# BGGN213 – Foundations of Bioinformatics

# Find-A-Gene 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 its 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: Argonaute-1 (AGO1)

**Accession**: NP\_001246314.1

**Species**: Drosophila melanogaster

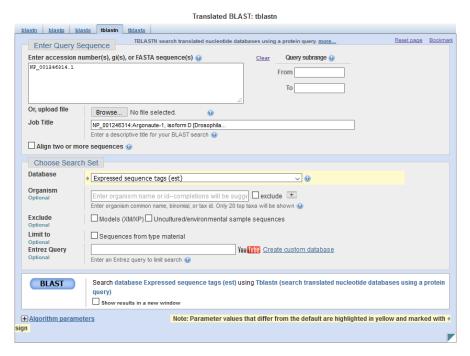
[Q2] Perform a BLAST search against a DNA database, such as a database consisting of genomic DNA or ESTs. The BLAST server can be at NCBI or elsewhere. Include details of the BLAST method used, database searched and any limits applied (e.g. Organism).

Method: TBLASTN (NCBI)

**Database**: Expressed Sequence Tags (est)

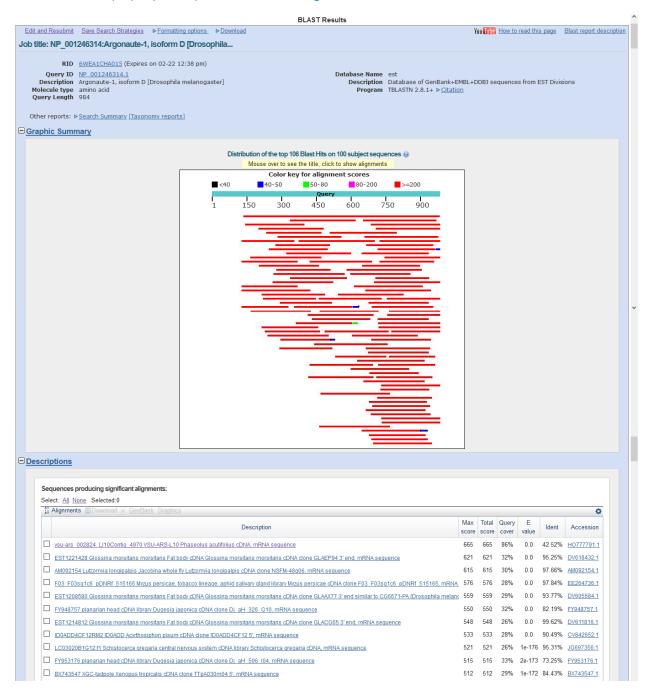
Organism: Any

Also include the output of that BLAST search in your document. If appropriate, change the font to Courier New size 10 so that the results are displayed neatly. You can also screen capture a BLAST output. 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. It should be labeled a "genomic clone" or "mRNA sequence", etc. - but include no functional annotation.

Chosen match: Accession HO777791.1, a 3644 base pair cDNA clone from *Phaseolus acutifolius* (Tepary bean). See below for alignment details.



## ■Download ∨ GenBank Graphics

vsu-ars\_002824\_LI10Contig\_4970 VSU-ARS-L10 Phaseolus acutifolius cDNA, mRNA sequence Sequence ID: <u>H0777791.1</u> Length: 3644 Number of Matches: 1

Score 1		Expect Method	Identities	Positives	Gaps	Frame
		7) 0.0 Compositional matrix adjust.				
Query	•	PRRPNLGREGRPIVLRANHFQVTMPRGYVHHYDII			.95	
Sbjct	925		I P+ R VNR +	+E +V Y +	.104	
Query	196	-FGVLKPVFDGRNNLYTRDPLPIGNERLELEVTL			48	
Sbjct	1105	G P +DGR +LYT PLP ++ + + HLGKRLPAYDGRKSLYTAGPLPFISKEFRITLID		+V IK A+ KVVIKLAARAD 1	.284	
Query	249	LFNLEEALEGRIRQIPYDAILALDVVMRHLPSMT	YTPVGRSFFSSPEGY	YHPLGGGREVW 3	808	
Sbjct	1285	L +L L+G+ P +A+ LD+V+R LP+ 1 LHHLGLFLQGKQTDAPQEALQVLDIVLRELPTTR			.464	
Query	309	FGFHQSVRPSQWKMMLNIDVSATAFYKAQPVIDF1 GF+QS+RP+Q + LNID+S+TAF + PVIDF-			68	
Sbjct	1465	RGFYÖSIRPTÖMGLSLNIDMSSTAFIEPLPVIDF			.635	
Query	369	KEIKGLKIEITHCGQMRRKYRVCNVTRRPAQMQSI K ++G+K+E+TH G MRRKYR+ +T + + +1			28	
Sbjct	1636	KALRGIKVEVTHRGNMRRKYRISGLTSQATRELT	FPVD-ERGTMKSV	VEYFYETYGFV 1	.806	
Query	429	LRYPHLPCLQVGQEHKHTYLPLEVCNIVAGQRCII +++ PCLQVG + YLP+EVC IV GQR I			88	
Sbjct	1807	IQHTQWPCLÖVGNTQRPNYLPMEVCKIVEGÖRYSI			.986	
Query	489	NNLVKRADFNNDSYVQEFGLTISNSMMEVRGRVL			48	
Sbjct	1987	MQTVYHNAYHEDPYAKEFGIKISEKLAQVEARIL	PAPWLKYHDTGR	EKDCL 2	139	
Query	549	VSLASPNQGVWDMRGKQFFTGVEIRIWAIACFAP( P G W+M K+ G + W C			808	
Sbjct	2140	PQVGQWNMMNKKMVNGGTVNNWFCINF			298	
Query	609	IIGQPCFCKYATGPDQVEPMFRYLKITFPG: +P + PDQVE + RY K G			61	
Sbjct	2299	FNPEPVVPPVSARPDÕVEKVLKTRYHDAKNKLQGI			478	
Query	662	TVLGMATQCVQAKNVNKTSPQTLSNLCLKINVKLOT LG+ +QC K+V K S Q L+N+ LKINVK+O	GGINSILVPSIR GG N++LV ++	PKVFNEPVIFL 7 P V + P I	18	
Sbjct	2479	TDLGLVSQCCLTKHVFKMSKQYLANVALKINVKV	GGRNTVLVDALSRRI	PLVGDRPTIIF 2	658	
Query	719	GADVTHPPAGDNKKPSIAAVVGSMDAHPSRYAGADVTHP G++ PSIAAVV S D +P ++YA			66	
Sbjct	2659	GADVTHPHPGEDSSPSIAAVVASQD-YPEITKYA	GLVCAÕAHRÕELIÕD	LFKQWQDPVRG 2	835	
Query	767	SSMVRELLIMFYKSTGGYKPHRIILYRDGVSI M++ELLI F ++TG KP RII YRDGVSI	EGQFPHVLQHELTAI EGQF VL EL AI	REACIKLEPEY 8 R+AC LEP Y	123	
Sbjct	2836	M++ELLI F ++TG KP RII YRDGVSi TVTGGMIKELLISFRRATG-QKPQRIIFYRDGVSi	EGŐFYQVLLFELDAI	RKACASLEPNY 3	012	
Query	824	RPGITFIVVQKRHHTRLFCAEKKEQSGKSGNI +P +TF+VVQKRHHTRLF + ++S +SGNI			81	
Sbjct	3013	QPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNI	LPGTVVDSKICHPTE	FDFYLCSHAGI 3	192	
Query	882	QGTSRPSHYHVLWDDNHFDSDELQCLTYQLCHTY QGTSRP+HYHVLWD+N+F +D LQ LT LC+TY	VRCTRSVSIPAPAYY RCTRSVSI PAYY	AHLVAFRARYH 9 AHL AFRAR++	)41	
Sbjct	3193	ÖGTSRP+HYHVLWD+N+F +D LÖ LT ~LC+TY ÖGTSRPAHYHVLWDENNFTADALÖTLTNNLCYTYA	ARCTRSVSIVPPAYY	AHLAAFRARFY 3	372	

