# Find-A-Gene - A. Geffre - BGGN213 Spring 2019

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06/03/2019

Find-a-gene project requires us to use a fovorite gene to find homologous sequences from unannotated organisms. (e.g. BLAST favorite seq - find a potential homolog from an under-annotated species, etc.)

# Question 1

> 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.

Protein of Interest: PGRP-S2; peptidoglycan recognition protein S2 (*Apis mellifera*)

NP_001157188.1 peptidoglycan-recognition protein S2 precursor [Apis mellifera]
MTKLIAVLFLLVNCQILFCSVHETPVRPRIISRSEWGARKPTTTIRALAQNPPPFVIIHHSATDSCI
TQA
ICNARVRSFQNYHIDEKGWGDIGYQFLVGEDGNIYEGRGWDKHGAHSISYNSKSIGICIIGNFV
GHTPNA AAIEATKNLISYGVAIGKIQSNYTLLGHRQTTRTSCPGDSLYELIKTWPHWSSI

Accession Number: Gene ID: 412484 Gene: NM_001163716.1 Protein: NP_001157188.1

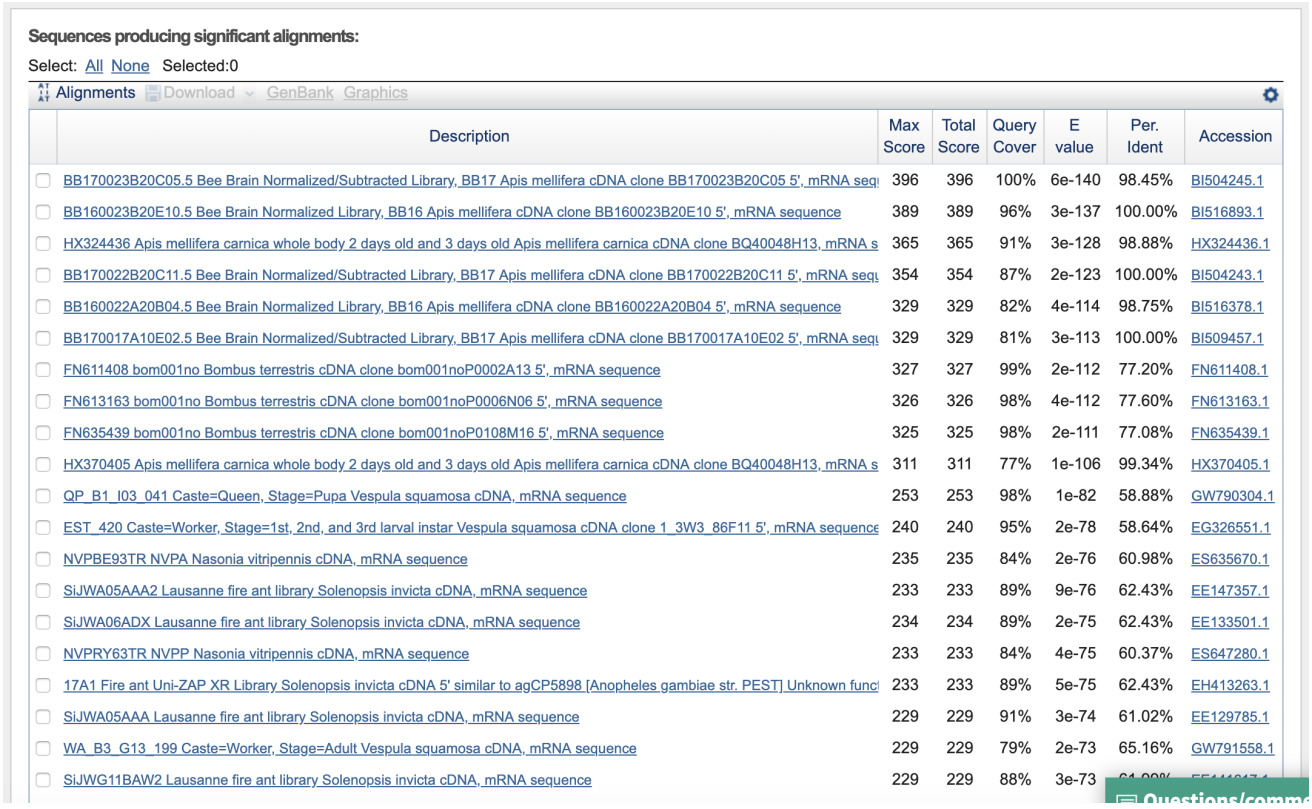
This protein is related to anti-viral immune response in honey bees (Nazzi F, Brown SP, Annoscia D, et al. Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. PLoS Pathog. 2012;8(6):e1002735. doi:10.1371/journal.ppat.1002735).

# Question 2

> 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). tBLASTn search ID: DA9TFDNZ014, searching est (Database of GenBank+EMBL+DDBJ sequences from EST Division) database.

> 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.

An overview of the top hits:



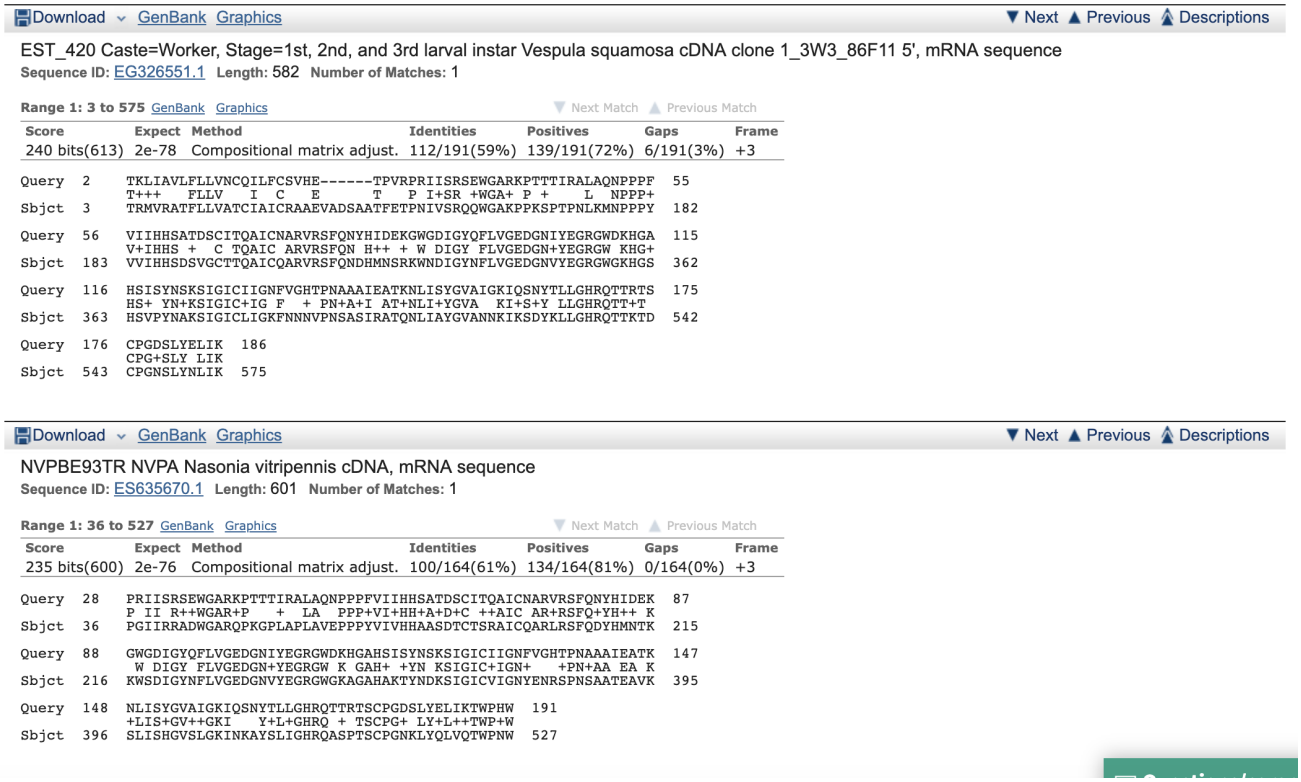
**Sequences producing significant alignments:**

Select: All None Selected:0

Alignments Download GenBank Graphics

| Description | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|---|---|
| BB170023B20C05.5 Bee Brain Normalized/Subtracted Library, BB17 Apis mellifera cDNA clone BB170023B20C05 5', mRNA seq | 396 | 396 | 100% | 6e-140 | 98.45% | BI504245.1 |
| BB160023B20E10.5 Bee Brain Normalized Library, BB16 Apis mellifera cDNA clone BB160023B20E10 5', mRNA sequence | 389 | 389 | 96% | 3e-137 | 100.00% | BI516893.1 |
| HX324436 Apis mellifera carnica whole body 2 days old and 3 days old Apis mellifera carnica cDNA clone BQ40048H13, mRNA s | 365 | 365 | 91% | 3e-128 | 98.88% | HX324436.1 |
| BB170022B20C11.5 Bee Brain Normalized/Subtracted Library, BB17 Apis mellifera cDNA clone BB170022B20C11 5', mRNA seq | 354 | 354 | 87% | 2e-123 | 100.00% | BI504243.1 |
| BB160022A20B04.5 Bee Brain Normalized Library, BB16 Apis mellifera cDNA clone BB160022A20B04 5', mRNA sequence | 329 | 329 | 82% | 4e-114 | 98.75% | BI516378.1 |
| BB170017A10E02.5 Bee Brain Normalized/Subtracted Library, BB17 Apis mellifera cDNA clone BB170017A10E02 5', mRNA seq | 329 | 329 | 81% | 3e-113 | 100.00% | BI509457.1 |
| FN611408 bom001no Bombus terrestris cDNA clone bom001noP0002A13 5', mRNA sequence | 327 | 327 | 99% | 2e-112 | 77.20% | FN611408.1 |
| FN613163 bom001no Bombus terrestris cDNA clone bom001noP0006N06 5', mRNA sequence | 326 | 326 | 98% | 4e-112 | 77.60% | FN613163.1 |
| FN635439 bom001no Bombus terrestris cDNA clone bom001noP0108M16 5', mRNA sequence | 325 | 325 | 98% | 2e-111 | 77.08% | FN635439.1 |
| HX370405 Apis mellifera carnica whole body 2 days old and 3 days old Apis mellifera carnica cDNA clone BQ40048H13, mRNA s | 311 | 311 | 77% | 1e-106 | 99.34% | HX370405.1 |
| QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence | 253 | 253 | 98% | 1e-82 | 58.88% | GW790304.1 |
| EST_420 Caste=Worker, Stage=1st, 2nd, and 3rd larval instar Vespula squamosa cDNA clone 1_3W3_86F11 5', mRNA sequence | 240 | 240 | 95% | 2e-78 | 58.64% | EG326551.1 |
| NVPBE93TR NVPA Nasonia vitripennis cDNA, mRNA sequence | 235 | 235 | 84% | 2e-76 | 60.98% | ES635670.1 |
| SiJWA05AAA2 Lausanne fire ant library Solenopsis invicta cDNA, mRNA sequence | 233 | 233 | 89% | 9e-76 | 62.43% | EE147357.1 |
| SiJWA06ADX Lausanne fire ant library Solenopsis invicta cDNA, mRNA sequence | 234 | 234 | 89% | 2e-75 | 62.43% | EE133501.1 |
| NVPRY63TR NVPP Nasonia vitripennis cDNA, mRNA sequence | 233 | 233 | 84% | 4e-75 | 60.37% | ES647280.1 |
| 17A1 Fire ant Uni-ZAP XR Library Solenopsis invicta cDNA 5' similar to agCP5898 [Anopheles gambiae str. PEST] Unknown funct | 233 | 233 | 89% | 5e-75 | 62.43% | EH413263.1 |
| SiJWA05AAA Lausanne fire ant library Solenopsis invicta cDNA, mRNA sequence | 229 | 229 | 91% | 3e-74 | 61.02% | EE129785.1 |
| WA_B3_G13_199 Caste=Worker, Stage=Adult Vespula squamosa cDNA, mRNA sequence | 229 | 229 | 79% | 2e-73 | 65.16% | GW791558.1 |
| SiJWG11BAW2 Lausanne fire ant library Solenopsis invicta cDNA, mRNA sequence | 229 | 229 | 88% | 3e-73 | | |

Some select hits of interest:



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EST_420 Caste=Worker, Stage=1st, and 3rd larval instar Vespula squamosa cDNA clone 1_3W3_86F11 5', mRNA sequence

