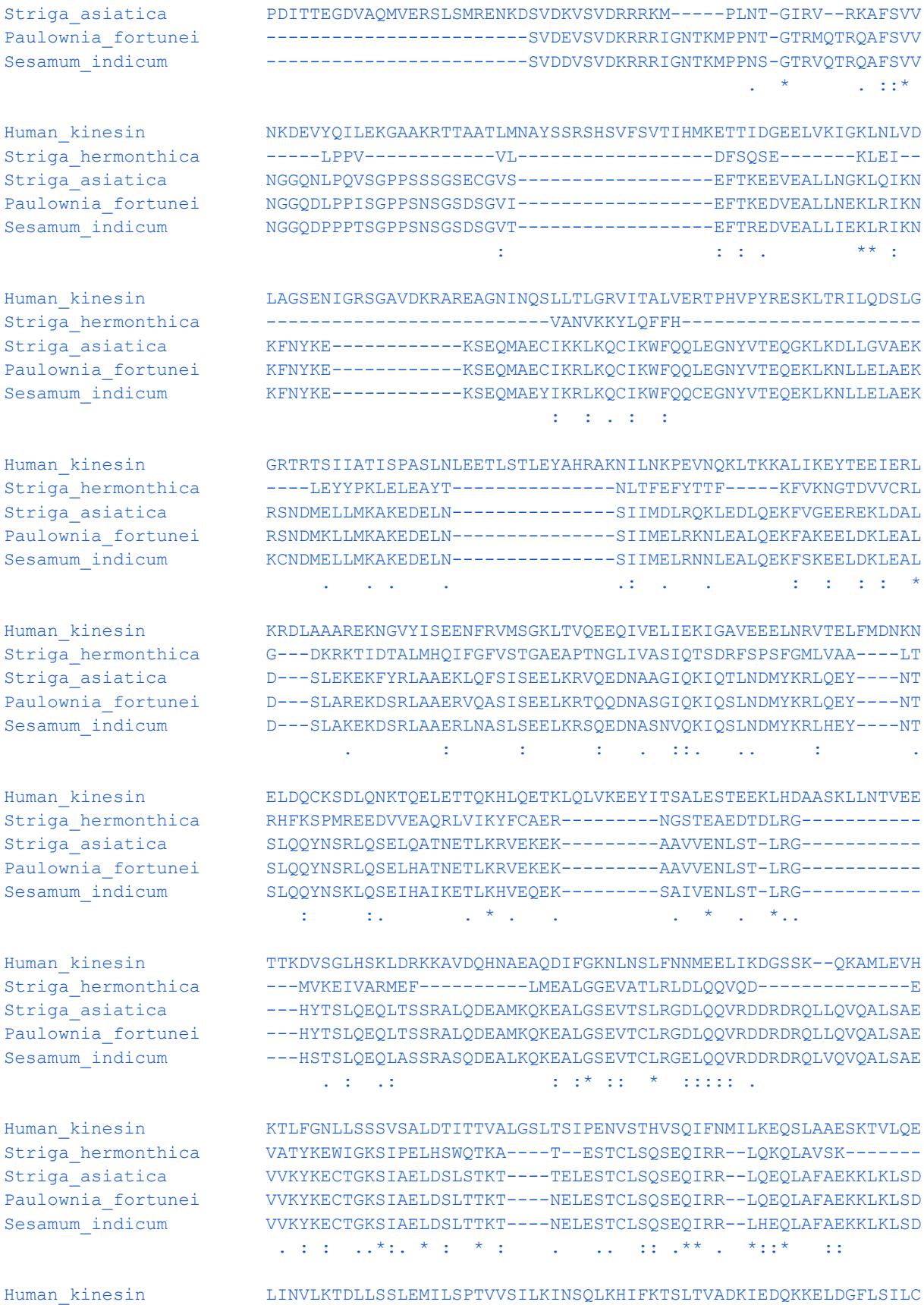
Alignment: Obtained using MUSCLE at EBI (3.8): CLUSTAL multiple sequence alignment

```
Human_kinesin           MASQPNSSAKKKEEKGKNIQVVVRCRPFNLAERKASAHSIVECDPVRKEVSVRTGGLADK
Striga_hermonthica      ------------------------------------------------------------
Striga_asiatica         MSSSKQLDIQFEDHRQNQNKWLCPLREDVFAAFISKDNPTVHNIFGAASSLFSPFLFGKF
Paulownia_fortunei      MAS------------RNQNK----------------------------------------
Sesamum_indicum         MAS------------KNQNK----------------------------------------

Human_kinesin           SSRKTYTFDMVFGASTKQIDVYRSVVCPILDEVIMGYNCTIFAYGQTGTGKTFTM-----
Striga_hermonthica      ------------------------------------------------------------
Striga_asiatica         FDPSDAFPLWEFDPQALLPNNFNSSEHETVDWFRMENGYVLRAQLPNGTSQNTIQVCIAN
Paulownia_fortunei      -PPSS--------------------------------------------------------
Sesamum_indicum         -PPSS--------------------------------------------------------

Human_kinesin           ---------------EGERSPNEEYTWEEDPLAGIIPRTLHQIFEKLTDNGTEFSVKVSL
Striga_hermonthica      ------------------------------------------------------------
Striga_asiatica         GKILEIYGQWKQQRESKTKDWKSSHWWEHGFVRRLELPEQADWRKLEAHVKNELVLEIKV
Paulownia_fortunei      ------------------------------------PSHSKY-----------------
Sesamum_indicum         ------------------------------------PSHSKY-----------------

Human_kinesin           LEIYNEELF---------------DLLNPSSDVSERLQMFDDPRNKRGVIIKGLEEITVH
Striga_hermonthica      ----------------------------------------MRSA-GRIYTRLSKFSV-
```