Query 942 LVEKEHDSGEGSHQSGC-----SEDRTPGAMARAITVHA---DTKKVMYF 983 + + DS GS SG R PGA A + A + K+VM++
Sbjct 3373 MEPETSDS--GSMTSGAVAGRGMXGGMGRSTRAPGANAAVRPLPALKENVKRVMFY 3534

[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:

>Phaseolus acutifolius protein (sequence taken from BLAST result)
PLRPGKGSYGIKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRESHLGKRLPAYDGRKSLYT
AGPLPFISKEFRITLIDDDEGAAGGQRRDREFKVVIKLAARADLHHLGLFLQGKQTDAPQEALQVLDIVLRELPTTR
YCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVTQLLNRDVSARPLSDA
DRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMKSVVEYFYETYGFVIQHTQWPCLQVGNT
QRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERERDIMQTVYHNAYHEDPYAKEFGIKISEKLAQVEA
RILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNGGTVNNWFCINFSRSVQDSVARGFCYELAQMCYISGMAFNPE
PVVPPVSARPDQVEKVLKTRYHDAKNKLQGRELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQ
YLANVALKINVKVGGRNTVLVDALSRRIPLVGDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCA
QAHRQELIQDLFKQWQDPVRGTVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLE
PNYQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWD
ENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVAGRGMXGGMGRSTRA
PGANAAVRPLPALKENVKRVMFY

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: Phaseolus protein

**Species**: Phaseolus acutifolius

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;

Spermatophyta; Magnoliophyta; eudicotyledons; Gunneridae; Pentapetalae; rosids; fabids; Fabales; Fabaceae; Papilionoideae; 50 kb inversion clade; NPAAA

clade; indigoferoid/millettioid clade; Phaseoleae; Phaseolus.

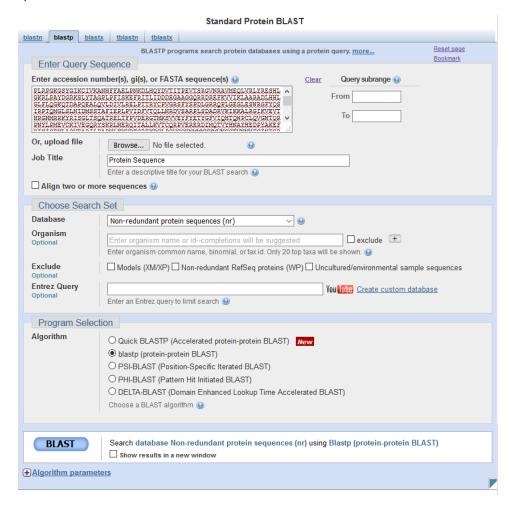
[Q4] Prove that this gene, and its corresponding protein, are novel. For the purposes of this project, "novel" is defined as follows. Take the protein sequence (your answer to [Q3]), and use it as a query in a blastp search of the nr database at NCBI.

- 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:

Searching this *Phaseolus* protein using BLASTP against non-redundant (NR) database reveals no hits with 100% identity. Although some results are close to 100% identity (the top hit has 99.89% identity), none of them are found in *Phaseolus acutifolius*. The top hit is found in *Phaseolus vulgaris*, and the other hits all appear to be found in different genera. (See BLASTP setup, BLASTP hits, and alignment of the top hit with the query below.)

#### **BLASTP** setup



#### **BLASTP** hits

#### Sequences producing significant alignments:



# Alignment of top hit

### BDownload ∨ GenPept Graphics

hypothetical protein PHAVU\_004G142900g [Phaseolus vulgaris]

Sequence ID: XP\_007152592.1 Length: 1063 Number of Matches: 1

▶ See 1 more title(s)

Score			ept Graphics  Method	Identities	Positives	Gaps
	bits(46	95) 0.0	Compositional matrix ad			
Query	1		GIKCIVKANHFFAELPNKDLHQ	YDVTITPEVTSRGVNRA	VMEQLVRLYRES	60
Sbjct	193		GIKCIVKANHFFAELPNKDLHQ: GIKCIVKANHFFAELPNKDLHQ:			252
)uery	61		DGRKSLYTAGPLPFISKEFRITI DGRKSLYTAGPLPFISKEFRITI			120
Sbjct	253	HLGKRLPAY	DGRKSLYTAGPLPFISKEFRITI	LIDDDEGAAGGÖRRDRE	FKVVIKLAARAD	312
)uery	121		GKOTDAPOEALOVLDIVLRELP: GKOTDAPOEALOVLDIVLRELP:			180
bjct	313	LHHLGLFLQ	GKÕTDAPÕEALÕVLDIVLRELP:	TTRYCPVGRSFYSPDLG	RRÕPLGEGLESW	372
Query	181		TQMGLSLNIDMSSTAFIEPLPV: TQMGLSLNIDMSSTAFIEPLPV:			240
Sbjct	373		TQMGLSLNIDMSSTAFIEPLPV:			432
Query	241		RGNMRRKYRISGLTSQATRELTI RGNMRRKYRISGLTSQATRELTI			300
Sbjct	433		RGNMRRKYRISGLTSQATRELTI			492
Query	301		RPNYLPMEVCKIVEGQRYSKRL1 RPNYLPMEVCKIVEGQRYSKRL1			360
Bbjct	493		RPNYLPMEVCKIVEGÕRYSKRLI			552
uery)	361		KEFGIKISEKLAQVEARILPAPI KEFGIKISEKLAQVEARILPAPI			420
bjct	553		KEFGIKISEKLAQVEARILPAPI			612
Query	421		INFSRSVQDSVARGFCYELAQM INFSRSVQDSVARGFCYELAQM			480
Sbjct	613		INFSRSVÕDSVARGFCYELAÕM			672
Query	481		LQGRELDLLIVILPDNNGSLYGI LQGRELDLLIVILPDNNGSLYGI			540
Sbjct	673		LÕGRELDLLIVILPDNNGSLYG			732
Query	541		VKVGGRNTVLVDALSRRIPLVGI VKVGGRNTVLVDALSRRIPLVGI			600
Sbjct	733		VKVGGRNTVLVDALSRRIPLVGI			792
Query	601		YAGLVCAQAHRQELIQDLFKQW YAGLVCAQAHRQELIQDLFKQW			660
Sbjct	793	SQDYPEITK	YAGLVCAĞAHRÖELIĞDLFKÖW(	DPVRGTVTGGMIKELI	ISFRRATGOKPO	852
)uery	661		SEGQFYQVLLFELDAIRKACASI SEGQFYQVLLFELDAIRKACASI			720
Sbjct	853		SEGQFYQVLLFELDAIRKACASI			912
uery)	721	KSSVDRSGN	ILPGTVVDSKICHPTEFDFYLCS ILPGTVVDSKICHPTEFDFYLCS	SHAGIQGTSRPAHYHVI	WDENNFTADALQ	780
bjct	913		ILPGTVVDSKICHPTEFDFYLC:			972
uery)	781		YARCTRSVSIVPPAYYAHLAAFI			840
bjct	973		YARCTRSVSIVPPAYYAHLAAFI YARCTRSVSIVPPAYYAHLAAFI			1032
Query	841		ANAAVRPLPALKENVKRVMFY ANAAVRPLPALKENVKRVMFY	870		
Sbjct	1033		ANAAVRPLPALKENVKRVMFY ANAAVRPLPALKENVKRVMFY	1062		