**Sequence ID:** EG326551.1 **Length:** 582 **Number of Matches:** 1

**Range 1: 3 to 575** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Method | Identities | Positives | Gaps | Frame |
|---|---|---|---|---|---|---|
| 240 bits(613) | 2e-78 | Compositional matrix adjust. | 112/191(59%) | 139/191(72%) | 6/191(3%) | +3 |

```
Query  2    TKLIAVLFLLVNCQILFCSVHE------TPVRPRIISRSEWGARKPTTTIRALAQNPPPF  55
            T+++   FLLV   I  C   E      T   P I+SR +WGA+ P +    L  NPPP+
Sbjct  3    TRMVRATFLLVATCIAICRAAEVADSAATFETPNIVSRQQWGAKPPKSPTPNLKMNPPPY  182

Query  56   VIIHHSATDSCITQAICNARVRSFQNYHIDEKGWGDIGYQFLVGEDGNIYEGRGWDKHGA  115
            V+IHHS +  C TQAIC ARVRSFQN H++ + W DIGY FLVGEDGN+YEGRGW KHG+
Sbjct  183  VVIHHSDSVGCTTQAICQARVRSFQNDHMNSRKWNDIGYNFLVGEDGNVYEGRGWGKHGS  362

Query  116  HSISYNSKSIGICIIGNFVGHTPNAAAIEATKNLISYGVAIGKIQSNYTLLGHRQTTRTS  175
            HS+ YN+KSIGIC+IG F  + PN+A+I AT+NLI+YGVA  KI+S+Y LLGHRQTT+T
Sbjct  363  HSVPYNAKSIGICLIGKFNNNVPNSASIRATQNLIAYGVANNKIKSDYKLLGHRQTTKTD  542

Query  176  CPGDSLYELIK  186
            CPG+SLY LIK
Sbjct  543  CPGNSLYNLIK  575
```



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NVPBE93TR NVPA Nasonia vitripennis cDNA, mRNA sequence

**Sequence ID:** ES635670.1 **Length:** 601 **Number of Matches:** 1

**Range 1: 36 to 527** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Method | Identities | Positives | Gaps | Frame |
|---|---|---|---|---|---|---|
| 235 bits(600) | 2e-76 | Compositional matrix adjust. | 100/164(61%) | 134/164(81%) | 0/164(0%) | +3 |

```
Query  28   PRIISRSEWGARKPTTTIRALAQNPPPFVIIHHSATDSCITQAICNARVRSFQNYHIDEK  87
            P II R++WGAR+P    +  LA  PPP+VI+HH+A+D+C ++AIC AR+RSFQ+YH++ K
Sbjct  36   PGIIRRADWGARQPKGPLAPLAVEPPPYVIVHHAASDTCTSRAICQARLRSFQDYHMNTK  215

Query  88   GWGDIGYQFLVGEDGNIYEGRGWDKHGAHSISYNSKSIGICIIGNFVGHTPNAAAIEATK  147
             W DIGY FLVGEDGN+YEGRGW K GAH+ +YN KSIGIC+IGN+   +PN+AA EA K
Sbjct  216  KWSDIGYNFLVGEDGNVYEGRGWGKAGAHAKTYNDKSIGICVIGNYENRSPNSAATEAVK  395

Query  148  NLISYGVAIGKIQSNYTLLGHRQTTRTSCPGDSLYELIKTWPHW  191
            +LIS+GV++GKI   Y+L+GHRQ + TSCPG+ LY+L++TWP+W
Sbjct  396  SLISHGVSLGKINKAYSLIGHRQASPTSCPGNKLYQLVQTWPNW  527
```

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.

I am interested in FN611408.1 (*Bombus terrestris)*; GW790304.1, (*Vespula squamosa*); EE147357.1 (*Solenopsis invicta*), ES647280.1 (*Nasonia vitripens*) etc.

All of these sequences appear to be hitherto unannotated mRNAs, all of which belong to Hymenopterans. Vespula squamosa is likely the most under-annotated, as it is not a model organism or agriculturally-relevant species.

# Question 3

> 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

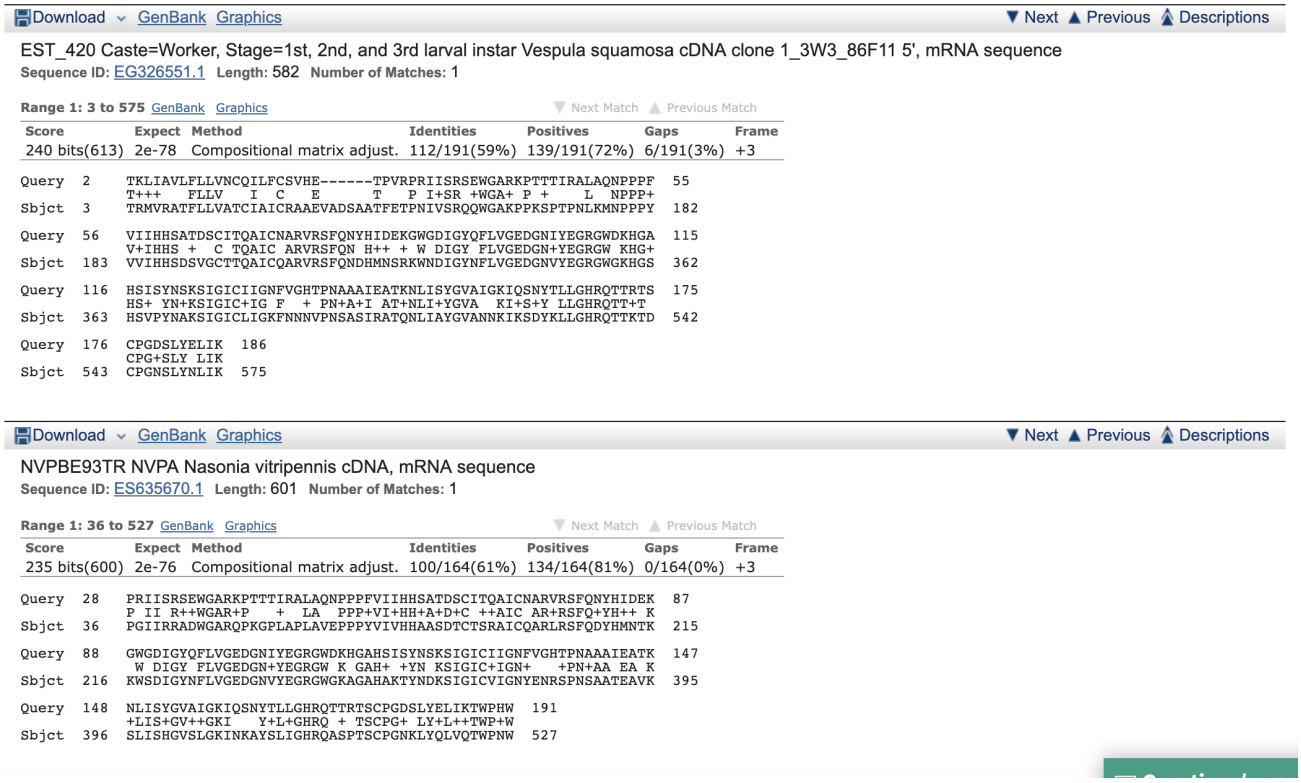
> may not start with a methionine if you don't have the complete coding region. Make sure the sequence you provide includes a header/subject line and is in traditional FASTA format. Here, tell me the name of the novel protein, and the species from which it derives. It is very unlikely (but still definitely 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.

## The Target Sequence

Below follows the FASTA sequence for the cDNA clone from *V. squamosa*: GW790304.1 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence

CGAGAATGGTACGAGCAACGTTTCTACTTGTTGCAACGTGCATTGCAATTTGCCGTGCAGC
GGAAGTTGCAGATTCGGCT
GCAACCTTTGAAACACCTAATATCGTTTCAAGACAACAATGGGGAGCTAAACCGCCGAAAA
GCCCTACGCCTAATCTTAA
AATGAATCCACCTCCTTACGTTGTTATTCATCATTCGGATTCAGTTGGTTGTACCACTCAAGC
AATTTGTCAGGCTAGAG
TTAGAAGTTTTCAGAATGATCATATGAACTCGAGAAAATGGAATGACATCGGCTACAACTTTT
TGGTCGGTGAAGATGGT
AATGTTTACGAAGGTCGTGGCTGGGGTAAACATGGCTCCCATTCAGTCCCGTACAATGCCA
AGAGTATCGGTATTTGCCT
TATTGGTAAATTTAACAATAACGTACCGAATTCAGCGAGTATTCGAGCAACACAAAATTTGATA
GCTTACGGAGTAGCTA
ACAACAAAATCAAATCCGATTATAAGCTTCTTGGCCATCGACAAACCACTAAAACTGATTGT
CCTGGAAATTCTCTATAC
AATTTGATTAAAACATGGCCTCACTGGACCGACACGCCATAAGAAATATAATCGTTATGCGAT
TAAATTAATCTACCAAG
TATAACGTCTTATTGTTTTGGACTCGACGATCATTGGAAATTAACGATCATCGGATCGATAACT
TTTGTTGACTTTTCTT
TAATTAACGATGGATACCTTCAAATTCGATTTTCGTGCGAACATTTTTACTTGATGTTTCGTAA
AGGAAGAG

BLAST gives this translated protein sequence:



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EST_420 Caste=Worker, Stage=1st, 2nd, and 3rd larval instar Vespula squamosa cDNA clone 1_3W3_86F11 5', mRNA sequence

**Sequence ID:** EG326551.1 **Length:** 582 **Number of Matches:** 1

**Range 1: 3 to 575** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Method | Identities | Positives | Gaps | Frame |
|---|---|---|---|---|---|---|
| 240 bits(613) | 2e-78 | Compositional matrix adjust. | 112/191(59%) | 139/191(72%) | 6/191(3%) | +3 |

```
Query  2    TKLIAVLFLLVNCQILFCSVHE------TPVRPRIISRSEWGARKPTTTIRALAQNPPPF  55
            T+++   FLLV   I  C   E      T   P I+SR +WGA+ P +    L  NPPP+
Sbjct  3    TRMVRATFLLVATCIAICRAAEVADSAATFETPNIVSRQQWGAKPPKSPTPNLKMNPPPY  182

Query  56   VIIHHSATDSCITQAICNARVRSFQNYHIDEKGWGDIGYQFLVGEDGNIYEGRGWDKHGA  115
            V+IHHS +  C TQAIC ARVRSFQN H++ + W DIGY FLVGEDGN+YEGRGW KHG+
Sbjct  183  VVIHHSDSVGCTTQAICQARVRSFQNDHMNSRKWNDIGYNFLVGEDGNVYEGRGWGKHGS  362

Query  116  HSISYNSKSIGICIIGNFVGHTPNAAAIEATKNLISYGVAIGKIQSNYTLLGHRQTTRTS  175
            HS+ YN+KSIGIC+IG F  + PN+A+I AT+NLI+YGVA  KI+S+Y LLGHRQTT+T
Sbjct  363  HSVPYNAKSIGICLIGKFNNNVPNSASIRATQNLIAYGVANNKIKSDYKLLGHRQTTKTD  542

Query  176  CPGDSLYELIK  186
            CPG+SLY LIK
Sbjct  543  CPGNSLYNLIK  575
```



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NVPBE93TR NVPA Nasonia vitripennis cDNA, mRNA sequence

**Sequence ID:** ES635670.1 **Length:** 601 **Number of Matches:** 1

**Range 1: 36 to 527** GenBank Graphics ▼ Next Match ▲ Previous Match

| Score | Expect | Method | Identities | Positives | Gaps | Frame |
|---|---|---|---|---|---|---|
| 235 bits(600) | 2e-76 | Compositional matrix adjust. | 100/164(61%) | 134/164(81%) | 0/164(0%) | +3 |

```
Query  28   PRIISRSEWGARKPTTTIRALAQNPPPFVIIHHSATDSCITQAICNARVRSFQNYHIDEK  87
            P II R++WGAR+P    +  LA  PPP+VI+HH+A+D+C ++AIC AR+RSFQ+YH++ K
Sbjct  36   PGIIRRADWGARQPKGPLAPLAVEPPPYVIVHHAASDTCTSRAICQARLRSFQDYHMNTK  215

Query  88   GWGDIGYQFLVGEDGNIYEGRGWDKHGAHSISYNSKSIGICIIGNFVGHTPNAAAIEATK  147
             W DIGY FLVGEDGN+YEGRGW K GAH+ +YN KSIGIC+IGN+   +PN+AA EA K
Sbjct  216  KWSDIGYNFLVGEDGNVYEGRGWGKAGAHAKTYNDKSIGICVIGNYENRSPNSAATEAVK  395

Query  148  NLISYGVAIGKIQSNYTLLGHRQTTRTSCPGDSLYELIKTWPHW  191
            +LIS+GV++GKI   Y+L+GHRQ + TSCPG+ LY+L++TWP+W
Sbjct  396  SLISHGVSLGKINKAYSLIGHRQASPTSCPGNKLYQLVQTWPNW  527
```

# EMBOSS protein translation

I put this sequence through EMBOSS Transeq (EBI) for the following translations (please note, to avoid confusion with RMD, I have substituted the "*" code for stop codons with "x"): GW790304.1_1 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence

REWYEQRFYLLQRALQFAVQRKLQIRLQPLKHLISFQDNNGELNRRKALRLILKxIHLLT
LLFIIRIQLVVPLKQFVRLELEVFRMIIxTRENGMTSATTFWSVKMVMFTKVVAGVNMAP
IQSRTMPRVSVFALLVNLTITYRIQRVFEQHKIxxLTExLTTKSNPIISFLAIDKPLKLI
VLEILYTIxLKHGLTGPTRHKKYNRYAIKLIYQVxRLIVLDSTIIGNxRSSDRxLLLTFL
xLTMDTFKFDFRANIFTxCFVKEE

GW790304.1_2 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence

ENGTSNVSTCCNVHCNLPCSGSCRFGCNLxNTxYRFKTTMGSxTAEKPYAxSxNESTSLR
CYSSFGFSWLYHSSNLSGxSxKFSExSYELEKMExHRLQLFGRxRWxCLRRSWLGxTWLP
FSPVQCQEYRYLPYWxIxQxRTEFSEYSSNTKFDSLRSSxQQNQIRLxASWPSTNHxNxL
SWKFSIQFDxNMASLDRHAIRNIIVMRLNxSTKYNVLLFWTRRSLEINDHRIDNFCxLFF
NxRWIPSNSIFVRTFLLDVSxRKX

**GW790304.1_3 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence**
RMVRATFLLVATCIAICRAAEVADSAATFETPNIVSRQQWGAKPPKSPTPNLKMNPPPYV
VIHHSDSVGCTTQAICQARVRSFQNDHMNSRKWNDIGYNFLVGEDGNVYEGRGWGKHGSH
SVPYNAKSIGICLIGKFNNNVPNSASIRATQNLIAYGVANNKIKSDYKLLGHRQTTKTDC
PGNSLYNLIKTWPHWTDTPxEIxSLCDxINLPSITSYCFGLDDHWKLTIIGSITFVDFSL
INDGYLQIRFSCEHFYLMFRKGRX

(This appears to be the best option to go with; longest ORF.)