Striga_asiatica PDITTEGDVAQMVERSLSMRENKDSVDKVSVDRRRKM-----PLNT-GIRV--RKAFSVV
Paulownia_fortunei ----------SVDEVSVDKRRRIGNTKMPPNT-GTRMQTRQAFSVV
Sesamum_indicum ----------SVDDVSVDKRRRIGNTKMPPNS-GTRVQTRQAFSVV
Human_kinesin NKDEVYQILEKGAAKRTTAATLMNAYSSRSHSVFSVTIHMKETTIDGEELVKIGKLNLVD
Striga_hermonthica -----LPPV----------VL----------DFSQSE-------KLEI--
Striga_asiatica NGGQNLPQVSGPPSSSGSECGVS----------EFTKEEVEALLNGKLQIKN
Paulownia_fortunei NGGQDLPPISGPPSNSGSDSGVI----------EFTKEDVEALLNEKLRIKN
Sesamum_indicum NGGQDPPPTSGPPSNSGSDSGVT----------EFTREDVEALLIEKLRIKN
Human_kinesin LAGSENIGRSGAVDKRAREAGNINQSLLTLGRVITALVERTPHVPYRESKLTRILQDSLG
Striga_hermonthica ----------VANVKKYLQFFH----------
Striga_asiatica KFNYKE----------KSEQMAECIKKLKQCIKWFQQLEGNYVTEQGKLKDLLGVAEK
Paulownia_fortunei KFNYKE----------KSEQMAECIKRLKQCIKWFQQLEGNYVTEQEKLKNLLELAEK
Sesamum_indicum KFNYKE----------KSEQMAEYIKRLKQCIKWFQQCEGNYVTEQEKLKNLLELAEK
Human_kinesin GRTRTSIIATISPASLNLEETLSTLEYAHRAKNILNKPEVNQKLTKKALIKEYTEEIERL
Striga_hermonthica ----LEYYPKLELEAYT----------NLTFEFYTTF-----KFVKNGTDVVCRL
Striga_asiatica RSNDMELLMKAKEDELN----------SIIMDLRQKLEDLQEKFVGEEREKLDAL
Paulownia_fortunei RSNDMKLLMKAKEDELN----------SIIMELRKNLEALQEKFAKEELDKLEAL
Sesamum_indicum KCNDMELLMKAKEDELN----------SIIMELRNNLEALQEKFSKEELDKLEAL
Human_kinesin KRDLAAAREKNGVYISEENFRVMSGKLTVQEEQIVELIEKIGAVEEELNRVTELFMDNKN
Striga_hermonthica G---DKRKTIDTALMHQIFGFVSTGAEAPTNGLIVASIQTSDRFSPSFGMLVAA----LT
Striga_asiatica D---SLEKEKFYRLAAEKLQFSISEELKRVQEDNAAGIQKIQTLNDMYKRLQEY----NT
Paulownia_fortunei D---SLAREKDSRLAAERVQASISEELKRTQQDNASGIQKIQSLNDMYKRLQEY----NT
Sesamum_indicum D---SLAKEKDSRLAAERLNASLSEELKRSQEDNASNVQKIQSLNDMYKRLHEY----NT
Human_kinesin ELDQCKSDLQNKTQELETTQKHLQETKLQLVKEEYITSALESTEEKLHDAASKLLNTVEE
Striga_hermonthica RHFKSPMREEDVVEAQRLVIKYFCAER---------NGSTEAEDTDLRG----------
Striga_asiatica SLQQYNSRLQSELQATNETLKRVEKEK---------AAVVENLST-LRG----------
Paulownia_fortunei SLQQYNSRLQSELHATNETLKRVEKEK---------AAVVENLST-LRG----------
Sesamum_indicum SLQQYNSKLQSEIHAIKETLKHVEQEK---------SAIVENLST-LRG----------
Human_kinesin TTKDVSGLHSKLDRKKAVDQHNAEAQDIFGKNLNSLFNNMEELIKDGSSK--QKAMLEVH
Striga_hermonthica ---MVKEIVARMEF----------LMEALGGEVATLRLDLQQVQD----------E
Striga_asiatica ---HYTSLQEQLTSSRALQDEAMKQKEALGSEVTSLRGDLQQVRDDRDRQLLQVQALSAE
Paulownia_fortunei ---HYTSLQEQLTSSRALQDEAMKQKEALGSEVTCLRGDLQQVRDDRDRQLLQVQALSAE
Sesamum_indicum ---HSTSLQEQLASSRASQDEALKQKEALGSEVTCLRGELQQVRDDRDRQLVQVQALSAE
Human_kinesin KTLFGNLLSSSVSALDTITTVALGSLTSIPENVSTHVSQIFNMILKEQSLAAESKTVLQE
Striga_hermonthica VATYKEWIGKSIPELHSWQTKA----T--ESTCLSQSEQIRR--LQKQLAVSK-------
Striga_asiatica VVKYKECTGKSIAELDSLSTKT----TELESTCLSQSEQIRR--LQEQLAFAEKKLKLSD
Paulownia_fortunei VVKYKECTGKSIAELDSLTTKT----NELESTCLSQSEQIRR--LQEQLAFAEKKLKLSD
Sesamum_indicum VVKYKECTGKSIAELDSLTTKT----NELESTCLSQSEQIRR--LHEQLAFAEKKLKLSD
Human_kinesin LINVLKTDLLSSLEMILSPTVVSILKINSQLKHIFKTSLTVADKIEDQKKELDGFLSILC