[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.

#### Re-labeled sequences for alignment:

(15 total sequences; all of the plants are from the legume family except cork oak and peach.)

>Fruit\_fly (original query; Drosophila melanogaster Argonaute-1)
PRRPNLGREGRPIVLRANHFQVTMPRGYVHHYDINIQPDKCPRKVNREIIETMVHAYSKIFGVLKPVFDG
RNNLYTRDPLPIGNERLELEVTLPGEGKDRIFRVTIKWQAQVSLFNLEEALEGRTRQIPYDAILALDVVM
RHLPSMTYTPVGRSFFSSPEGYYHPLGGGREVWFGFHQSVRPSQWKMMLNIDVSATAFYKAQPVIDFMCE
VLDIRDINEQRKPLTDSQRVKFTKEIKGLKIEITHCGQMRRKYRVCNVTRRPAQMQSFPLQLENGQTVEC
TVAKYFLDKYRMKLRYPHLPCLQVGQEHKHTYLPLEVCNIVAGQRCIKKLTDMQTSTMIKATARSAPDRE
REINNLVKRADFNNDSYVQEFGLTISNSMMEVRGRVLPPPKLQYGGRVSTGLTGQQLFPPQNKVSLASPN
QGVWDMRGKQFFTGVEIRIWAIACFAPQRTVREDALRNFTQQLQKISNDAGMPIIGQPCFCKYATGPDQV
EPMFRYLKITFPGlqlvvvvlPGKTPVYAEVKRVGDTVLGMATQCVQAKNVNKTSPQTLSNLCLKINVKL
GGINSILVPSIRPKVFNEPVIFLGADVTHPPAGDNKKPSIAAVVGSMDAHPSRYAATVRVQQHRQEIIQE
LSSMVRELLIMFYKSTGGYKPHRIILYRDGVSEGQFPHVLQHELTAIREACIKLEPEYRPGITFIVVQKR
HHTRLFCAEKKEQSGKSGNIPAGTTVDVGITHPTEFDFYLCSHQGIQGTSRPSHYHVLWDDNHFDSDELQ
CLTYQLCHTYVRCTRSVSIPAPAYYAHLVAFRARYHLVEKEHDSGEGSHQSGCSEDRTPGAMARAITVHA
DTKKVMYF

>Tepary\_bean (novel protein; from Phaseolus acutifolius cDNA)
PLRPGKGSYGIKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRESHLGKRLPAYD
GRKSLYTAGPLPFISKEFRITLIDDDEGAAGGQRRDREFKVVIKLAARADLHHLGLFLQGKQTDAPQEAL
QVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLP
VIDFVTQLLNRDVSARPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTM
KSVVEYFYETYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVER
ERDIMQTVYHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVN
GGTVNNWFCINFSRSVQDSVARGFCYELAQMCYISGMAFNPEPVVPPVSARPDQVEKVLKTRYHDAKNKL
QGRELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLV
DALSRRIPLVGDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQ
WQDPVRGTVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPV
TFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWD
ENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVAGRGMXGG
MGRSTRAPGANAAVRPLPALKENVKRVMFY

>Mung\_bean XP\_014512211.1:190-1059 protein argonaute 1 [Vigna radiata var. radiata]
PLRPGKGSYGIKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRESHLGKRLPAYD

GRKSLYTAGPLPFISKEFRITLIDDDEGAAGGQRRDREFKVVIKLAARADLHHLGLFLQGRQTDAPQEAL

QVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLP VIDFVTQLLNRDVSARPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTM KSVVEYFYETYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVER ERDIMQTVYHNAYHEDPYAQEFGIKISEKLAQVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVN GGTVNHWFCINFSRSVQDSVARGFCYELAQMCYISGMAFNPEPVVPPVSARPDQVEKVLKTRYHDAKNKL QGRELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLV DALSRRIPLVGDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQ WQDPVRGTVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPV TFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWD ENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVAGRGMGGG IGRSTRAPGANAAVRPLPALKENVKRVMFY

>Pigeon\_pea KYP61687.1:183-1053 Protein argonaute [Cajanus cajan]
PLRPGKGSYGIKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVKLYRESHLGKRLPAYD
GRKSLYTAGPLPFLSKEFRITLVDDDEGAGGQRRDREFKVVIKLAARADLHHLGLFLQGRQTDAPQEALQ
VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGDGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV
IDFVTQLLNRDVSTRPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK
SVVEYFYETYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERE
RDIMQTVHHNAYHDDPYAKEFGIKISEKLAQVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG
GTVNNWFCVNFSRNVQDTVARGFCYELAQMCYISGMAFNPEPVVPPVSARPDQVEKVLKTRYHDAKNKLQ
GRELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVD
ALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQW
QDPVRGTVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT
FVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE
NNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVAGRGMGGGG
GLGRSTRAPGASAAVRPLPALKENVKRVMFY

>Jequirity\_bean XP\_027359534.1:181-1050 protein argonaute 1 [Abrus precatorius]

PLRPGKGSYGTRCIVKANHFFAELPNKDLHQYDVTITPEVPSRGVNRAVMEQLVRLYRESHLGKRLPAYD GRKSLYTAGPLPFISKEFRITLIDDDEGAGGQRRDREFKVVIKFAARADLHHLGLFLQGRQTDAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IDFVTQLLNRDVSSRPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFYDTYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERE RDIMQTVHHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNNWFCINFSRNVQDSVARGFCYELAQMCYISGMAFNPEPVVPPLSARPDQVEKVLKTRYHDAKNKLQ GRELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVD AISRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQW QDPVRGTLTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT FVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE NNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVAGRGMGGGG AGRSTRAPGASAAVRPLPALKENVKRVMFY

>Soybean XP\_003534084.1:185-1057 protein argonaute 1 [Glycine max] PLRPGKGSYGTKCVVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRESHLGKRLPAYD GRKSLYTAGPLPFMSKEFRIVLADDDEGAGGQRRDREFKVVIKLAARADLHHLGLFLQGRQTDAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IDFVNQLLNRDVSARPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFYETYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERE RDIMQTVHHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNNWFCINFSRNVQDSVARGFCYELAQMCYISGMAFTPEPVVPPVSARPDQVEKVLKTRYHDAKNKLQ GKELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVD ALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQW QDPVRGTVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT FVVVQKRHHTRLFASNHHDKSSFDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE NNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVAGRGMGGGG GGGVGRSTRAPGANAAVRPLPALKENVKRVMFY