GW790304.1_4 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence
LFLYETSSKNVRTKIEFEGIHRxLKKSQQKLSIRxSLISNDRRVQNNKTLYLVDxFNRIT
IIFLMACRSSEAMFxSNCIENFQDNQFxWFVDGQEAYNRIxFCCxLLRKLSNFVLLEYSL
NSVRYCxIYQxGKYRYSWHCTGLNGSHVYPSHDLRKHYHLHRPKSCSRCHSIFSSSYDHS
ENFxLxPDKLLEWYNQLNPNDExQRKEVDSFxDxAxGFSAVxLPIVVLKRYxVFQRLQPN
LQLPLHGKLQCTLQQVETLLVPFS

GW790304.1_5 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence
LPLRNIKxKCSHENRIxRYPSLIKEKSTKVIDPMIVNFQxSSSPKQxDVILGRLIxSHND
YISYGVSVQxGHVLIKLYREFPGQSVLVVCRWPRSLxSDLILLLATPxAIKFCVARILAE
FGTLLLNLPIRQIPILLALYGTEWEPCLPQPRPSxTLPSSPTKKLxPMSFHFLEFIxSFx
KLLTLAxQIAxVVQPTESExxITTxGGGFILRLGVGLFGGLAPHCCLETILGVSKVAAES
ATSAARQIAMHVATSRNVARTILX

GW790304.1_6 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence
SSFTKHQVKMFARKSNLKVSIVNxRKVNKSYRSDDRxFPMIVESKTIRRYTWxINLIAxR
LYFLWRVGPVRPCFNQIVxRISRTISFSGLSMAKKLIIGFDFVVSYSVSYQILCCSNTRx
IRYVIVKFTNKANTDTLGIVRDxMGAMFTPATTFVNITIFTDQKVVADVIPFSRVHMIIL
KTSNSSLTNCLSGTTNxIRMMNNNVRRWIHFKIRRRAFRRFSSPLLSxNDIRCFKGCSRI
CNFRCTANCNARCNKxKRCSYHSR

# Question 4

> 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 not is not actually homologous to the original query. You should probably start over.

The BLASTp results from the selected sequence above follow below:

| Description | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|---|---|---|---|---|---|---|
| PREDICTED: peptidoglycan-recognition protein SC2-like [Polistes dominula] | 293 | 293 | 68% | 3e-97 | 74.03% | XP_015177188.1 |
| PREDICTED: peptidoglycan recognition protein 3-like [Polistes canadensis] | 276 | 451 | 64% | 9e-88 | 74.10% | XP_014602399.1 |
| peptidoglycan-recognition protein SC2 isoform X1 [Ooceraea biroi] | 264 | 264 | 73% | 5e-86 | 62.44% | XP_011344962.1 |
| PREDICTED: peptidoglycan-recognition protein SC2-like [Trachymyrmex cornetzi] | 264 | 264 | 67% | 5e-86 | 65.54% | XP_018369940.1 |
| PREDICTED: peptidoglycan-recognition protein SC2-like [Atta cephalotes] | 262 | 262 | 66% | 2e-85 | 65.34% | XP_012059667.1 |
| Peptidoglycan-recognition protein SC2 [Acromyrmex echinatior] | 263 | 263 | 64% | 2e-85 | 66.67% | EGI64536.1 |
| PREDICTED: peptidoglycan-recognition protein SC2-like [Atta colombica] | 260 | 260 | 66% | 9e-85 | 64.77% | XP_018046500.1 |
| peptidoglycan-recognition protein SB1-like [Pogonomyrmex barbatus] | 260 | 260 | 73% | 2e-84 | 59.28% | XP_011631523.1 |
| peptidoglycan-recognition protein SC2 isoform X2 [Ooceraea biroi] | 258 | 258 | 63% | 5e-84 | 68.86% | XP_011344963.1 |
| peptidoglycan-recognition protein precursor [Myrmica ruginodis] | 257 | 257 | 64% | 2e-83 | 65.09% | ACT66860.1 |
| peptidoglycan-recognition protein precursor [Myrmica lobicornis] | 256 | 256 | 67% | 3e-83 | 63.84% | ACT66861.1 |
| Peptidoglycan recognition protein 3 [Trachymyrmex cornetzi] | 262 | 412 | 64% | 4e-83 | 67.25% | KYN14761.1 |
| peptidoglycan-recognition protein precursor [Myrmica brevispinosa] | 255 | 255 | 64% | 6e-83 | 65.09% | ACT66868.1 |
| peptidoglycan-recognition protein precursor [Myrmica alaskensis] | 255 | 255 | 63% | 7e-83 | 64.88% | ACT66869.1 |
| peptidoglycan-recognition protein precursor [Myrmica sulcinodis] | 254 | 254 | 65% | 1e-82 | 64.16% | ACT66863.1 |
| peptidoglycan-recognition protein precursor [Myrmica fracticornis] | 254 | 254 | 64% | 2e-82 | 64.50% | ACT66867.1 |
| PREDICTED: peptidoglycan-recognition protein LF-like [Habropoda laboriosa] | 263 | 447 | 63% | 2e-82 | 70.91% | XP_017789839.1 |
| peptidoglycan-recognition protein precursor [Myrmica sabuleti] | 254 | 254 | 65% | 2e-82 | 63.22% | ACT66865.1 |
| peptidoglycan-recognition protein SC2 [Bombus terrestris] | 254 | 254 | 73% | 4e-82 | 61.14% | XP_012170795.1 |

The top hits are not exact matches, but appear to be related to the function from the original *A. mellifera* gene, as well as other closely related species, suggesting there is homology among these sequences, and potentially similar function in this *V. squamosa* sequence.

# Question 5

> 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.

## Input Sequences

List of sequences used for multiple alignment using MUSCLE (EBI); the first if the original Apis mellifera reference sequence, the second is the presumed Vespula squamosa sequence, and the other 9 are sequences from other Hymenoptera.

- NP_001157188.1 peptidoglycan-recognition protein S2 precursor [Apis mellifera]
  MTKLIAVLFLLVNCQILFCSVHETPVRPRIISRSEWGARKPTTTIRALAQNPPPFVIIHHSATD
  SCITQA
  ICNARVRSFQNYHIDEKGWGDIGYQFLVGEDGNIYEGRGWDKHGAHSISYNSKSIGICIIG
  NFVGHTPNA
  AAIEATKNLISYGVAIGKIQSNYTLLGHRQTTRTSCPGDSLYELIKTWPHWSSI
- GW790304.1_3 QP_B1_I03_041 Caste=Queen, Stage=Pupa Vespula squamosa cDNA, mRNA sequence
  RMVRATFLLVATCIAICRAAEVADSAATFETPNIVSRQQWGAKPPKSPTPNLKMNPPPYV
  VIHHSDSVGCTTQAICQARVRSFQNDHMNSRKWNDIGYNFLVGEDGNVYEGRGWGKH
  GSH
  SVPYNAKSIGICLIGKFNNNVPNSASIRATQNLIAYGVANNKIKSDYKLLGHRQTTKTDC
  PGNSLYNLIKTWPHWTDTPxEIxSLCDxINLPSITSYCFGLDDHWKLTIIGSITFVDFSL
  INDGYLQIRFSCEHFYLMFRKGRx
- XP_011344962.1 peptidoglycan-recognition protein SC2 isoform X1 [Ooceraea biroi]
  MYIARRTTVLIFAAVYVTLVAQEAAARPNTGGQIIPNIISRAQWGAKSPKSPLSNLAKKPAP
  YVIIHHST
  DTGCETQALCQAKVRGFQNYHMNSKGWTDIGYNFLVGEDGNVYEGRGWGKKGSHSK
  PFNGKSIGICIIGD
  YSNRTPKPAAVQAVSKLIAYGVSNDEIKSDYILLGHRQTGQTTCPGNSLYGMIKSWPHWQ
  SSA
- XP_011631523.1 peptidoglycan-recognition protein SB1-like [Pogonomyrmex barbatus]
  MYITKRTTLIFATVYVTLIVQETVATSPNIISRSEWGARAPKSRAPNLKLKPAPYVLIHHSTGS
  GCETQA
  LCQLKVRQFQNEHMNTKGWSDIGYNFLVGEDGNVYEGRGWGKQGAHSIPFNKKSIGICI
  IGDYRKRTPNA
  MAVQAVANLIAQGVQNGEIKSDYKLLGHRQTWPTICPGDSLYTMIKSWPHWSERE
- XP_012170795.1 peptidoglycan-recognition protein SC2 [Bombus terrestris]
  MTKLIAVILLLASCQVLFCAVHKTPVRPSIISRSEWGANAPKSTLRNLAEEPAPFVIIHHSAS
  DSCTTRA
  ICQARVRSFQNHHMNQKGWNDIGYNFLVGEDGNIYEGRGWGKHGAHSTPYNSKSIGIC