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Striga_hermonthica      --------------------------------------------------ELKGNIRVFC
Striga_asiatica         ISAMETRSEFEEQKTTILELQNCLADAESKIVE----GEKLRKKLHNTILELKGNIRVFC
Paulownia_fortunei      MSAMETRSEFEEQKTFILELQNRLADAELKIVE----GEKLRKKLHNTILELKGNIRVFC
Sesamum_indicum         MSAMETRSEFEEQKTIISQLQNRLADAESKIVE----GEQLRKKLHNTILELKGNIRVFC
                                                             **.* : ::*

Human_kinesin           NNLHELQENTICSLVESQKQCGNLTEDLKTIKQTHSQELCKLMNLWTERFCALEEKCENI
Striga_hermonthica      RVRPFLSDDGVGN-------------NAKVVSFPTSPEHWDEALIWHTTGKSFLSLSIKC
Striga_asiatica         RVRPLLSDDGVGN-------------DAKVVSFPISTETLGRGIDLAQNGQKHSFTFDKV
Paulownia_fortunei      RVRPLLSDDGVGI-------------DAKVVSFPTSMEALGRGIDLTQNGQKLSFTFDKV
Sesamum_indicum         RVRPLLSDDGVGA-------------DTKVVSFPTSMEAQGRGIDLTQNGQKLSFTFDKV
                        .    *.:: :               : *.:. . * *                   :