>Velvet\_bean RDY09546.1:232-1100 Protein argonaute 1, partial [Mucuna pruriens]

PLRPGKGSYGTKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRESHLGKRLPAYD GRKSLYTAGALPFISKEFRITLIDDDEGAGQQRRDREFKVVIKLAARADLHHLGLFLQGRQTDAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IDFVTQLLNRDVSSRPLSDSDRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFYETYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERE RDIMQTVHHNAYHDDPYAKEFGIKISEKLAQVEARILPPPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNNWFCINFSRNVQDSVARGFCYELAQMCYISGMAFNPEPVVPPVSSRPDQVEKVLKTRYHDAKSKLQ GRDLDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVD ALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQW QDPVRGTVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT FVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE NNFTADGLQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAIGRGMGGGGA GRSTRAPGANAAVRPLPALKENVKRTSSY

>Chickpea XP\_012567214.1:225-1093 protein argonaute 1 [Cicer arietinum] PLRPGKGSCGRKCVVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRESHLGKRLPAYD GRKSLYTAGPLPFISKDFRITLVDDDDGTGGQRRDREFKVVIKLAARADLHHLGLFLEGRQTDAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IDFVTQLLNRDVSTRPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFYETYGFVIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPLDRE RDIMQTVHHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNNWFCVNFSRNVQDSVARGFCCELAQMCYISGMAFNPEPVVPPLSARPDQVDKVLKTRYQDAKNKLQ GRELDLLIVILPDNNGSLYGDLKRICETELGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVD ALSRRIPLVSDRPTIIFGADVTHPHPGEDMSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQW QDPVRGTLTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT FVVVQKRHHTRLFASDHRDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE NNFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVSGRGMGGGV GRSTRAPGANAAVRPLPALKENVKRVMFY

>Red\_clover PNY07943.1:221-1089 protein argonaute 1-like [Trifolium pratense]
PLRPGKGSTGKRCIVKANHFIAELPKKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRDSHLGKRLPAYD
GRKSLYTAGPLPFISKDFRITLVDEDDGSGSQRRDREFKVVIKLAARADLHHLGLFLEGRQTDAPQEALQ
VLDIVLRELPTSRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV
IEFVTQLLNRDVSARPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK
SVVEYFYETYGFSIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPLDRE
RDIMQTVHHNAYHDDPYAKEFGIKISDKLAQVEARILPPPWLKYHDTGKEKDCLPQVGQWNMMNKKMVNG
GTVNNWFCVNFSRNVQDSVARGFCSELAHMCYVSGMAFNPEPVVPPLSARPDQVEKVLKTRYHDAKNKLQ
GRELDLLIVILPDNNGSLYGDLKRICETDLGVVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLID
ALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEVTKYAGLVCAQAHRQELIQDLFKQW
QDPARGTLTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT
FVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE
NNFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVSRGGMGAAA
GRSTRAPGANAAVRPLPALKENVKRVMFY

>Barrelclover KEH26855.1:245-1114 argonaute protein 1A [Medicago truncatula] PLRPGKGSYGRKTLVKANHFFAELPKKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRDSHLGKRLPAYD GRKSLYTAGPLPFISKDFRITLVDEDDGSGGQRRDREFKVVIKLAARADLHHLGLFLEGRQTDAPQEALQ VLDIVLRELPTSRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IEFVTQLLNRDVSARPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRIAGLTSQATRELTFPVDERGTMK SVVEYFFETYGFVIQHTQWPCLQVGNPQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPLDRE RDIMQTVHHNAYHEDPYAKEFGIKISDRLAQVEARILPAPWLKYNDTGREKDCLPQVGQWNMMNKKMFNG GSVKYWLCVNFSRTVQDSVARGFCYELAQMCYVSGMEFNAEPVVPALTARPDQVEKVLKNRYHDAKSKMP KDKELDLLIVILPDNNGSLYGDLKRICETDLGVVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLV DALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQ

WQDPVRGTLTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPV TFVVVQKRHHTRLFASNHQDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWD ENNFSADGLQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAVSRGGAGAA VGRSTRAPGANAAVRPLPALKDNVKKVMFY

>Wild\_peanut XP\_016203325.1:189-1055 protein argonaute 1 isoform X2 [Arachis ipaensis]

PLRPGKGSYGTKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEELVRLYRESHLGKRLPAYD GRKSLYTAGPLPFVSKEFRIMLIDEDDGTGAQRREREFKVVIKLAARADLHHLGLFLQGRQTDAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IEFVTQLLNRDVSSRPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFSETYGFHIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERE RDIMQTVHHNAYDQDPYAKEFGIKISEKLAQVEARILPPPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNHWFCINFSRNVQDSVARTFCYELAQMCQVSGMAFNLDPVVPPVSARPDQVEKVLKTRYYDAKNKLP GKDLDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVYKMSKQYLANVALKINVKVGGRNTVLVD ALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQW QDPNRGQVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT FVVVQKRHHTRLFASNHHDRNSIDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE NNFTADALQMLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAAAGRGMGAGR STRAPGASAAVRPLPSLKDNVKRVMFY

>Peanut XP\_025701446.1:190-1056 protein argonaute 1-like [Arachis hypogaea] PLRPGKGSYGTKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEELVRLYRESHLGKRLPAYD GRKSLYTAGPLPFVSKEFRIMLIDEDDGTGAQRREREFKVVIKLAARADLHHLGLFLQGRQTDAPQEALQ VLDIVLRELPTTRYSPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IEFVTQLLNRDVSTRPLSDADRVKIKKALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFSETYGFHIQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERE RDIMQTVHHNAYDQDPYAKEFGIKISEKLAQVEARILPPPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNHWFCINFSRNVQDSVARTFCYELAQMCQVSGMAFNLDPVVPPVSARPDQVEKVLKTRYYDAKNKLP GKDLDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVYKMSKQYLANVALKINVKVGGRNTVLVD ALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQW QDPNRGQVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPNYQPPVT FVVVQKRHHTRLFASNHHDRNSIDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWDE NNFTADALQMLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAAAGRGMGAGR STRAPGASAAVRPLPSLKDNVKRVMFY

>Castor\_bean XP\_002526275.1:191-1062 protein argonaute 1 [Ricinus communis] PLRPGKGSTGIRCIVKANHFFAELPDKDLHQYDVTITPEVTSRGVNRAVMEQLVKLYRESHLGKRLPAYD GRKSLYTAGPLPFISKEFKITLIDEDDGSGGQRREREFRVVIKLAARADLHHLGLFLQGRQADAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IDFVNQLLNRDVSSRPLSDADRVKIKKALRGVKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFYETYGFVIQHTQWPCLQVGNQQRPNYLPMEVCKVVEGQRYSKRLNERQITALLKVTCQRPQERE RDIMQTVHHNAYGNDPYAKEFGIKISEKLASVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNNWICINFSRNVQDSVARGFCYELAQMCYISGMAFNPEPVLPPVSARPEQVEKVLKTRYHDAMTKLQ QGKELDLLIVILPDNNGSLYGELKRICETDLGLVSQCCLTKHVFRMNKQYLANVALKINVKVGGRNTVLV DALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEVTKYAGLVCAQAHRQELIQDLFKE WQDPVRGRVTGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLYELDAIRKACASLEPNYQPPV TFVVVQKRHHTRLFANNHNDRNAVDKSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLWD ENKFTADGLQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGPVGGRGGMGG GAGARSTRGPAASAAVRPLPALKENVKRVMFY