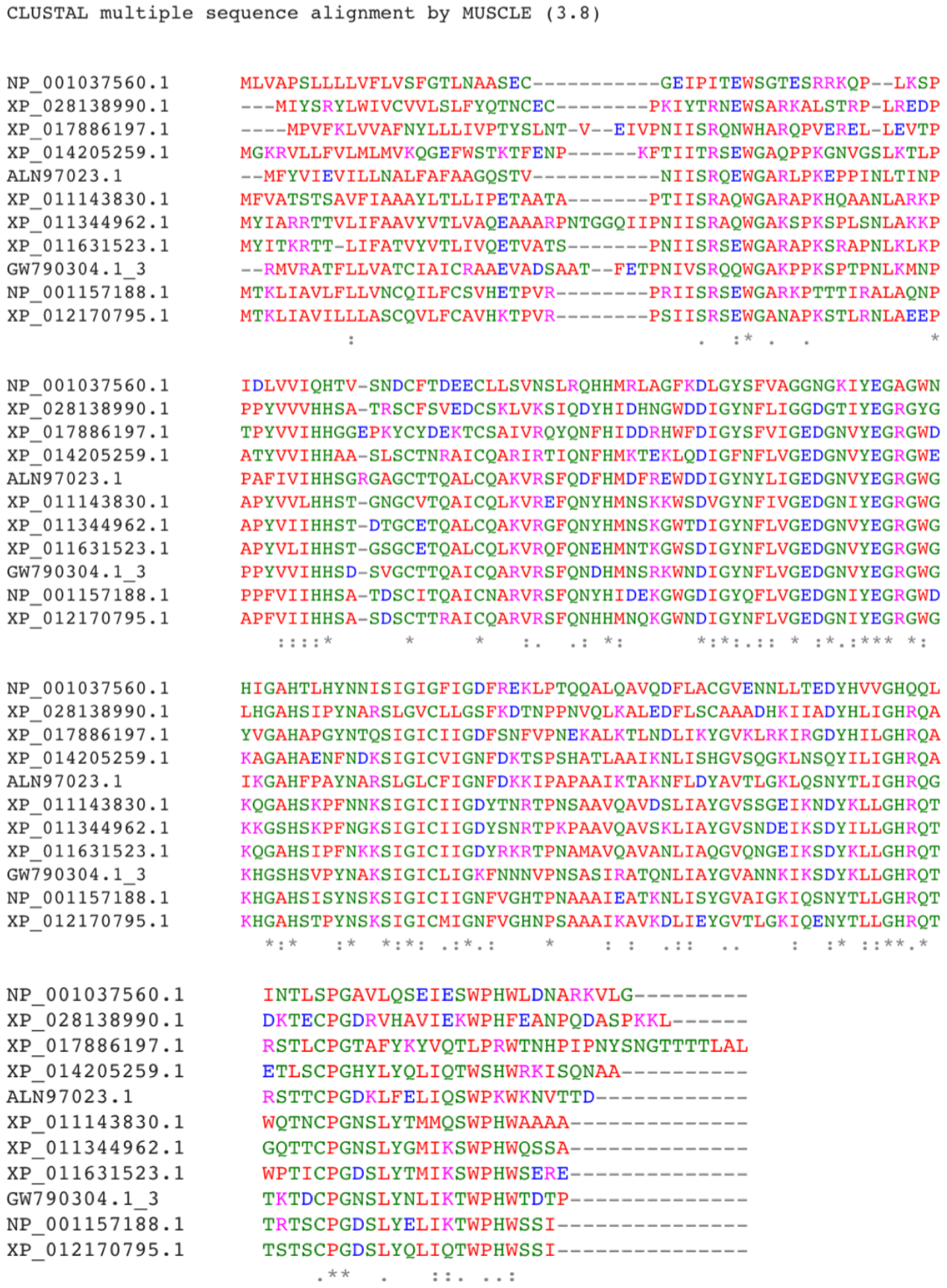
MIGNFVGHNPSA
AAIKAVKDLIEYGVTLGKIQENYTLLGHRQTTSTSCPGDSLYQLIQTWPHWSSI

- XP_011143830.1 peptidoglycan-recognition protein SC2 isoform X1 [Harpegnathos saltator]
  MFVATSTSAVFIAAAYLTLLIPETAATAPTIISRAQWGARAPKHQAANLARKPAPYVVLHHST
  GNGCVTQ
  AICQLKVREFQNYHMNSKKWSDVGYNFIVGEDGNIYEGRGWGKQGAHSKPFNNKSIGI
  CIIGDYTNRTPN
  SAAVQAVDSLIAYGVSSGEIKNDYKLLGHRQTWQTNCPGNSLYTMMQSWPHWAAAA
- XP_014205259.1 peptidoglycan-recognition protein SC2-like [Copidosoma floridanum]
  MGKRVLLFVLMLMVKQGEFWSTKTFENPKFTIITRSEWGAQPPKGNVGSLKTLPATYVVI
  HHAASLSCTN
  RAICQARIRTIQNFHMKTEKLQDIGFNFLVGEDGNVYEGRGWEKAGAHAENFNDKSIGIC
  VIGNFDKTSP
  SHATLAAIKNLISHGVSQGKLNSQYILIGHRQAETLSCPGHYLYQLIQTWSHWRKISQNAA
- XP_017886197.1 peptidoglycan-recognition protein SB1-like [Ceratina calcarata]
  MPVFKLVVAFNYLLLIVPTYSLNTVEIVPNIISRQNWHARQPVERELLEVTPTPYVVIHHGGE
  PKYCYDE
  KTCSAIVRQYQNFHIDDRHWFDIGYSFVIGEDGNVYEGRGWDYVGAHAPGYNTQSIGICII
  GDFSNFVPN
  EKALKTLNDLIKYGVKLRKIRGDYHILGHRQARSTLCPGTAFYKYVQTLPRWTNHPIPNYS
  NGTTTTLAL
- XP_028138990.1 peptidoglycan-recognition protein 2-like [Diabrotica virgifera virgifera]
  MIYSRYLWIVCVVLSLFYQTNCECPKIYTRNEWSARKALSTRPLREDPPPYVVVHHSATRS
  CFSVEDCSK
  LVKSIQDYHIDHNGWDDIGYNFLIGGDGTIYEGRGYGLHGAHSIPYNARSLGVCLLGSFK
  DTNPPNVQLK
  ALEDFLSCAAADHKIIADYHLIGHRQADKTECPGDRVHAVIEKWPHFEANPQDASPKKL
- NP_001037560.1 peptidoglycan recognition protein S2 precursor [Bombyx mori]
  MLVAPSLLLLVFLVSFGTLNAASECGEIPITEWSGTESRRKQPLKSPIDLVVIQHTVSNDCFT
  DEECLLS
  VNSLRQHHMRLAGFKDLGYSFVAGGNGKIYEGAGWNHIGAHTLHYNNISIGIGFIGDFRE
  KLPTQQALQA
  VQDFLACGVENNLLTEDYHVVGHQQLINTLSPGAVLQSEIESWPHWLDNARKVLG
- ALN97023.1 peptidoglycan recognition protein S2 [Microplitis mediator]
  MFYVIEVILLNALFAFAAGQSTVNIISRQEWGARLPKEPPINLTINPPAFIVIHHSGRGAGCTT
  QALCQA
  KVRSFQDFHMDFREWDDIGYNYLIGEDGNVYEGRGWGIKGAHFPAYNARSLGLCFIGNF

DKKIPAPAAIK
TAKNFLDYAVTLGKLQSNYTLIGHRQGRSTTCPGDKLFELIQSWPKWKNVTTD

## MUSCLE Output

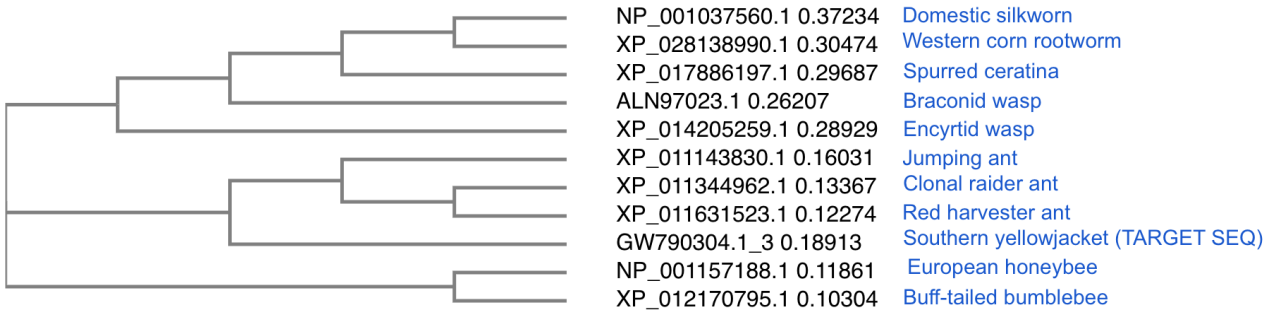
I ran the MUSCLE with default parameters to produce the following ClustalW output:
CLUSTAL multiple sequence alignment by MUSCLE (3.8).


CLUSTAL multiple sequence alignment by MUSCLE (3.8)
NP_001037560.1 MLVAPSLLLLVFLVSFGTLNAASEC----------GEIPITEWSGTESRRKQP--LKSP
XP_028138990.1 ---MIYSRYLWIVCVVLSLFYQTNCEC--------PKIYTRNEWSARKALSTRP-LREDP
XP_017886197.1 ----MPVFKLVVAFNYLLLIVPTYSLNT-V--EIVPNIISRQNWHARQPVEREL-LEVTP
XP_014205259.1 MGKRVLLFVLMLMVKQGEFWSTKTFENP------KFTIITRSEWGAQPPKGNVGSLKTLP
ALN97023.1 --MFYVIEVILLNALFAFAAGQSTV----------NIISRQEWGARLPKEPPINLTINP
XP_011143830.1 MFVATSTSAVFIAAAYLTLLIPETAATA-------PTIISRAQWGARAPKHQAANLARKP
XP_011344962.1 MYIARRTTVLIFAAVYVTLVAQEAAARPNTGGQIIPNIISRAQWGAKSPKSPLSNLAKKP
XP_011631523.1 MYITKRTT-LIFATVYVTLIVQETVATS-------PNIISRSEWGARAPKSRAPNLKLKP
GW790304.1_3 --RMVRATFLLVATCIAICRAAEVADSAAT--FETPNIVSRQQWGAKPPKSPTPNLKMNP
NP_001157188.1 MTKLIAVLFLLVNCQILFCSVHETPVR--------PRIISRSEWGARKPTTTIRALAQNP
XP_012170795.1 MTKLIAVILLLASCQVLFCAVHKTPVR--------PSIISRSEWGANAPKSTLRNLAEEP
NP_001037560.1 IDLVVIQHTV-SNDCFTDEECLLSVNSLRQHHMRLAGFKDLGYSFVAGGNGKIYEGAGWN
XP_028138990.1 PPYVVVHHSA-TRSCFSVEDCSKLVKSIQDYHIDHNGWDDIGYNFLIGGDGTIYEGRGYG
XP_017886197.1 TPYVVIHHGGEPKYCYDEKTCSAIVRQYQNFHIDDRHWFDIGYSFVIGEDGNVYEGRGWD
XP_014205259.1 ATYVVIHHAA-SLSCTNRAICQARIRTIQNFHMKTEKLQDIGFNFLVGEDGNVYEGRGWE
ALN97023.1 PAFIVIHHSGRGAGCTTQALCQAKVRSFQDFHMDFREWDDIGYNYLIGEDGNVYEGRGWG
XP_011143830.1 APYVVLHHST-GNGCVTQAICQLKVREFQNYHMNSKKWSDVGYNFIVGEDGNIYEGRGWG
XP_011344962.1 APYVIIHHST-DTGCETQALCQAKVRGFQNYHMNSKGWTDIGYNFLVGEDGNVYEGRGWG
XP_011631523.1 APYVLIHHST-GSGCETQALCQLKVRQFQNEHMNTKGWSDIGYNFLVGEDGNVYEGRGWG
GW790304.1_3 PPYVVIHHSD-SVGCTTQAICQARVRSFQNDHMNSRKWNDIGYNFLVGEDGNVYEGRGWG
NP_001157188.1 PPFVIIHHSA-TDSCITQAICNARVRSFQNYHIDEKGWGDIGYQFLVGEDGNIYEGRGWD
XP_012170795.1 APFVIIHHSA-SDSCTTRAICQARVRSFQNHHMNQKGWNDIGYNFLVGEDGNIYEGRGWG
NP_001037560.1 HIGAHTLHYNNISIGIGFIGDFREKLPTQQALQAVQDFLACGVENNLLTEDYHVVGHQQL
XP_028138990.1 LHGAHSIPYNARSLGVCLLGSFKDTNPPNVQLKALEDFLSCAAADHKIIADYHLIGHRQA
XP_017886197.1 YVGAHAPGYNTQSIGICIIGDFSNFVPNEKALKTLNDLIKYGVKLRKIRGDYHILGHRQA
XP_014205259.1 KAGAHAENFNDKSIGICVIGNFDKTSPSHATLAAIKNLISHGVSQGKLNSQYILIGHRQA
ALN97023.1 IKGAHFPAYNARSLGLCFIGNFDKKIPAPAAIKTAKNFLDYAVTLGKLQSNYTLIGHRQG
XP_011143830.1 KQGAHSKPFNNKSIGICIIGDYTNRTPNSAAVQAVDSLIAYGVSSGEIKNDYKLLGHRQT
XP_011344962.1 KKGSHSKPFNGKSIGICIIGDYSNRTPKPAAVQAVSKLIAYGVSNDEIKSDYILLGHRQT
XP_011631523.1 KQGAHSIPFNKKSIGICIIGDYRKRTPNAMAVQAVANLIAQGVQNGEIKSDYKLLGHRQT
GW790304.1_3 KHGSHSVPYNAKSIGICLIGKFNNNVPNSASIRATQNLIAYGVANNKIKSDYKLLGHRQT
NP_001157188.1 KHGAHSISYNSKSIGICIIGNFVGHTPNAAAIEATKNLISYGVAIGKIQSNYTLLGHRQT
XP_012170795.1 KHGAHSTPYNSKSIGICMIGNFVGHNPSAAAIKAVKDLIEYGVTLGKIQENYTLLGHRQT
NP_001037560.1 INTLSPGAVLQSEIESWPHWLDNARKVLG---------
XP_028138990.1 DKTECPGDRVHAVIEKWPHFEANPQDASPKKL------
XP_017886197.1 RSTLCPGTAFYKYVQTLPRWTNHPIPNYSNGTTTTLAL
XP_014205259.1 ETLSCPGHYLYQLIQTWSHWRKISQNAA----------
ALN97023.1 RSTTCPGDKLFELIQSWPKWKNVTTD------------
XP_011143830.1 WQTNCPGNSLYTMMQSWPHWAAAA--------------
XP_011344962.1 GQTTCPGNSLYGMIKSWPHWQSSA--------------
XP_011631523.1 WPTICPGDSLYTMIKSWPHWSERE--------------
GW790304.1_3 TKTDCPGNSLYNLIKTWPHWTDTP--------------
NP_001157188.1 TRTSCPGDSLYELIKTWPHWSSI---------------
XP_012170795.1 TSTSCPGDSLYQLIQTWPHWSSI---------------

# Question 6

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 ported the output from the previous question to Simple Phylogeny (EBI) and asked it to make me a cladogram using default parameters.




Below is a key describing each branch in order:

| Accession | Scientific name | Common name | General Detail |
|---|---|---|---|
| NP_001037560.1 | [B. mori] | Domestic silkworm | (Moth) |
| XP_028138990.1 | [D. virgifera virgifera] | Western corn rootworm | (Beetle) |
| XP_017886197.1 | [C. calcarata] | Spurred ceratina | (Apid bee) |
| ALN97023.1 | [M. mediator] | NCN | (Braconid wasp) |
| XP_014205259.1 | [C. floridanum] | NCN | (Encyrtid wasp) |
| XP_011143830.1 | [H. saltator] | Jumping ant | (Ant) |
| XP_011344962.1 | [O. biroi] | Clonal raider ant | (Ant) |
| XP_011631523.1 | [P. barbatus] | Red harvester ant | (Ant) |
| GW790304.1_3 | [V. squamosa, Target] | Southern yellowjacket | (Vespid wasp) |

| Accession | Scientific name | Common name | General Detail |
|---|---|---|---|
| NP_001157188.1 | [A. mellifera] | European honeybee | (Apid bee) |
| XP_012170795.1 | [B. terrestris] | Buff-tailed bumblebee | (Apid bee) |

As an interesting note, we see that the largely solitary hymenoptera (the Ceratina and parasitic wasps) cluster with the outgroup moth and beetle, whereas the highly social lineages (the ants, bumblebees, yellowjackets and honey bees) cluster together. It's not particularly relevant for this project, but it's cool to know that this supports the current hypothesis that social living deeply affects immune response in Hymenoptera, notably a reduction in function of individual immunes genes in favor of social behaviors that support group immunity.