Human_kinesin           QKPLSSVQENIQQKSKDIVNKMTFHSQKFCADSDGFSQELRNFNQEGTKLVEESVKHSDK
Striga_hermonthica      SCP-------------MLLRKMFLW---------------RSHSLFRV--------HLMA
Striga_asiatica         FMPDASQEDVFVEISQLVQSALDGY--KVCIFAYGQTGSGKTHTMMGK-------PGLPD
Paulownia_fortunei      FLPDASQEDVFVEISQLVQSALDGY--KVCIFAYGQTGSGKTYTMMGK-------PGPPD
Sesamum_indicum         FVPDASQEDVFVEISQLVQSALDGY--KVCIFAYGQTGSGKTYTMMGK-------PAPID
                          *             :   :                 ....

Human_kinesin           LNGNLEKISQETEQRCESLNTRTVYFSEQW-----VSSLNEREQELHNLLEVVSQCCEAS
Striga_hermonthica      IRG---------------LHGYMVRQAQGWKYDMRISMLEIYNETIRDLLAPNRTCSDAS
Striga_asiatica         QKGLIPRSLEQV------FETRQILQAQGWKYDMQVSMLEIYNETIRDLLAP--------
Paulownia_fortunei      QKGLIPRSLEQV------FETRQILQAQGWKYEMQVSMLEIYNETIRDLLAPNRSGFDAS
Sesamum_indicum         QKGLIPRSLEQV------FETRQILQAQGWKYGMQVSMLEIYNETIRDLLAPNRSGFDAS
                         .*              :    :  :: *     :* *:  :: :.:**

Human_kinesin           SSDITEKSDGRKAAHEKQHNIF-LDQMTIDEDKLIAQNLE-LNETIKIGLTKLN-----C
Striga_hermonthica      RAENAGKQYAIKHDANGNTQVFDLTVVDVQSSKEVSYLLERAAQSRSVGKTQMNEQSSRS
Striga_asiatica         ------------------------------------------------------------
Paulownia_fortunei      RAETGGKQYAIKHDANGNTHVSDLTIVDVRSSKEVSYLLDRAAQSRSVGKTQMNEQSSRS
Sesamum_indicum         RAENAGKQYSIKHDANGNTHVSDLTIVDVHSSKEVSYLLDRAAQSRSVGKTQMNEQSSRS

Human_kinesin           FLEQDLKLDIPTGTTPQRKSYLYPSTLVRTEPREHLLDQLKRKQPELLMMLNCSENNKEE
Striga_hermonthica      HFVFTLRIMGFNENTDQQVC-VLNLIDLAGSERLSKSGSTGNQLKETQAINKSLSSLSDV
Striga_asiatica         ------------------------------------------------------------
Paulownia_fortunei      HFVFTMRIMGFNESTDQQVQGVLNLIDLAGSERLSKSGSTGDRLKETQAINKSLSSLSDV
Sesamum_indicum         HFVFTLRITGFNESTDQQVQGVLNLIDLAGSERLSKSGSTGDRLKETQAINKSLSSLSDV

Human_kinesin           TIPDVDVEEAVLGQYTEEPLSQEPSVDAGVDCSSIGGVPFFQHKKSHGKDKENRGINTLE
Striga_hermonthica      IFALAKKEEHVPYRNSKLTYLLQPCL--GGDSKTLMFVNVSPDHSLEGE-----SLCSLR
Striga_asiatica         ------------------------------------------------------------
Paulownia_fortunei      IFALAKKEEHVPYRNSKLTYLLQPCL--GGDSKTLMFVNVSPDPSSVGE-----SLCSLR
Sesamum_indicum         IFALAKKEEHVPYRNSKLTYLLQPCL--GGDSKTLMFVNVSPDPSSVGE-----SLCSLR