>Cork\_oak XP\_023880904.1:197-1067 protein argonaute 1 [Quercus suber] PLRPGKGSTGTKCMVKANHFFAELPDKDLHQYDVSITPEVTSRGVNRAVMEQLVKLYKETHLGKRLPAYD GRKSLYTAGPLPFLSKEFKIPLIDEDDGSGGQRREREFKVVIKLAARADLHHLGLFLQGRQADAPQEALQ VLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IEFVTQLLNRDVTSRPLSDADRVKIKKALRGVKVEVTHRGNMRRKYRISGLTSQATRELTFPVDERGTMK SVVEYFYETYGFAIQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPQERE

RDIMOTVEHNAYHNDPYAKEFGIKISERLASVEARILPAPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GTVNNWICINFSRNVQDSVARGFCYELAQMCYISGMAFNPEPVLPPLSARPDQVERVLKTRYHDAMTKIQ PQGKELDLLIVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFRMSKQYLANVALKINVKVGGRNTVL VDALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEVTKYAGLVCAQAHRQELIQDLFK TWODPVRGTVSGGMIKELLISFRRATGOKPKRIIFYRDGVSEGOFYOVLLYELDAIRKACASLEPNYOPP VTFVVVOKRHHTRLFANNHHDRNAVDKSGNILPGTVVDSKICHPTEFDFYLCSHAGIOGTSRPAHYHVLW DENKFTADGLOSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAAAGRGMGG AGPRSTRPPGANAAVRPLPALKENVKRVMFY

>Peach XP 007210410.1:201-1068 protein argonaute 1 [Prunus persica] PLRPGKGSTGIRCTVKANHFFAELPDKDLHQYDVTITPEVTSRGVNRAVMEQLVKLYRESHLGKRLPAYD GRKSLYTAGPLPFLSKEFKIILIDEDDGPGGORREREFRVVIKFAARADLHHLGLFLOGROADAPOEALO VLDIVLRELPTSRYCPVGRSFYAPDLGRRQSLGEGLESWRGFYQSIRPTQMGLSLNIDMSSTAFIEPLPV IEFVTOLLNRDVTHRPLSDSDRVKIKKALRGVKVEVTHRGNMRRKYRISGLTSOATRELTFPVDERGTMK  ${\tt SVVEYFYETYGFVIQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPHDRE}$ QDIMRTVRHNAYHEDPYAKEFGIKISENLAQVEARILPPPWLKYHDTGREKDCLPQVGQWNMMNKKMVNG GKVNNWICINFSRNVQDSVARGFCSELAQMCYISGMAFNPEPVLPPISARPDQVEKVLKTRYHDAMTKLR VQGKELDLLVVILPDNNGSLYGDLKRICETDLGLVSQCCLTKHVFRMSKQYLANVALKINVKVGGRNTVL VDALSRRIPLVSDRPTIIFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFK TWQDPARGTVSGGMIKELLISFRRATGQKPQRIIFYRDGVSEGQFYQVLLYELDAIRKACASLEPNYQPP VTFVVVQKRHHTRLFANNHHDRNTVDRSGNILPGTVVDSKICHPTEFDFYLCSHAGIQGTSRPAHYHVLW DENKFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARFYMEPETSDSGSMTSGAPGRGGMGA RSTRAPGANAAVRPLPALKENVKRVMFY

#### Alignment:

(Obtained using MUSCLE version 3.8 at EBI)

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

Fruit fly Barrelclover Wild peanut Peanut. Red clover Chickpea Soybean Tepary bean Mung bean Peach Castor\_bean Cork oak

Fruit fly Soybean

PRRPNLGREGRPIVLRANHFOVTMPRGYVHHYDINIOPDKCPRKVNREIIETMVHAYSKI PLRPGKGSYGRKTLVKANHFFAELPKKDLHOYDVTITPEVTSRGVNRAVMEOLVRLYRDS PLRPGKGSYGTKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEELVRLYRES PLRPGKGSYGTKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEELVRLYRES PLRPGKGSTGKRCIVKANHFIAELPKKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRDS PLRPGKGSCGRKCVVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRES PLRPGKGSYGTKCVVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRES PLRPGKGSYGIKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRES
PLRPGKGSYGIKCIVKANHFFAELPNKDLHQYDVTITPEVTSRGVNRAVMEQLVRLYRES PLRPGKGSTGIRCTVKANHFFAELPDKDLHQYDVTITPEVTSRGVNRAVMEQLVKLYRES PLRPGKGSTGIRCIVKANHFFAELPDKDLHQYDVTITPEVTSRGVNRAVMEQLVKLYRES PLRPGKGSTGTKCMVKANHFFAELPDKDLHQYDVSITPEVTSRGVNRAVMEQLVKLYKET 

Fruit\_fly -FGVLKPVFDGRNNLYTRDPLPIGNERLELEVTLPGEG------KDRIFRVTIKWQAQVS
Barrelclover HLGKRLPAYDGRKSLYTAGPLPFISKDFRITLVDEDDG-SGGQRRDREFKVVIKLAARAD
Wild\_peanut HLGKRLPAYDGRKSLYTAGPLPFVSKEFRIMLIDEDDG-TGAQRREREFKVVIKLAARAD
Peanut HLGKRLPAYDGRKSLYTAGPLPFVSKEFRIMLIDEDDG-TGAQRREREFKVVIKLAARAD
Red\_clover HLGKRLPAYDGRKSLYTAGPLPFISKDFRITLVDEDDG-SGSQRRDREFKVVIKLAARAD
Chickpea HLGKRLPAYDGRKSLYTAGPLPFISKDFRITLVDDDDG-TGGQRRDREFKVVIKLAARAD
Velvet\_bean HLGKRLPAYDGRKSLYTAGALPFISKEFRITLIDDDEG-AGQQRRDREFKVVIKLAARAD
Jequirity\_bean HLGKRLPAYDGRKSLYTAGPLPFISKEFRITLIDDDEG-AGGQRRDREFKVVIKFAARAD
Pigeon\_pea HLGKRLPAYDGRKSLYTAGPLPFISKEFRITLVDDDEG-AGGQRRDREFKVVIKLAARAD -FGVLKPVFDGRNNLYTRDPLPIGNERLELEVTLPGEG-----KDRIFRVTIKWQAQVS HLGKRLPAYDGRKSLYTAGPLPFMSKEFRIVLADDDEG-AGGQRRDREFKVVIKLAARAD

Tepary\_bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut Peanut Red clover Chickpea Velvet bean Jequirity bean Pigeon pea Soybean Tepary bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut Peanut Red clover Chickpea Velvet bean Jequirity bean Pigeon pea Soybean Tepary bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut Peanut Red clover Chickpea Velvet bean Soybean Tepary\_bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover

HLGKRLPAYDGRKSLYTAGPLPFISKEFRITLIDDDEGAAGGORRDREFKVVIKLAARAD HLGKRLPAYDGRKSLYTAGPLPFISKEFRITLIDDDEGAAGGQRRDREFKVVIKLAARAD HLGKRLPAYDGRKSLYTAGPLPFLSKEFKIILIDEDDG-PGGQRREREFRVVIKFAARAD HLGKRLPAYDGRKSLYTAGPLPFISKEFKITLIDEDDG-SGGQRREREFRVVIKLAARAD HLGKRLPAYDGRKSLYTAGPLPFLSKEFKIPLIDEDDG-SGGQRREREFKVVIKLAARAD \* \* \* \* \* \*

LFNLEEALEGRTRQIPYDAILALDVVMRHLPSMTYTPVGRSFFSSPEGYYHPLGGGREVW LHHLGLFLEGRQTDAPQEALQVLDIVLRELPTSRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLQGRQTDAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLQGRQTDAPQEALQVLDIVLRELPTTRYSPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLEGRQTDAPQEALQVLDIVLRELPTSRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLEGROTDAPOEALOVLDIVLRELPTTRYCPVGRSFYSPDLGRROPLGEGLESW LHHLGLFLOGROTDAPOEALOVLDIVLRELPTTRYCPVGRSFYSPDLGRROPLGEGLESW  $\verb|LHHLGLFLQGRQTDAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW|$ LHHLGLFLQGRQTDAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGDGLESW LHHLGLFLQGRQTDAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLQGKQTDAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLQGRQTDAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLQGRQADAPQEALQVLDIVLRELPTSRYCPVGRSFYAPDLGRRQSLGEGLESW LHHLGLFLQGRQADAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW LHHLGLFLQGRQADAPQEALQVLDIVLRELPTTRYCPVGRSFYSPDLGRRQPLGEGLESW \*.:\* \*:\*. : \* :\*: .\*\*: \* \*\*\*\*\*\*::. \* :.\*\* \* \*

FGFHQSVRPSQWKMMLNIDVSATAFYKAQPVIDFMCEVLDIRDINEQRKPLTDSQRVKFT RGFYOSIRPTOMGLSLNIDMSSTAFIEPLPVIEFVTOLLN-RDVSA--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIEFVTQLLN-RDVSS--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIEFVTQLLN-RDVST--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIEFVTQLLN-RDVSA--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVTQLLN-RDVST--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVTQLLN-RDVSS--RPLSDSDRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVTQLLN-RDVSS--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVTQLLN-RDVST--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVNQLLN-RDVSA--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIDFVTQLLN-RDVSA--RPLSDADRVKIK RGFYOSIRPTOMGLSLNIDMSSTAFIEPLPVIDFVTOLLN-RDVSA--RPLSDADRVKIK RGFYQSIRPTQMGLSLNIDMSSTAFIEPLPVIEFVTQLLN-RDVTH--RPLSDSDRVKIK RGFYOSIRPTOMGLSLNIDMSSTAFIEPLPVIDFVNOLLN-RDVSS--RPLSDADRVKIK RGFYOSIRPTOMGLSLNIDMSSTAFIEPLPVIEFVTOLLN-RDVTS--RPLSDADRVKIK 

KEIKGLKIEITHCGQMRRKYRVCNVTRRPAQMQSFPLQLENGQTVECTVAKYFLDKYRMK KALRGIKVEVTHRGNMRRKYRIAGLTSQATRELTFPVD-ERGTMK--SVVEYFFETYGFV KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFSETYGFH KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFSETYGFH KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFS KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV Jequirity\_bean KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYDTYGFV Pigeon pea KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGIKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGVKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGVKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFV KALRGVKVEVTHRGNMRRKYRISGLTSQATRELTFPVD-ERGTMK--SVVEYFYETYGFA :\*.:\*\* :.\* :

> LRYPHLPCLQVGQEHKHTYLPLEVCNIVAGQRCIKKLTDMQTSTMIKATARSAPDREREI IQHTQWPCLQVGNPQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPLDRERDI

Wild\_peanut
Peanut
Red\_clover
Chickpea
Velvet\_bean
Jequirity\_bean
Pigeon\_pea
Soybean
Tepary\_bean
Mung\_bean
Peach
Castor\_bean
Cork oak

Fruit\_fly
Barrelclover
Wild\_peanut
Peanut
Red\_clover
Chickpea
Velvet\_bean
Jequirity\_bean
Pigeon\_pea
Soybean
Tepary\_bean
Mung\_bean
Peach
Castor\_bean
Cork oak

Fruit\_fly
Barrelclover
Wild\_peanut
Peanut
Red\_clover
Chickpea
Velvet\_bean
Jequirity\_bean
Pigeon\_pea
Soybean
Tepary\_bean
Mung\_bean
Peach
Castor\_bean
Cork oak

Fruit\_fly
Barrelclover
Wild\_peanut
Peanut
Red\_clover
Chickpea
Velvet\_bean
Jequirity\_bean
Pigeon\_pea
Soybean
Tepary bean

IQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERERDI
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IQHTQWPCLQVGNTQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPVERERDI
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IQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPQERERDI
IQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPQERERDI
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IQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPQERERDI
IQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPQERERDI
IQHTQWPCLQVGNQQRPNYLPMEVCKIVEGQRYSKRLNERQITALLKVTCQRPQERERDI

NNLVKRADFNNDSYVQEFGLTISNSMMEVRGRVLPPPKLQYGGRVSTGLTGQQLFPPQNK MQTVHHNAYHEDPYAKEFGIKISDRLAQVEARILPAPWLKYN---DTGREKDCL----MQTVHHNAYDQDPYAKEFGIKISEKLAQVEARILPPPWLKYH---DTGREKDCL----MQTVHHNAYDQDPYAKEFGIKISEKLAQVEARILPPPWLKYH---DTGREKDCL----MQTVHHNAYHDDPYAKEFGIKISDKLAQVEARILPPPWLKYH---DTGKEKDCL-----MQTVHHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYH---DTGREKDCL-----MQTVHHNAYHDDPYAKEFGIKISEKLAQVEARILPPPWLKYH---DTGREKDCL-----MQTVHHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYH---DTGREKDCL-----MQTVHHNAYHDDPYAKEFGIKISEKLAQVEARILPAPWLKYH---DTGREKDCL-----MQTVHHNAYHEDPYAKEFGIKISEKLAQVEARILPAPWLKYH---DTGREKDCL-----MOTVYHNAYHEDPYAKEFGIKISEKLAOVEARILPAPWLKYH---DTGREKDCL-----MQTVYHNAYHEDPYAQEFGIKISEKLAQVEARILPAPWLKYH---DTGREKDCL-----MRTVRHNAYHEDPYAKEFGIKISENLAQVEARILPPPWLKYH---DTGREKDCL-----MQTVHHNAYGNDPYAKEFGIKISEKLASVEARILPAPWLKYH---DTGREKDCL-----MQTVEHNAYHNDPYAKEFGIKISERLASVEARILPAPWLKYH---DTGREKDCL----.\*\* : \* 