# Question 7

For Question 7, we will make a heatmap of the alignment we ran of the V. squamosa predicted PGRP S2 with the base sequence from A. mellifera, and the 9 other PRGP-associated sequences.

## Making the alignment in R

```
pgrp <- read.fasta("aln-fasta.txt") # This file contains aligned sequences
head(pgrp)
```

```
## $id
##  [1] "NP_001037560.1" "XP_028138990.1" "XP_017886197.1" "XP_014205259.1"
##  [5] "ALN97023.1"     "XP_011143830.1" "XP_011344962.1" "XP_011631523.1"
##  [9] "GW790304.1_3"   "NP_001157188.1" "XP_012170795.1"
##
## $ali
##                 [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11]
## NP_001037560.1 "M"  "L"  "V"  "A"  "P"  "S"  "L"  "L"  "L"  "L"   "V"
## XP_028138990.1 "-"  "-"  "-"  "M"  "I"  "Y"  "S"  "R"  "Y"  "L"   "W"
## XP_017886197.1 "-"  "-"  "-"  "-"  "M"  "P"  "V"  "F"  "K"  "L"   "V"
## XP_014205259.1 "M"  "G"  "K"  "R"  "V"  "L"  "L"  "F"  "V"  "L"   "M"
## ALN97023.1     "-"  "-"  "M"  "F"  "Y"  "V"  "I"  "E"  "V"  "I"   "L"
## XP_011143830.1 "M"  "F"  "V"  "A"  "T"  "S"  "T"  "S"  "A"  "V"   "F"
## XP_011344962.1 "M"  "Y"  "I"  "A"  "R"  "R"  "T"  "T"  "V"  "L"   "I"
## XP_011631523.1 "M"  "Y"  "I"  "T"  "K"  "R"  "T"  "T"  "-"  "L"   "I"
## GW790304.1_3   "-"  "-"  "R"  "M"  "V"  "R"  "A"  "T"  "F"  "L"   "L"
## NP_001157188.1 "M"  "T"  "K"  "L"  "I"  "A"  "V"  "L"  "F"  "L"   "L"
## XP_012170795.1 "M"  "T"  "K"  "L"  "I"  "A"  "V"  "I"  "L"  "L"   "L"
##                [,12] [,13] [,14] [,15] [,16] [,17] [,18] [,19] [,20] [,21]
```

```
## NP_001037560.1 "F"   "L"   "V"   "S"   "F"   "G"   "T"   "L"   "N"   "A"
## XP_028138990.1 "I"   "V"   "C"   "V"   "V"   "L"   "S"   "L"   "F"   "Y"
## XP_017886197.1 "V"   "A"   "F"   "N"   "Y"   "L"   "L"   "L"   "I"   "V"
## XP_014205259.1 "L"   "M"   "V"   "K"   "Q"   "G"   "E"   "F"   "W"   "S"
## ALN97023.1     "L"   "N"   "A"   "L"   "F"   "A"   "F"   "A"   "A"   "G"
## XP_011143830.1 "I"   "A"   "A"   "A"   "Y"   "L"   "T"   "L"   "L"   "I"
## XP_011344962.1 "F"   "A"   "A"   "V"   "Y"   "V"   "T"   "L"   "V"   "A"
## XP_011631523.1 "F"   "A"   "T"   "V"   "Y"   "V"   "T"   "L"   "I"   "V"
## GW790304.1_3   "V"   "A"   "T"   "C"   "I"   "A"   "I"   "C"   "R"   "A"
## NP_001157188.1 "V"   "N"   "C"   "Q"   "I"   "L"   "F"   "C"   "S"   "V"
## XP_012170795.1 "A"   "S"   "C"   "Q"   "V"   "L"   "F"   "C"   "A"   "V"
##                [,22] [,23] [,24] [,25] [,26] [,27] [,28] [,29] [,30] [,31]
## NP_001037560.1 "A"   "S"   "E"   "C"   "-"   "-"   "-"   "-"   "-"   "-"
## XP_028138990.1 "Q"   "T"   "N"   "C"   "E"   "C"   "-"   "-"   "-"   "-"
## XP_017886197.1 "P"   "T"   "Y"   "S"   "L"   "N"   "T"   "-"   "V"   "-"
## XP_014205259.1 "T"   "K"   "T"   "F"   "E"   "N"   "P"   "-"   "-"   "-"
## ALN97023.1     "Q"   "S"   "T"   "V"   "-"   "-"   "-"   "-"   "-"   "-"
## XP_011143830.1 "P"   "E"   "T"   "A"   "A"   "T"   "A"   "-"   "-"   "-"
## XP_011344962.1 "Q"   "E"   "A"   "A"   "A"   "R"   "P"   "N"   "T"   "G"
## XP_011631523.1 "Q"   "E"   "T"   "V"   "A"   "T"   "S"   "-"   "-"   "-"
## GW790304.1_3   "A"   "E"   "V"   "A"   "D"   "S"   "A"   "A"   "T"   "-"
## NP_001157188.1 "H"   "E"   "T"   "P"   "V"   "R"   "-"   "-"   "-"   "-"
## XP_012170795.1 "H"   "K"   "T"   "P"   "V"   "R"   "-"   "-"   "-"   "-"
##                [,32] [,33] [,34] [,35] [,36] [,37] [,38] [,39] [,40] [,41]
## NP_001037560.1 "-"   "-"   "-"   "-"   "-"   "G"   "E"   "I"   "P"   "I"
## XP_028138990.1 "-"   "-"   "-"   "-"   "P"   "K"   "I"   "Y"   "T"   "R"
## XP_017886197.1 "-"   "E"   "I"   "V"   "P"   "N"   "I"   "I"   "S"   "R"
## XP_014205259.1 "-"   "-"   "-"   "K"   "F"   "T"   "I"   "I"   "T"   "R"
## ALN97023.1     "-"   "-"   "-"   "-"   "-"   "N"   "I"   "I"   "S"   "R"
## XP_011143830.1 "-"   "-"   "-"   "-"   "P"   "T"   "I"   "I"   "S"   "R"
## XP_011344962.1 "G"   "Q"   "I"   "I"   "P"   "N"   "I"   "I"   "S"   "R"
## XP_011631523.1 "-"   "-"   "-"   "-"   "P"   "N"   "I"   "I"   "S"   "R"
## GW790304.1_3   "-"   "F"   "E"   "T"   "P"   "N"   "I"   "V"   "S"   "R"
## NP_001157188.1 "-"   "-"   "-"   "-"   "P"   "R"   "I"   "I"   "S"   "R"
## XP_012170795.1 "-"   "-"   "-"   "-"   "P"   "S"   "I"   "I"   "S"   "R"
##                [,42] [,43] [,44] [,45] [,46] [,47] [,48] [,49] [,50] [,51]
## NP_001037560.1 "T"   "E"   "W"   "S"   "G"   "T"   "E"   "S"   "R"   "R"
## XP_028138990.1 "N"   "E"   "W"   "S"   "A"   "R"   "K"   "A"   "L"   "S"
## XP_017886197.1 "Q"   "N"   "W"   "H"   "A"   "R"   "Q"   "P"   "V"   "E"
## XP_014205259.1 "S"   "E"   "W"   "G"   "A"   "Q"   "P"   "P"   "K"   "G"
## ALN97023.1     "Q"   "E"   "W"   "G"   "A"   "R"   "L"   "P"   "K"   "E"
## XP_011143830.1 "A"   "Q"   "W"   "G"   "A"   "R"   "A"   "P"   "K"   "H"
## XP_011344962.1 "A"   "Q"   "W"   "G"   "A"   "K"   "S"   "P"   "K"   "S"
## XP_011631523.1 "S"   "E"   "W"   "G"   "A"   "R"   "A"   "P"   "K"   "S"
## GW790304.1_3   "Q"   "Q"   "W"   "G"   "A"   "K"   "P"   "P"   "K"   "S"
## NP_001157188.1 "S"   "E"   "W"   "G"   "A"   "R"   "K"   "P"   "T"   "T"
## XP_012170795.1 "S"   "E"   "W"   "G"   "A"   "N"   "A"   "P"   "K"   "S"
##                [,52] [,53] [,54] [,55] [,56] [,57] [,58] [,59] [,60] [,61]
## NP_001037560.1 "K"   "Q"   "P"   "-"   "-"   "L"   "K"   "S"   "P"   "I"
## XP_028138990.1 "T"   "R"   "P"   "-"   "L"   "R"   "E"   "D"   "P"   "P"
## XP_017886197.1 "R"   "E"   "L"   "-"   "L"   "E"   "V"   "T"   "P"   "T"
```

```
## XP_014205259.1 "N"   "V"   "G"   "S"   "L"   "K"   "T"   "L"   "P"   "A"
## ALN97023.1     "P"   "P"   "I"   "N"   "L"   "T"   "I"   "N"   "P"   "P"
## XP_011143830.1 "Q"   "A"   "A"   "N"   "L"   "A"   "R"   "K"   "P"   "A"
## XP_011344962.1 "P"   "L"   "S"   "N"   "L"   "A"   "K"   "K"   "P"   "A"
## XP_011631523.1 "R"   "A"   "P"   "N"   "L"   "K"   "L"   "K"   "P"   "A"
## GW790304.1_3   "P"   "T"   "P"   "N"   "L"   "K"   "M"   "N"   "P"   "P"
## NP_001157188.1 "T"   "I"   "R"   "A"   "L"   "A"   "Q"   "N"   "P"   "P"
## XP_012170795.1 "T"   "L"   "R"   "N"   "L"   "A"   "E"   "E"   "P"   "A"
##                [,62] [,63] [,64] [,65] [,66] [,67] [,68] [,69] [,70] [,71]
## NP_001037560.1 "D"   "L"   "V"   "V"   "I"   "Q"   "H"   "T"   "V"   "-"
## XP_028138990.1 "P"   "Y"   "V"   "V"   "V"   "H"   "H"   "S"   "A"   "-"
## XP_017886197.1 "P"   "Y"   "V"   "V"   "I"   "H"   "H"   "G"   "G"   "E"
## XP_014205259.1 "T"   "Y"   "V"   "V"   "I"   "H"   "H"   "A"   "A"   "-"
## ALN97023.1     "A"   "F"   "I"   "V"   "I"   "H"   "H"   "S"   "G"   "R"
## XP_011143830.1 "P"   "Y"   "V"   "V"   "L"   "H"   "H"   "S"   "T"   "-"
## XP_011344962.1 "P"   "Y"   "V"   "I"   "I"   "H"   "H"   "S"   "T"   "-"
## XP_011631523.1 "P"   "Y"   "V"   "L"   "I"   "H"   "H"   "S"   "T"   "-"
## GW790304.1_3   "P"   "Y"   "V"   "V"   "I"   "H"   "H"   "S"   "D"   "-"
## NP_001157188.1 "P"   "F"   "V"   "I"   "I"   "H"   "H"   "S"   "A"   "-"
## XP_012170795.1 "P"   "F"   "V"   "I"   "I"   "H"   "H"   "S"   "A"   "-"
##                [,72] [,73] [,74] [,75] [,76] [,77] [,78] [,79] [,80] [,81]
## NP_001037560.1 "S"   "N"   "D"   "C"   "F"   "T"   "D"   "E"   "E"   "C"
## XP_028138990.1 "T"   "R"   "S"   "C"   "F"   "S"   "V"   "E"   "D"   "C"
## XP_017886197.1 "P"   "K"   "Y"   "C"   "Y"   "D"   "E"   "K"   "T"   "C"
## XP_014205259.1 "S"   "L"   "S"   "C"   "T"   "N"   "R"   "A"   "I"   "C"
## ALN97023.1     "G"   "A"   "G"   "C"   "T"   "T"   "Q"   "A"   "L"   "C"
## XP_011143830.1 "G"   "N"   "G"   "C"   "V"   "T"   "Q"   "A"   "I"   "C"
## XP_011344962.1 "D"   "T"   "G"   "C"   "E"   "T"   "Q"   "A"   "L"   "C"
## XP_011631523.1 "G"   "S"   "G"   "C"   "E"   "T"   "Q"   "A"   "L"   "C"
## GW790304.1_3   "S"   "V"   "G"   "C"   "T"   "T"   "Q"   "A"   "I"   "C"
## NP_001157188.1 "T"   "D"   "S"   "C"   "I"   "T"   "Q"   "A"   "I"   "C"
## XP_012170795.1 "S"   "D"   "S"   "C"   "T"   "T"   "R"   "A"   "I"   "C"
##                [,82] [,83] [,84] [,85] [,86] [,87] [,88] [,89] [,90] [,91]
## NP_001037560.1 "L"   "L"   "S"   "V"   "N"   "S"   "L"   "R"   "Q"   "H"
## XP_028138990.1 "S"   "K"   "L"   "V"   "K"   "S"   "I"   "Q"   "D"   "Y"
## XP_017886197.1 "S"   "A"   "I"   "V"   "R"   "Q"   "Y"   "Q"   "N"   "F"
## XP_014205259.1 "Q"   "A"   "R"   "I"   "R"   "T"   "I"   "Q"   "N"   "F"
## ALN97023.1     "Q"   "A"   "K"   "V"   "R"   "S"   "F"   "Q"   "D"   "F"
## XP_011143830.1 "Q"   "L"   "K"   "V"   "R"   "E"   "F"   "Q"   "N"   "Y"
## XP_011344962.1 "Q"   "A"   "K"   "V"   "R"   "G"   "F"   "Q"   "N"   "Y"
## XP_011631523.1 "Q"   "L"   "K"   "V"   "R"   "Q"   "F"   "Q"   "N"   "E"
## GW790304.1_3   "Q"   "A"   "R"   "V"   "R"   "S"   "F"   "Q"   "N"   "D"
## NP_001157188.1 "N"   "A"   "R"   "V"   "R"   "S"   "F"   "Q"   "N"   "Y"
## XP_012170795.1 "Q"   "A"   "R"   "V"   "R"   "S"   "F"   "Q"   "N"   "H"
##                [,92] [,93] [,94] [,95] [,96] [,97] [,98] [,99] [,100]
## NP_001037560.1 "H"   "M"   "R"   "L"   "A"   "G"   "F"   "K"   "D"
## XP_028138990.1 "H"   "I"   "D"   "H"   "N"   "G"   "W"   "D"   "D"
## XP_017886197.1 "H"   "I"   "D"   "D"   "R"   "H"   "W"   "F"   "D"
## XP_014205259.1 "H"   "M"   "K"   "T"   "E"   "K"   "L"   "Q"   "D"
## ALN97023.1     "H"   "M"   "D"   "F"   "R"   "E"   "W"   "D"   "D"
## XP_011143830.1 "H"   "M"   "N"   "S"   "K"   "K"   "W"   "S"   "D"
```

```
## XP_011344962.1 "H"   "M"   "N"   "S"   "K"   "G"   "W"   "T"   "D"
## XP_011631523.1 "H"   "M"   "N"   "T"   "K"   "G"   "W"   "S"   "D"
## GW790304.1_3   "H"   "M"   "N"   "S"   "R"   "K"   "W"   "N"   "D"
## NP_001157188.1 "H"   "I"   "D"   "E"   "K"   "G"   "W"   "G"   "D"
## XP_012170795.1 "H"   "M"   "N"   "Q"   "K"   "G"   "W"   "N"   "D"
##                [,101] [,102] [,103] [,104] [,105] [,106] [,107] [,108]
## NP_001037560.1 "L"    "G"    "Y"    "S"    "F"    "V"    "A"    "G"
## XP_028138990.1 "I"    "G"    "Y"    "N"    "F"    "L"    "I"    "G"
## XP_017886197.1 "I"    "G"    "Y"    "S"    "F"    "V"    "I"    "G"
## XP_014205259.1 "I"    "G"    "F"    "N"    "F"    "L"    "V"    "G"
## ALN97023.1     "I"    "G"    "Y"    "N"    "Y"    "L"    "I"    "G"
## XP_011143830.1 "V"    "G"    "Y"    "N"    "F"    "I"    "V"    "G"
## XP_011344962.1 "I"    "G"    "Y"    "N"    "F"    "L"    "V"    "G"
## XP_011631523.1 "I"    "G"    "Y"    "N"    "F"    "L"    "V"    "G"
## GW790304.1_3   "I"    "G"    "Y"    "N"    "F"    "L"    "V"    "G"
## NP_001157188.1 "I"    "G"    "Y"    "Q"    "F"    "L"    "V"    "G"
## XP_012170795.1 "I"    "G"    "Y"    "N"    "F"    "L"    "V"    "G"
##                [,109] [,110] [,111] [,112] [,113] [,114] [,115] [,116]
## NP_001037560.1 "G"    "N"    "G"    "K"    "I"    "Y"    "E"    "G"
## XP_028138990.1 "G"    "D"    "G"    "T"    "I"    "Y"    "E"    "G"
## XP_017886197.1 "E"    "D"    "G"    "N"    "V"    "Y"    "E"    "G"
## XP_014205259.1 "E"    "D"    "G"    "N"    "V"    "Y"    "E"    "G"
## ALN97023.1     "E"    "D"    "G"    "N"    "V"    "Y"    "E"    "G"
## XP_011143830.1 "E"    "D"    "G"    "N"    "I"    "Y"    "E"    "G"
## XP_011344962.1 "E"    "D"    "G"    "N"    "V"    "Y"    "E"    "G"
## XP_011631523.1 "E"    "D"    "G"    "N"    "V"    "Y"    "E"    "G"
## GW790304.1_3   "E"    "D"    "G"    "N"    "V"    "Y"    "E"    "G"
## NP_001157188.1 "E"    "D"    "G"    "N"    "I"    "Y"    "E"    "G"
## XP_012170795.1 "E"    "D"    "G"    "N"    "I"    "Y"    "E"    "G"
##                [,117] [,118] [,119] [,120] [,121] [,122] [,123] [,124]
## NP_001037560.1 "A"    "G"    "W"    "N"    "H"    "I"    "G"    "A"
## XP_028138990.1 "R"    "G"    "Y"    "G"    "L"    "H"    "G"    "A"
## XP_017886197.1 "R"    "G"    "W"    "D"    "Y"    "V"    "G"    "A"
## XP_014205259.1 "R"    "G"    "W"    "E"    "K"    "A"    "G"    "A"
## ALN97023.1     "R"    "G"    "W"    "G"    "I"    "K"    "G"    "A"
## XP_011143830.1 "R"    "G"    "W"    "G"    "K"    "Q"    "G"    "A"
## XP_011344962.1 "R"    "G"    "W"    "G"    "K"    "K"    "G"    "S"
## XP_011631523.1 "R"    "G"    "W"    "G"    "K"    "Q"    "G"    "A"
## GW790304.1_3   "R"    "G"    "W"    "G"    "K"    "H"    "G"    "S"
## NP_001157188.1 "R"    "G"    "W"    "D"    "K"    "H"    "G"    "A"
## XP_012170795.1 "R"    "G"    "W"    "G"    "K"    "H"    "G"    "A"
##                [,125] [,126] [,127] [,128] [,129] [,130] [,131] [,132]
## NP_001037560.1 "H"    "T"    "L"    "H"    "Y"    "N"    "N"    "I"
## XP_028138990.1 "H"    "S"    "I"    "P"    "Y"    "N"    "A"    "R"
## XP_017886197.1 "H"    "A"    "P"    "G"    "Y"    "N"    "T"    "Q"
## XP_014205259.1 "H"    "A"    "E"    "N"    "F"    "N"    "D"    "K"
## ALN97023.1     "H"    "F"    "P"    "A"    "Y"    "N"    "A"    "R"
## XP_011143830.1 "H"    "S"    "K"    "P"    "F"    "N"    "N"    "K"
## XP_011344962.1 "H"    "S"    "K"    "P"    "F"    "N"    "G"    "K"
## XP_011631523.1 "H"    "S"    "I"    "P"    "F"    "N"    "K"    "K"
## GW790304.1_3   "H"    "S"    "V"    "P"    "Y"    "N"    "A"    "K"
```

```
## NP_001157188.1 "H"    "S"    "I"    "S"    "Y"    "N"    "S"    "K"
## XP_012170795.1 "H"    "S"    "T"    "P"    "Y"    "N"    "S"    "K"
##                [,133] [,134] [,135] [,136] [,137] [,138] [,139] [,140]
## NP_001037560.1 "S"    "I"    "G"    "I"    "G"    "F"    "I"    "G"
## XP_028138990.1 "S"    "L"    "G"    "V"    "C"    "L"    "L"    "G"
## XP_017886197.1 "S"    "I"    "G"    "I"    "C"    "I"    "I"    "G"
## XP_014205259.1 "S"    "I"    "G"    "I"    "C"    "V"    "I"    "G"
## ALN97023.1     "S"    "L"    "G"    "L"    "C"    "F"    "I"    "G"
## XP_011143830.1 "S"    "I"    "G"    "I"    "C"    "I"    "I"    "G"
## XP_011344962.1 "S"    "I"    "G"    "I"    "C"    "I"    "I"    "G"
## XP_011631523.1 "S"    "I"    "G"    "I"    "C"    "I"    "I"    "G"
## GW790304.1_3   "S"    "I"    "G"    "I"    "C"    "L"    "I"    "G"
## NP_001157188.1 "S"    "I"    "G"    "I"    "C"    "I"    "I"    "G"
## XP_012170795.1 "S"    "I"    "G"    "I"    "C"    "M"    "I"    "G"
##                [,141] [,142] [,143] [,144] [,145] [,146] [,147] [,148]
## NP_001037560.1 "D"    "F"    "R"    "E"    "K"    "L"    "P"    "T"
## XP_028138990.1 "S"    "F"    "K"    "D"    "T"    "N"    "P"    "P"
## XP_017886197.1 "D"    "F"    "S"    "N"    "F"    "V"    "P"    "N"
## XP_014205259.1 "N"    "F"    "D"    "K"    "T"    "S"    "P"    "S"
## ALN97023.1     "N"    "F"    "D"    "K"    "K"    "I"    "P"    "A"
## XP_011143830.1 "D"    "Y"    "T"    "N"    "R"    "T"    "P"    "N"
## XP_011344962.1 "D"    "Y"    "S"    "N"    "R"    "T"    "P"    "K"
## XP_011631523.1 "D"    "Y"    "R"    "K"    "R"    "T"    "P"    "N"
## GW790304.1_3   "K"    "F"    "N"    "N"    "N"    "V"    "P"    "N"
## NP_001157188.1 "N"    "F"    "V"    "G"    "H"    "T"    "P"    "N"
## XP_012170795.1 "N"    "F"    "V"    "G"    "H"    "N"    "P"    "S"
##                [,149] [,150] [,151] [,152] [,153] [,154] [,155] [,156]
## NP_001037560.1 "Q"    "Q"    "A"    "L"    "Q"    "A"    "V"    "Q"
## XP_028138990.1 "N"    "V"    "Q"    "L"    "K"    "A"    "L"    "E"
## XP_017886197.1 "E"    "K"    "A"    "L"    "K"    "T"    "L"    "N"
## XP_014205259.1 "H"    "A"    "T"    "L"    "A"    "A"    "I"    "K"
## ALN97023.1     "P"    "A"    "A"    "I"    "K"    "T"    "A"    "K"
## XP_011143830.1 "S"    "A"    "A"    "V"    "Q"    "A"    "V"    "D"
## XP_011344962.1 "P"    "A"    "A"    "V"    "Q"    "A"    "V"    "S"
## XP_011631523.1 "A"    "M"    "A"    "V"    "Q"    "A"    "V"    "A"
## GW790304.1_3   "S"    "A"    "S"    "I"    "R"    "A"    "T"    "Q"
## NP_001157188.1 "A"    "A"    "A"    "I"    "E"    "A"    "T"    "K"
## XP_012170795.1 "A"    "A"    "A"    "I"    "K"    "A"    "V"    "K"
##                [,157] [,158] [,159] [,160] [,161] [,162] [,163] [,164]
## NP_001037560.1 "D"    "F"    "L"    "A"    "C"    "G"    "V"    "E"
## XP_028138990.1 "D"    "F"    "L"    "S"    "C"    "A"    "A"    "A"
## XP_017886197.1 "D"    "L"    "I"    "K"    "Y"    "G"    "V"    "K"
## XP_014205259.1 "N"    "L"    "I"    "S"    "H"    "G"    "V"    "S"
## ALN97023.1     "N"    "F"    "L"    "D"    "Y"    "A"    "V"    "T"
## XP_011143830.1 "S"    "L"    "I"    "A"    "Y"    "G"    "V"    "S"
## XP_011344962.1 "K"    "L"    "I"    "A"    "Y"    "G"    "V"    "S"
## XP_011631523.1 "N"    "L"    "I"    "A"    "Q"    "G"    "V"    "Q"
## GW790304.1_3   "N"    "L"    "I"    "A"    "Y"    "G"    "V"    "A"
## NP_001157188.1 "N"    "L"    "I"    "S"    "Y"    "G"    "V"    "A"
## XP_012170795.1 "D"    "L"    "I"    "E"    "Y"    "G"    "V"    "T"
##                [,165] [,166] [,167] [,168] [,169] [,170] [,171] [,172]
```

```
## NP_001037560.1 "N"    "N"    "L"    "L"    "T"    "E"    "D"    "Y"
## XP_028138990.1 "D"    "H"    "K"    "I"    "I"    "A"    "D"    "Y"
## XP_017886197.1 "L"    "R"    "K"    "I"    "R"    "G"    "D"    "Y"
## XP_014205259.1 "Q"    "G"    "K"    "L"    "N"    "S"    "Q"    "Y"
## ALN97023.1     "L"    "G"    "K"    "L"    "Q"    "S"    "N"    "Y"
## XP_011143830.1 "S"    "G"    "E"    "I"    "K"    "N"    "D"    "Y"
## XP_011344962.1 "N"    "D"    "E"    "I"    "K"    "S"    "D"    "Y"
## XP_011631523.1 "N"    "G"    "E"    "I"    "K"    "S"    "D"    "Y"
## GW790304.1_3   "N"    "N"    "K"    "I"    "K"    "S"    "D"    "Y"
## NP_001157188.1 "I"    "G"    "K"    "I"    "Q"    "S"    "N"    "Y"
## XP_012170795.1 "L"    "G"    "K"    "I"    "Q"    "E"    "N"    "Y"
##                [,173] [,174] [,175] [,176] [,177] [,178] [,179] [,180]
## NP_001037560.1 "H"    "V"    "V"    "G"    "H"    "Q"    "Q"    "L"
## XP_028138990.1 "H"    "L"    "I"    "G"    "H"    "R"    "Q"    "A"
## XP_017886197.1 "H"    "I"    "L"    "G"    "H"    "R"    "Q"    "A"
## XP_014205259.1 "I"    "L"    "I"    "G"    "H"    "R"    "Q"    "A"
## ALN97023.1     "T"    "L"    "I"    "G"    "H"    "R"    "Q"    "G"
## XP_011143830.1 "K"    "L"    "L"    "G"    "H"    "R"    "Q"    "T"
## XP_011344962.1 "I"    "L"    "L"    "G"    "H"    "R"    "Q"    "T"
## XP_011631523.1 "K"    "L"    "L"    "G"    "H"    "R"    "Q"    "T"
## GW790304.1_3   "K"    "L"    "L"    "G"    "H"    "R"    "Q"    "T"
## NP_001157188.1 "T"    "L"    "L"    "G"    "H"    "R"    "Q"    "T"
## XP_012170795.1 "T"    "L"    "L"    "G"    "H"    "R"    "Q"    "T"
##                [,181] [,182] [,183] [,184] [,185] [,186] [,187] [,188]
## NP_001037560.1 "I"    "N"    "T"    "L"    "S"    "P"    "G"    "A"
## XP_028138990.1 "D"    "K"    "T"    "E"    "C"    "P"    "G"    "D"
## XP_017886197.1 "R"    "S"    "T"    "L"    "C"    "P"    "G"    "T"
## XP_014205259.1 "E"    "T"    "L"    "S"    "C"    "P"    "G"    "H"
## ALN97023.1     "R"    "S"    "T"    "T"    "C"    "P"    "G"    "D"
## XP_011143830.1 "W"    "Q"    "T"    "N"    "C"    "P"    "G"    "N"
## XP_011344962.1 "G"    "Q"    "T"    "T"    "C"    "P"    "G"    "N"
## XP_011631523.1 "W"    "P"    "T"    "I"    "C"    "P"    "G"    "D"
## GW790304.1_3   "T"    "K"    "T"    "D"    "C"    "P"    "G"    "N"
## NP_001157188.1 "T"    "R"    "T"    "S"    "C"    "P"    "G"    "D"
## XP_012170795.1 "T"    "S"    "T"    "S"    "C"    "P"    "G"    "D"
##                [,189] [,190] [,191] [,192] [,193] [,194] [,195] [,196]
## NP_001037560.1 "V"    "L"    "Q"    "S"    "E"    "I"    "E"    "S"
## XP_028138990.1 "R"    "V"    "H"    "A"    "V"    "I"    "E"    "K"
## XP_017886197.1 "A"    "F"    "Y"    "K"    "Y"    "V"    "Q"    "T"
## XP_014205259.1 "Y"    "L"    "Y"    "Q"    "L"    "I"    "Q"    "T"
## ALN97023.1     "K"    "L"    "F"    "E"    "L"    "I"    "Q"    "S"
## XP_011143830.1 "S"    "L"    "Y"    "T"    "M"    "M"    "Q"    "S"
## XP_011344962.1 "S"    "L"    "Y"    "G"    "M"    "I"    "K"    "S"
## XP_011631523.1 "S"    "L"    "Y"    "T"    "M"    "I"    "K"    "S"
## GW790304.1_3   "S"    "L"    "Y"    "N"    "L"    "I"    "K"    "T"
## NP_001157188.1 "S"    "L"    "Y"    "E"    "L"    "I"    "K"    "T"
## XP_012170795.1 "S"    "L"    "Y"    "Q"    "L"    "I"    "Q"    "T"
##                [,197] [,198] [,199] [,200] [,201] [,202] [,203] [,204]
## NP_001037560.1 "W"    "P"    "H"    "W"    "L"    "D"    "N"    "A"
## XP_028138990.1 "W"    "P"    "H"    "F"    "E"    "A"    "N"    "P"
## XP_017886197.1 "L"    "P"    "R"    "W"    "T"    "N"    "H"    "P"
```

```
## XP_014205259.1 "W"    "S"    "H"    "W"    "R"    "K"    "I"    "S"
## ALN97023.1     "W"    "P"    "K"    "W"    "K"    "N"    "V"    "T"
## XP_011143830.1 "W"    "P"    "H"    "W"    "A"    "A"    "A"    "A"
## XP_011344962.1 "W"    "P"    "H"    "W"    "Q"    "S"    "S"    "A"
## XP_011631523.1 "W"    "P"    "H"    "W"    "S"    "E"    "R"    "E"
## GW790304.1_3   "W"    "P"    "H"    "W"    "T"    "D"    "T"    "P"
## NP_001157188.1 "W"    "P"    "H"    "W"    "S"    "S"    "I"    "-"
## XP_012170795.1 "W"    "P"    "H"    "W"    "S"    "S"    "I"    "-"
##                [,205] [,206] [,207] [,208] [,209] [,210] [,211] [,212]
## NP_001037560.1 "R"    "K"    "V"    "L"    "G"    "-"    "-"    "-"
## XP_028138990.1 "Q"    "D"    "A"    "S"    "P"    "K"    "K"    "L"
## XP_017886197.1 "I"    "P"    "N"    "Y"    "S"    "N"    "G"    "T"
## XP_014205259.1 "Q"    "N"    "A"    "A"    "-"    "-"    "-"    "-"
## ALN97023.1     "T"    "D"    "-"    "-"    "-"    "-"    "-"    "-"
## XP_011143830.1 "-"    "-"    "-"    "-"    "-"    "-"    "-"    "-"
## XP_011344962.1 "-"    "-"    "-"    "-"    "-"    "-"    "-"    "-"
## XP_011631523.1 "-"    "-"    "-"    "-"    "-"    "-"    "-"    "-"
## GW790304.1_3   "-"    "-"    "-"    "-"    "-"    "-"    "-"    "-"
## NP_001157188.1 "-"    "-"    "-"    "-"    "-"    "-"    "-"    "-"
## XP_012170795.1 "-"    "-"    "-"    "-"    "-"    "-"    "-"    "-"
##                [,213] [,214] [,215] [,216] [,217] [,218]
## NP_001037560.1 "-"    "-"    "-"    "-"    "-"    "-"
## XP_028138990.1 "-"    "-"    "-"    "-"    "-"    "-"
## XP_017886197.1 "T"    "T"    "T"    "L"    "A"    "L"
## XP_014205259.1 "-"    "-"    "-"    "-"    "-"    "-"
## ALN97023.1     "-"    "-"    "-"    "-"    "-"    "-"
## XP_011143830.1 "-"    "-"    "-"    "-"    "-"    "-"
## XP_011344962.1 "-"    "-"    "-"    "-"    "-"    "-"
## XP_011631523.1 "-"    "-"    "-"    "-"    "-"    "-"
## GW790304.1_3   "-"    "-"    "-"    "-"    "-"    "-"
## NP_001157188.1 "-"    "-"    "-"    "-"    "-"    "-"
## XP_012170795.1 "-"    "-"    "-"    "-"    "-"    "-"
##
## $call
## read.fasta(file = "aln-fasta.txt")
```