Human_kinesin           RSKVEETTEHLVTKSRLPLRAQINL----
Striga_hermonthica      FAARVNACEIGVPRRQTNLRS--------
Striga_asiatica         -----------------------------
Paulownia_fortunei      FAARVNACEIGVPRRQTNLRSSDSRLSIG
Sesamum_indicum         FAARVNACEIGVPRRQTNLRSLDSRLSIG
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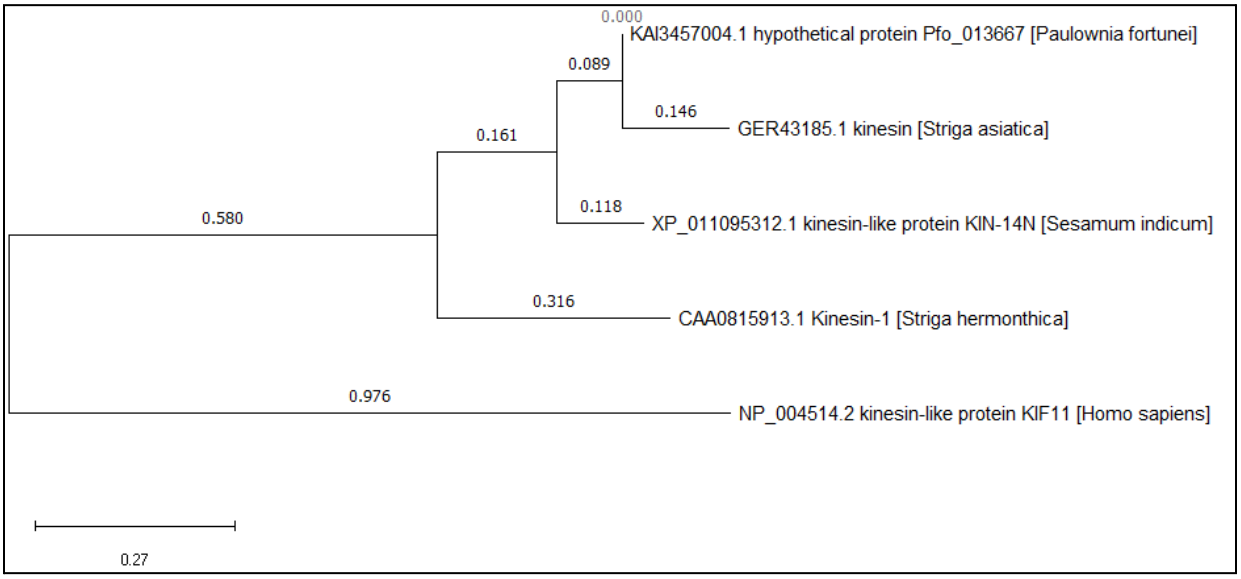
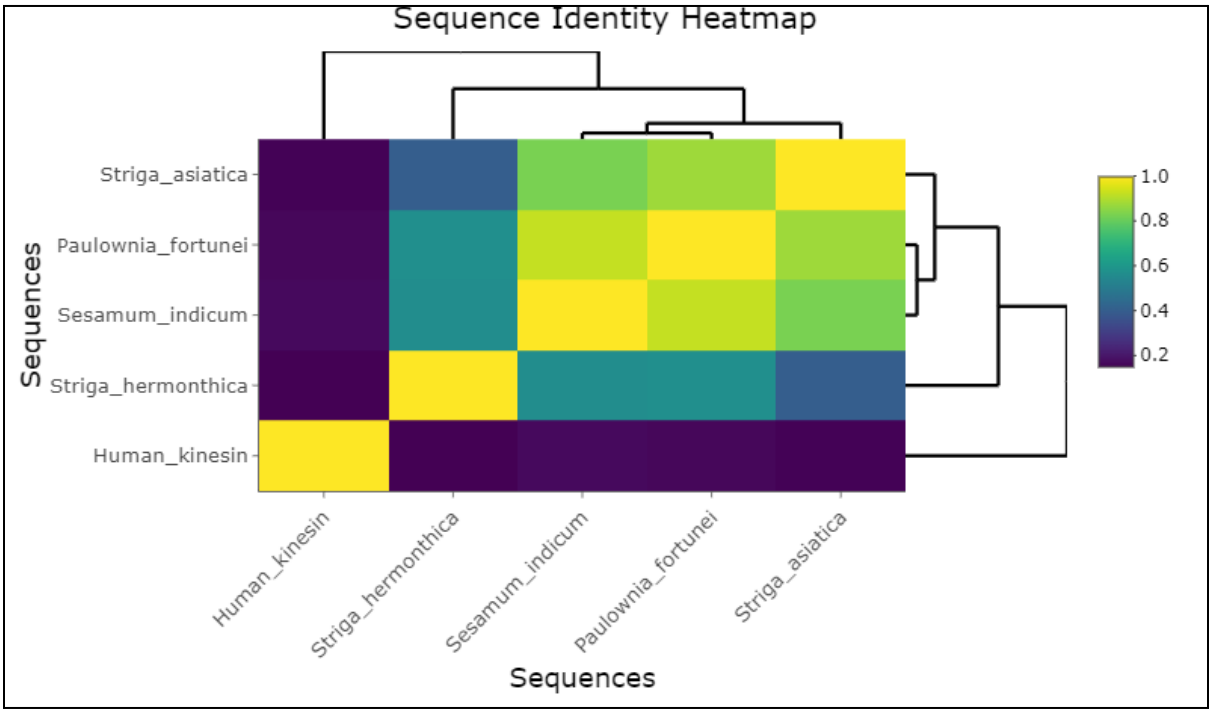
[Q6] Create a phylogenetic tree, using either a parsimony or distance-based approach. Bootstrapping and tree rooting are optional. Use “simple phylogeny” online from the EBI or any