VSLASPNQGVWDMRGKQFFTGVEIRIWAIACFAPQRTVREDALRNFTQQLQKISNDAGMP ----PQVGQWNMMNKKMFNGGSVKYW--LCVNFSRTVQDSVARGFCYELAQMCYVSGME ----PQVGQWNMMNKKMVNGGTVNHW--FCINFSRNVQDSVARTFCYELAQMCQVSGMA ----PQVGQWNMMNKKMVNGGTVNHW--FCINFSRNVQDSVARTFCYELAQMCQVSGMA ----PQVGQWNMMNKKMVNGGTVNNW--FCVNFSRNVQDSVARGFCSELAHMCYVSGMA ----POVGOWNMMNKKMVNGGTVNNW--FCVNFSRNVODSVARGFCCELAOMCYISGMA ----POVGOWNMMNKKMVNGGTVNNW--FCINFSRNVQDSVARGFCYELAQMCYISGMA ----POVGOWNMMNKKMVNGGTVNNW--FCINFSRNVQDSVARGFCYELAQMCYISGMA ----PQVGQWNMMNKKMVNGGTVNNW--FCVNFSRNVQDTVARGFCYELAQMCYISGMA ----POVGOWNMMNKKMVNGGTVNNW--FCINFSRNVQDSVARGFCYELAQMCYISGMA ----PQVGQWNMMNKKMVNGGTVNNW--FCINFSRSVQDSVARGFCYELAQMCYISGMA ----PQVGQWNMMNKKMVNGGTVNHW--FCINFSRSVQDSVARGFCYELAQMCYISGMA ----PQVGQWNMMNKKMVNGGKVNNW--ICINFSRNVQDSVARGFCSELAQMCYISGMA ----PQVGQWNMMNKKMVNGGTVNNW--ICINFSRNVQDSVARGFCYELAQMCYISGMA ----PQVGQWNMMNKKMVNGGTVNNW--ICINFSRNVQDSVARGFCYELAQMCYISGMA \*: \* \*:\* .\*:...\* :. \* \*. .\*.\*.: . \* \* :\* ::. :\*\*

IIGQPCFCKYATGPDQVEPMF--RY----LKIT--FPGLQLVVVVLPGKT-PVYAEVKRV
FNAEPVVPALTARPDQVEKVLKNRYHDAKSKMP-KDKELDLLIVILPDNNGSLYGDLKRI
FNLDPVVPPVSARPDQVEKVLKTRYYDAKNKLP--GKDLDLLIVILPDNNGSLYGDLKRI
FNLDPVVPPVSARPDQVEKVLKTRYYDAKNKLP--GKDLDLLIVILPDNNGSLYGDLKRI
FNPEPVVPPLSARPDQVEKVLKTRYHDAKNKLQ--GRELDLLIVILPDNNGSLYGDLKRI
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FNPEPVVPPVSARPDQVEKVLKTRYHDAKNKLQ--GRELDLLIVILPDNNGSLYGDLKRI

Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut Peanut Red clover Chickpea Velvet bean Jequirity bean Pigeon pea Soybean Tepary bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut Peanut Soybean Tepary bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut Peanut Red clover Chickpea Velvet bean Jequirity bean Pigeon pea Soybean Tepary bean Mung bean Peach Castor bean Cork oak

Fruit fly Barrelclover Wild peanut

FNPEPVVPPVSARPDOVEKVLKTRYHDAKNKLO--GRELDLLIVILPDNNGSLYGDLKRI FNPEPVLPPISARPDQVEKVLKTRYHDAMTKLRVQGKELDLLVVILPDNNGSLYGDLKRI FNPEPVLPPVSARPEQVEKVLKTRYHDAMTKLQ-QGKELDLLIVILPDNNGSLYGELKRI FNPEPVLPPLSARPDQVERVLKTRYHDAMTKIQPQGKELDLLIVILPDNNGSLYGDLKRI : :\* : \*:\*\*: :: \*\* 

GDTVLGMATQCVQAKNVNKTSPQTLSNLCLKINVKLGGINSILVPSIR---PKVFNEPVI CETDLGVVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSQCCLTKHVYKMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSQCCLTKHVYKMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGVVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLIDALSRRIPLVSDRPTI CETELGLVSOCCLTKHVFKMSKOYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSOCCLTKHVFKMSKOYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVDAISRRIPLVSDRPTI CETDLGLVSOCCLTKHVFKMSKOYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSOCCLTKHVFKMSKOYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVGDRPTI CETDLGLVSQCCLTKHVFKMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVGDRPTI CETDLGLVSQCCLTKHVFRMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSQCCLTKHVFRMNKQYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI CETDLGLVSQCCLTKHVFRMSKQYLANVALKINVKVGGRNTVLVDALSRRIPLVSDRPTI 

FLGADVTHPPAGDNKKPSIAAVVGSMD-AHPSRYAATVRVQQHRQEIIQEL------IFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQWQDPVR IFGADVTHPHPGEDSSPSIAAVVASODWPEITKYAGLVCAOAHROELIODLFKOWODPNR IFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKQWQDPNR IFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQWQDPVR IFGADVTHPHPGEDSSPSIAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQWQDPVR IFGADVTHIMIGESSOTSTAAVVASQDYPEITKYAGLVCAQAHRQELIQDLFKQWQDPVR IFGADVTHPHPGEDSSPSIAAVVASQDWPEITKYAGLVCAQAHRQELIQDLFKTWQDPVR IFGADVTHPHPGEDSSPSIAAVVASQDWPEVTKYAGLVCAQAHRQELIQDLFKEWQDPVR IFGADVTHPHPGEDSSPSIAAVVASODWPEVTKYAGLVCAOAHROELIODLFKTWODPVR 

> ----SSMVRELLIMFYKSTGGYKPHRIILYRDGVSEGQFPHVLQHELTAIREACIKLEPE GTLTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GQVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GQVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTLTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTLTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTLTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLFELDAIRKACASLEPN GTVSGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLYELDAIRKACASLEPN GRVTGGMIKELLISFRRATGQ-KPQRIIFYRDGVSEGQFYQVLLYELDAIRKACASLEPN GTVSGGMIKELLISFRRATGQ-KPKRIIFYRDGVSEGQFYQVLLYELDAIRKACASLEPN

YRPGITFIVVQKRHHTRLFCAEKKEQSG--KSGNIPAGTTVDVGITHPTEFDFYLCSHQG YQPPVTFVVVQKRHHTRLFASNHQDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG YQPPVTFVVVQKRHHTRLFASNHHDRNSIDRSGNILPGTVVDSKICHPTEFDFYLCSHAG Peanut Red clover Chickpea Soybean