To make our file accessible to the heatmap() function, we will need to convert the alignment to a sequence identity matrix, using the handy seqidentity() function from bio3d.

```
alnpgrp <- seqidentity(pgrp)
head(alnpgrp) # Perfect!
```

```
##                NP_001037560.1 XP_028138990.1 XP_017886197.1 XP_014205259.1
## NP_001037560.1          1.000          0.323          0.304          0.289
## XP_028138990.1          0.323          1.000          0.364          0.344
## XP_017886197.1          0.304          0.364          1.000          0.378
## XP_014205259.1          0.289          0.344          0.378          1.000
```

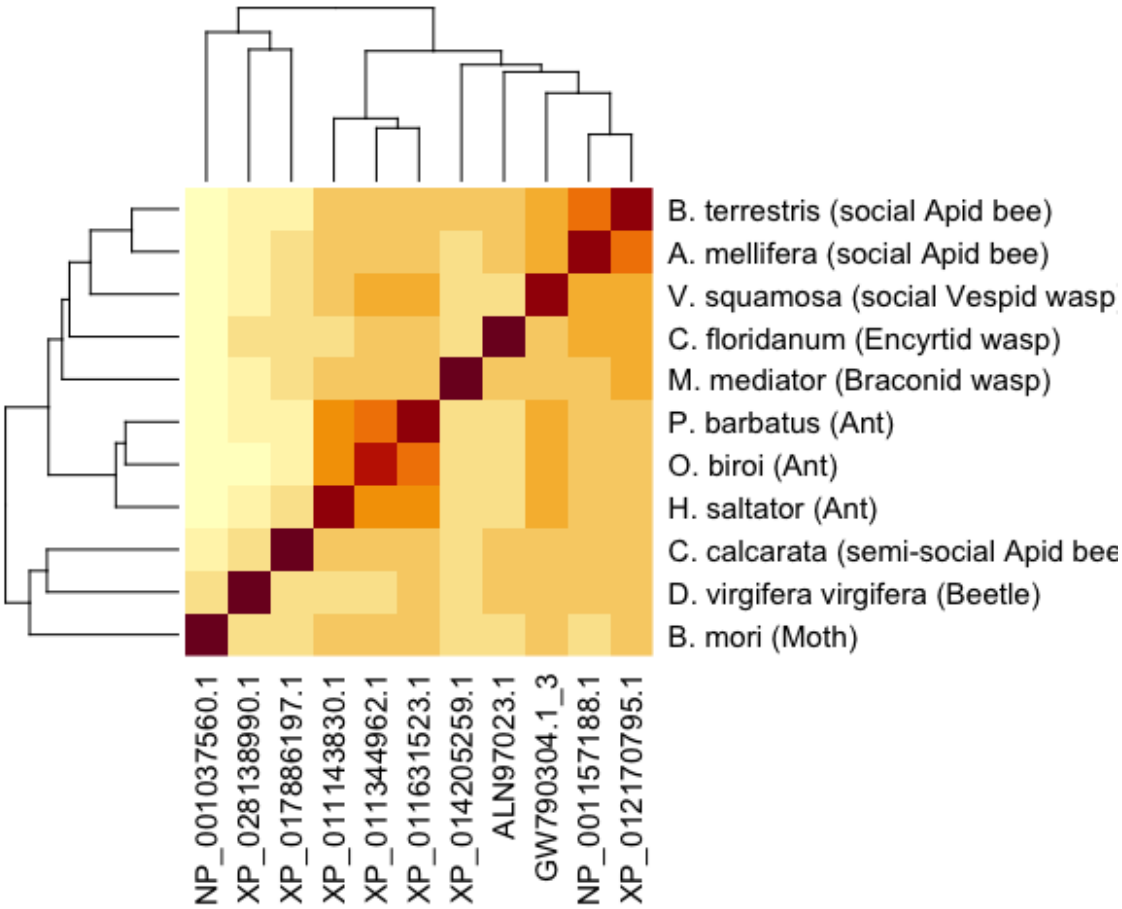
```
## ALN97023.1              0.300          0.395          0.426          0.438
## XP_011143830.1          0.353          0.356          0.435          0.429
##                ALN97023.1 XP_011143830.1 XP_011344962.1 XP_011631523.1
## NP_001037560.1      0.300          0.353          0.353          0.360
## XP_028138990.1      0.395          0.356          0.361          0.389
## XP_017886197.1      0.426          0.435          0.431          0.453
## XP_014205259.1      0.438          0.429          0.442          0.462
## ALN97023.1          1.000          0.432          0.474          0.481
## XP_011143830.1      0.432          1.000          0.704          0.703
##                GW790304.1_3 NP_001157188.1 XP_012170795.1
## NP_001037560.1        0.335          0.312          0.344
## XP_028138990.1        0.408          0.442          0.437
## XP_017886197.1        0.451          0.460          0.450
## XP_014205259.1        0.477          0.490          0.536
## ALN97023.1            0.500          0.513          0.534
## XP_011143830.1        0.572          0.521          0.546
```

```
rc <- rainbow(nrow(alnpgrp), start = 0, end = 1)
cc <- rainbow(ncol(alnpgrp), start = 0, end = 1)
Rnames <- cbind("B. mori (Moth)",
                "D. virgifera virgifera (Beetle)",
                "C. calcarata (semi-social Apid bee)",
                "M. mediator (Braconid wasp)",
                "C. floridanum (Encyrtid wasp)",
                "H. saltator (Ant)",
                "O. biroi (Ant)",
                "P. barbatus (Ant)",
                "V. squamosa (social Vespid wasp)",
                "A. mellifera (social Apid bee)",
                "B. terrestris (social Apid bee)"
                )
```

## Making the heatmap of alignment

Now, let's feed that into heatmap().

```
hm_pgrp <- heatmap(alnpgrp, labRow = Rnames, margins = c(9,9))
```




# Question 8

For question 8, we will use bio3d to search PDB for matching protein structure files. First, we will pick the sequence with greatest similarity to the others by asking R to generate maxima for each row in our identity matrix.