respected phylogeny program (such as MEGA, PAUP, or Phylip). Paste an image of your Cladogram or tree output in your report.

Imported sequences into MEGA, aligned with MUSCLE, and created neighbor-joining tree:



[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 it to your report. Do make sure your labels are visible and not cut at the figure margins.



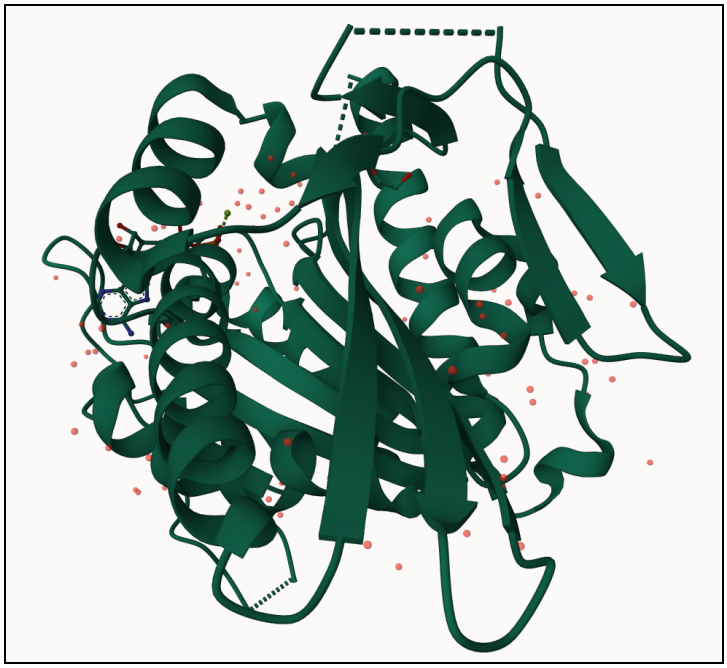
[Q8] Using R/Bio3D (or an online blast server if you prefer), search the main protein structure database for the most similar atomic resolution structures to your aligned sequences. List the top 3 unique hits (i.e. not hits representing different chains from the same structure) along with their Evalue 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 (experimental Technique), resolution (resolution), and source organism (source)

Paulownia fortunei has the highest percent matches aross all species earched.

PDB-ID	Technique	Resolution	Source	E-value	%Identity
3T0Q	X-ray diffraction	2.35	Eremothecium gossypii	1.25e-97	47.6
4GKR	X-ray diffraction	2.69	Candida glabrata	6.91e-99	46.6
2NCD	X-ray diffraction	2.50	Drosophila melanogaster	2.19e-93	44.2

[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?