YOPPVTFVVVOKRHHTRLFASNHHDRNSIDRSGNILPGTVVDSKICHPTEFDFYLCSHAG YQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG YQPPVTFVVVQKRHHTRLFASDHRDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG Velvet\_bean YQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG
Pigeon\_pea YQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG
Pigeon\_pea YQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG YQPPVTFVVVQKRHHTRLFASNHHDKSSFDRSGNILPGTVVDSKICHPTEFDFYLCSHAG Soybean YQPPVTFVVVQKRHHTRLFASNHHDKSSFDRSGNILPGTVVDSKICHPTEFDFYLCSHAG
Tepary\_bean YQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG
Mung\_bean YQPPVTFVVVQKRHHTRLFASNHHDKSSVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG
Peach YQPPVTFVVVQKRHHTRLFANNHHDRNTVDRSGNILPGTVVDSKICHPTEFDFYLCSHAG
Castor\_bean YQPPVTFVVVQKRHHTRLFANNHNDRNAVDKSGNILPGTVVDSKICHPTEFDFYLCSHAG
Cork\_oak YQPPVTFVVVQKRHHTRLFANNHHDRNAVDKSGNILPGTVVDSKICHPTEFDFYLCSHAG 

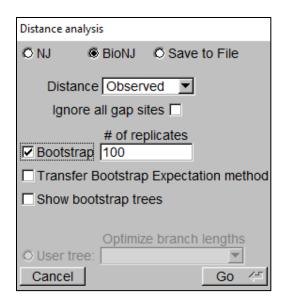
Fruit\_fly
Barrelclover
UgGTSRPAHYHVLWDDNHFDSDELQCLTYQLCHTYVRCTRSVSIPAPAYYAHLVAFRARY
Barrelclover
UlgGTSRPAHYHVLWDENNFSADGLQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
Wild\_peanut
Peanut
UgGTSRPAHYHVLWDENNFTADALQMLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
Red\_clover
Chickpea
UgGTSRPAHYHVLWDENNFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
Chickpea
UgGTSRPAHYHVLWDENNFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
Chickpea
UgGTSRPAHYHVLWDENNFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
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UgGTSRPAHYHVLWDENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADALQTLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADALQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
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UgGTSRPAHYHVLWDENNFTADGLQSLTNNLCYTYARCTRSVSIVPPAYYAHLAAFRARF
UgGTSRPAHYHVLWDENNFTADGLQSLTNNLCYTYARCTRSVSIVPAYYAHLAAFRARF
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UgGTSR

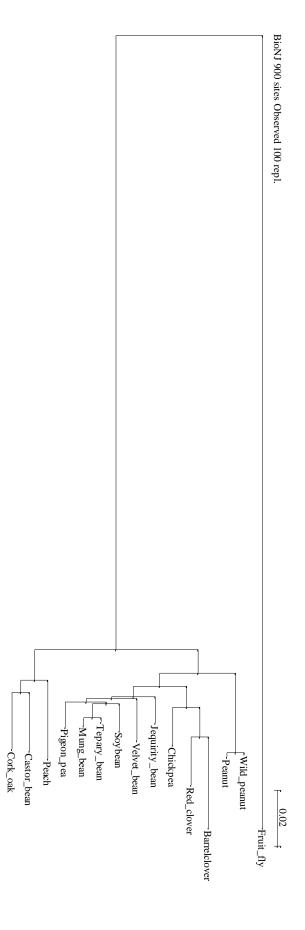
Castor bean Cork oak

YMEPETSDSGSMTSGPVGGRGG---MGGGAGARSTRGPAASAAVRPLPALKENVKRVMFY YMEPETSDSGSMTSGAAAGRGM----GGAGPRSTRPPGANAAVRPLPALKENVKRVMFY :: : \*\*\*. \* . \* \* . \* \* . : : : . \* . . :

[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.

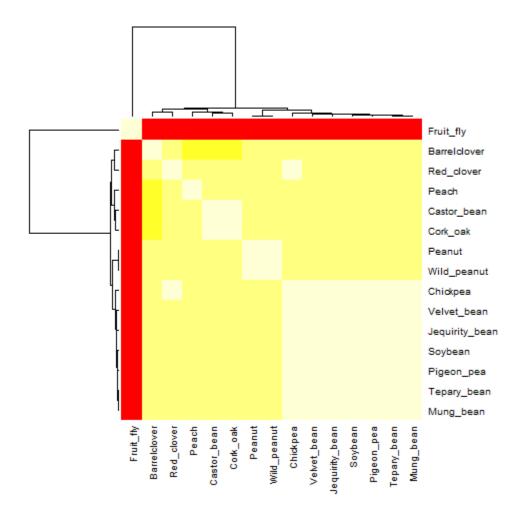
I imported the output from MUSCLE into SeaView to create a distance-based phylogenetic tree (using bootstrapping). See next page for distance analysis parameters and phylogenetic tree.





[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 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).

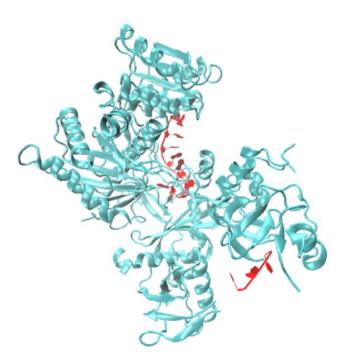
HINT: You can use a single sequence from your alignment or generate a consensus sequence from your alignment using the Bio3D function consensus(). The Bio3D functions blast.pdb(), plot.blast() and pdb.annotate() are likely to be of most relevance for completing this task. Note that the results of blast.pdb() 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
5VM9	X-RAY DIFFRACTION	3.28	Homo sapiens	0	44.549
3VNA	X-RAY DIFFRACTION	2	Arabidopsis thaliana	7.67E-84	84.459
5THE	X-RAY DIFFRACTION	2.1	Vanderwaltozyma polyspora	1.44E-52	33.506

[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?



#### 5VM9

This structure might be somewhat similar to the tepary bean argonaute, but since its identity is only 44.549%, it would probably have some differences.

In the figure to the left, the cyan cartoon represents the argonaute protein, and the red ribbon portion represents the guide RNA that is bound to argonaute.

[Q10] Perform a "Target" search of ChEMBEL (<a href="https://www.ebi.ac.uk/chembl/">https://www.ebi.ac.uk/chembl/</a>) 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?

ChEMBL has information about 6 binding assays (CHEMBL2311232), but no data on ligand efficiency.

https://www.ebi.ac.uk/chembl/target/inspect/CHEMBL2311232

The first listed binding assay discovered inhibitors of pre-miRNA degradation as well as enhancers of miRNA processing in a high-throughput screen of a few thousand small molecules. The overarching goal is to develop drugs that could be used in miRNA therapy to rescue global miRNA defects.

K. Asada, E. Canestrari, Z. Paroo, Bioorganic & Medicinal Chemistry Letters A druggable target for rescuing microRNA defects. *Bioorg. Med. Chem. Lett.* **26**, 4942–4946 (2016).

http://europepmc.org/abstract/MED/27641467

The remaining five binding assays detail xanthone derivatives (specifically, an aminoalkoxy-substituted thioxanthone derivative) inhibiting miRNA processing. They believe this molecule could be developed as a drug to treat miRNA related disease.

A. Murata, T. Fukuzumi, S. Umemoto, K. Nakatani, Bioorganic & Medicinal Chemistry Letters Xanthone derivatives as potential inhibitors of miRNA processing by human Dicer: Targeting secondary structures of pre-miRNA by small molecules. *Bioorg. Med. Chem. Lett.* **23**, 252–255 (2013).

http://europepmc.org/abstract/MED/23164709