3-D Molecular Structure of PDB structure with highest % identity: 3T0Q using Molstar:

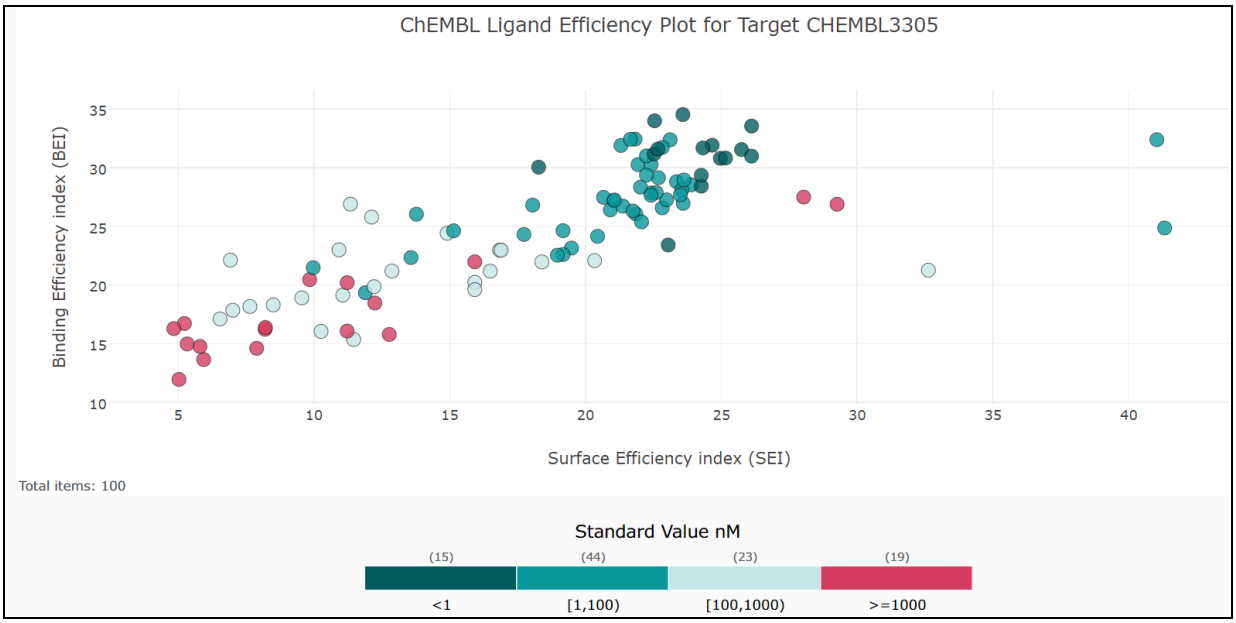


The above structure is the alpha chain of the “Motor Domain Structure of the Kar3-like kinesin from Eremothecium gossypii”. It is not likely to be similar in structure to the Striga hermonthica’s Kinesin-like protein, given its low sequence similarity (47.6%).

[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?

Based on a ChEMBEL Target search of novel protein sequence for striga hermonthica’s Kinesin-1 protein, the top search yielded:

https://www.ebi.ac.uk/chembl/target_report_card/CHEMBL3305/
CHEMBL3305, which details 26 Binding assays, 1 Functional Assay, and 7 ADME (absorption, distribution, metabolism, and excretion) Assays. There was ligand efficiency data available and displayed:



The ligand efficiency data is shown in this list: https://www.ebi.ac.uk/chembl/g/#browse/activities/filter/target_chembl_id%3ACHEMBL3305%20AND%20standard_type%3A(IC50%20OR%20Ki%20OR%20EC50%20OR%20Kd)%20AND%20_exists_%3Astandard_value%20AND%20_exists_%3Aligand_efficiency

One of the binding assays linked a journal article investigating the synthesis and biological component analysis of radiolabeled ligands for better identification and imaging of emerging androgen receptor-positive cancers and better targeting for therapy of the affected areas.

“Radiolabeled 5-Iodo-3′-O-(17β-succinyl-5α-androstan-3-one)-2′-deoxyuridine and Its 5′-Monophosphate for Imaging and Therapy of Androgen Receptor-Positive Cancers: Synthesis and Biological Evaluation”

Zbigniew P. Kortylewicz*, Jessica Nearman, and Janina Baranowska-Kortylewicz*

https://pubs.acs.org/doi/10.1021/jm9005803