## Find the most similar sequence

```
maxid <- apply(alnpgrp, 1, sum) # calculates the row sums; the largest ID scor
maxid
```

```
## NP_001037560.1 XP_028138990.1 XP_017886197.1 XP_014205259.1     ALN97023.1
##          4.273          4.819          5.152          5.285          5.493
## XP_011143830.1 XP_011344962.1 XP_011631523.1   GW790304.1_3 NP_001157188.1
##          6.051          6.235          6.343          6.155          6.226
## XP_012170795.1
##          6.366
```

From this, we see that XP_012170795.1, the sequence associated with B. terrestris, the buff-tailed bumblebee, has the most homology to the rest of the sequences. Let's use that one to search PDB for sequence files.

## Search PDB using *Bombus terrestris* PGRP protein sequence

```
# Read in the bombus sequence
bterr <- read.fasta("bterrestris.fasta")
# Use bio3d to run a blastp against the PDB
blastbterr <- blast.pdb(bterr, database = "pdb")
```

```
##  Searching ... please wait (updates every 5 seconds) RID = FF0R0FEX01R
##  .
##  Reporting 30 hits
```

```
# Now let's make a workable object, like a database
bterr_db <- cbind(blastbterr$hit.tbl$subjectids,
                  blastbterr$hit.tbl$identity,
                  blastbterr$hit.tbl$mismatches,
                  blastbterr$hit.tbl$gapopens,
                  blastbterr$hit.tbl$evalue
                  )
colnames(bterr_db) <- cbind("subjectids",
                            "identity",
                            "mismatches",
                            "gapopens",
                            "evalue"
                            )
bterr_db <- as.data.frame(bterr_db)
head(bterr_db)
```

```
##   subjectids identity mismatches gapopens   evalue
## 1     4Z8I_A   51.553         76        1 4.57e-58
## 2     4ZXM_A   51.553         76        1 6.17e-58
## 3     2F2L_X   46.012         87        1 4.31e-51
## 4     1SK4_A   45.122         87        1 6.89e-47
## 5     1SK3_A   45.181         88        1 7.65e-47
## 6     1OHT_A   45.783         87        2 8.43e-47
```

# Get background information about the protein

```
# Ask for the PDB annotations,
# including the stucture IDs and experimental validation info
bterrann <- pdb.annotate(blastbterr$hit.tbl$subjectids)
```

```
## Warning in pdb.annotate(blastbterr$hit.tbl$subjectids): ids should be
## standard 4 character PDB-IDs: trying first 4 characters...
```

```
bterrann$subjectids <- paste(bterr_db$subjectids)
```

```
# Let's squish everyhting together in a big data fram
whole_bterr <- merge.data.frame(bterrann, bterr_db)
# Quick fix to ensure R properly reads the e-value exponential notation.
whole_bterr$evalue <- as.numeric(as.character(whole_bterr$evalue))
```

Now we will pick out the required data from the whole merged data frames.

```
abbrev <- as.data.frame(cbind(whole_bterr$subjectids,
                whole_bterr$compound,
                whole_bterr$experimentalTechnique,
                whole_bterr$resolution,
                whole_bterr$source,
                whole_bterr$evalue))
colnames(abbrev) <- c("subjectids",
                      "compounds",
                      "experimentalTechnique",
                      "resolution",
                      "source",
                      "evalue")
abbrev$evalue <- as.numeric(as.character(abbrev$evalue))
# Fix the dumb problem with R converting evalue to a factor quick
sabbrev <- abbrev[order(abbrev$evalue),]

head(sabbrev)
```

```
##    subjectids                                          compounds
## 24     4Z8I_A                peptidoglycan recognition protein 3
## 25     4ZXM_A PGRP domain of peptidoglycan recognition protein 3
## 14     2F2L_X   Peptidoglycan recognition protein-LC isoform LCx
## 6      1SK4_A          Peptidoglycan recognition protein I-alpha
## 5      1SK3_A          Peptidoglycan recognition protein I-alpha
## 3      1OUT_A                                     CG14704 PROTEIN
```

```
## 3      1UHT_A                                      CG14704 PROTEIN
##    experimentalTechnique resolution                 source   evalue
## 24     X-RAY DIFFRACTION        2.7  Branchiostoma belcheri 4.57e-58
## 25     X-RAY DIFFRACTION        2.8  Branchiostoma belcheri 6.17e-58
## 14     X-RAY DIFFRACTION        2.1 Drosophila melanogaster 4.31e-51
## 6      X-RAY DIFFRACTION       1.65            Homo sapiens 6.89e-47
## 5      X-RAY DIFFRACTION        2.8            Homo sapiens 7.65e-47
## 3      X-RAY DIFFRACTION        2.0 Drosophila melanogaster 8.43e-47
```

# Question 9

> 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?

From the previous question, let's select the PDB entry with the lowest evalue: 4Z8I_A, a peptidoglycan receptor protein origianlly characterized in the lancelet *Branchiostoma belcheri*.

## Get the target PDB

```
pgrp_pdb <- read.pdb("4z8i")
```

```
##   Note: Accessing on-line PDB file
```

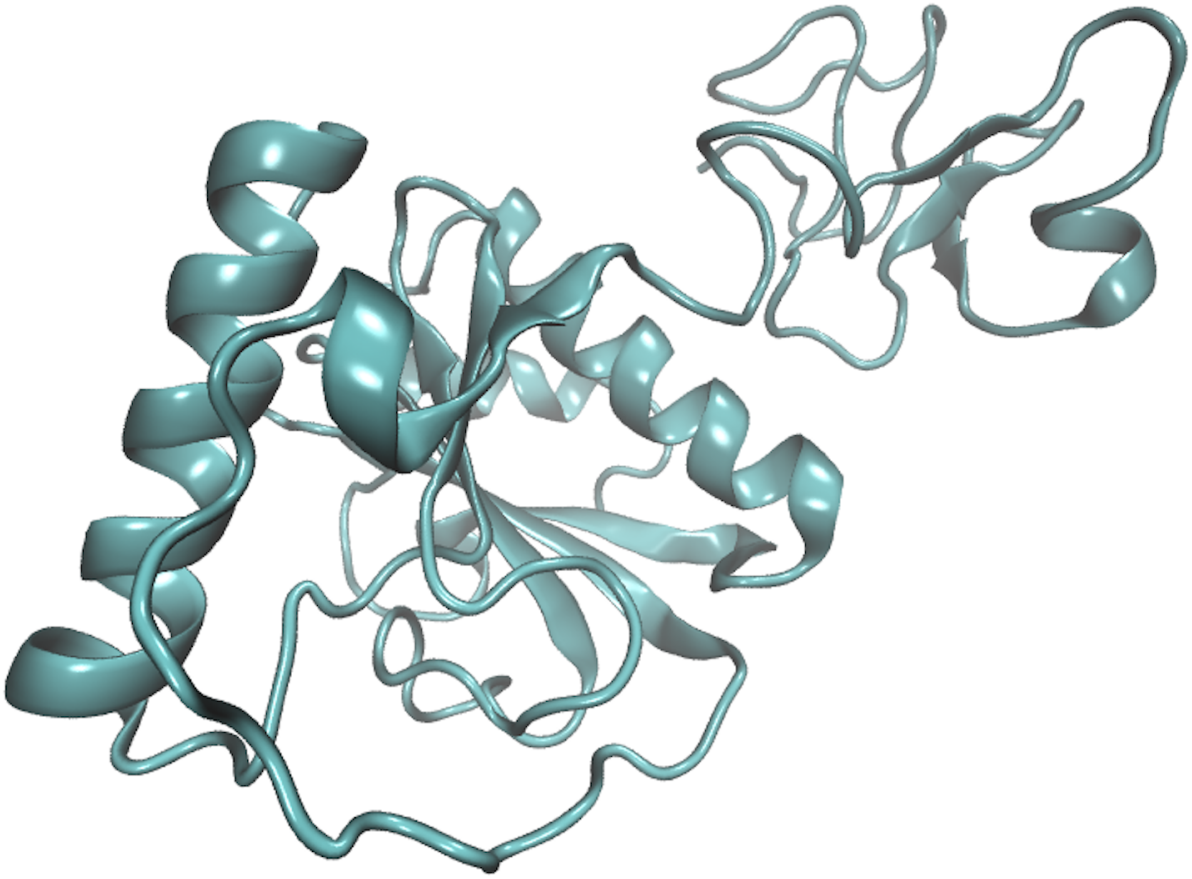
```
#write.pdb(pgrp_pdb, file = "bbelcheri_pgrp.pdb")
biounit(pgrp_pdb)
```

```
## $`AUTHOR.determined.monomer (1 chains)`
##
##  Call:  biounit(pdb = pgrp_pdb)
##
##    Total Models#: 1
##      Total Atoms#: 1766,  XYZs#: 5298  Chains#: 1  (values: A)
##
##      Protein Atoms#: 1691  (residues/Calpha atoms#: 224)
##      Nucleic acid Atoms#: 0  (residues/phosphate atoms#: 0)
##
##      Non-protein/nucleic Atoms#: 75  (residues: 75)
```

```
##      Non-protein/nucleic resid values: [ HOH (74), ZN (1) ]
##
##    Protein sequence:
##       QRWRSDGRCGPNYPAPDANPGECNPHAVDHCCSEWGWCGRETSHCTCSSCVDYSAGSSGT
##       CPRIVSKSEWGSRATNYNVFLSLPVPKVVIHHSAGATCSTQSSCSLQVRNIQNYHMDGRG
##       YSDIGYNFLVGNDGNVYEGRGWDRRGAHALNVNTESIGICFMGDFTSQKPTASAIAAAKS
##       LISCGVSLGKIRSGYSLYGHRDVGSTACPGNLLYDDIKSWGRYV
##
## + attr: atom, helix, sheet, seqres, xyz,
##         calpha, call, log
```

## View the VMD protein gummy

When I read my PMD file for the *B. belcerhi* PGRP, it gives me this cute looking gummy protein structure.



## Probability of similar structure

I compare the sequence from *V. squamosa* and *B. Belcheri* using seqaln().

```
compar <- read.fasta("q9_aln.txt")
seqaln(compar)
```

```
##                                  1        .         .         .         .
## [Truncated_Name:1]GW790304.1   RMVR-------------------ATFLLVATCIA---------
## [Truncated_Name:2]4Z8I:A|PDB   GSQRWRSDGRCGPNYPAPDANPGECNPHAVDHCCSEWGWCGRET
##                                   *                       *  *
##                                  1        .         .         .         .
##
##                                 51        .         .         .         .
## [Truncated_Name:1]GW790304.1   EVADSAA--TFETPNIVSRQQWGAKPPKSPTPNLKMN-PPPYVV
## [Truncated_Name:2]4Z8I:A|PDB   SCVDYSAGSSGTCPRIVSKSEWGSRATNY---NVFLSLPVPKVV
##                                   *  *  ^   * ***^  ** ^       *^ ^  * * **
##                                 51        .         .         .         .
##
##                                101        .         .         .         .
## [Truncated_Name:1]GW790304.1   VGCTTQAICQARVRSFQNDHMNSRKWNDIGYNFLVGEDGNVYEG
## [Truncated_Name:2]4Z8I:A|PDB   ATCSTQSSCSLQVRNIQNYHMDGRGYSDIGYNFLVGNDGNVYEG
##                                  *^**  *   **  ** **  * ^ ********* *******
##                                101        .         .         .         .
##
##                                151        .         .         .         .
## [Truncated_Name:1]GW790304.1   GSHSVPYNAKSIGICLIGKFNNNVPNSASIRATQNLIAYGVANN
## [Truncated_Name:2]4Z8I:A|PDB   GAHALNVNTESIGICFMGDFTSQKPTASAIAAAKSLISCGVSLG
##                                * * ^  *  ***** ^* *  ^ *    * *   **  **
##                                151        .         .         .         .
##
##                                201        .         .         .       239
## [Truncated_Name:1]GW790304.1   KLLGHRQTTKTDCPGNSLYNLIKTWPHWTDTP-------
## [Truncated_Name:2]4Z8I:A|PDB   SLYGHRDVGSTACPGNLLYDDIKSWGRYVGAAAHHHHHH
##                                 * ***    * **** **  **^* ^^
##                                201        .         .         .       239
##
## Call:
##   seqaln(aln = compar)
##
## Class:
##   fasta
##
## Alignment dimensions:
##   2 sequence rows; 239 position columns (196 non-gap, 43 gap)
##
## + attr: id, ali, call
```

```
alncompar <- seqidentity(compar)
head(alncompar)
```

```
##                                GW790304.1_3 4Z8I:A|PDBID|CHAIN|SEQUENCE
## GW790304.1_3                          1.000                        0.439
## 4Z8I:A|PDBID|CHAIN|SEQUENCE          0.439                        1.000
```

These two sequences (GW790304.1_3 is the *V. squamosa* and 4Z8I... is the *B. belcheri*) don't appear to be very related; they only have around %50 identity matching. Presumably the actual protein structure in *V. squamosa* looks different. Given the differences between immune responses in eusocial and solitary animals, perhaps this is not as surprising?

# Question 10

> 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?

When I search ChEMBL with the truncated *V. squamosa* sequence described in question 5, a single hit is returns. It appears to be a n-temrinal kinase protein (or something like that) characterized in *Mus musculus*.

| | |
|---|---|
| **ID:** | CHEMBL3721301 |
| **Type:** | SINGLE PROTEIN |
| **Preferred Name:** | N-terminal kinase-like protein |
| **Synonyms:** | 105 kDa kinase-like protein, Mitosis-associated kinase-like protein NTKL, N-terminal kinase-like protein, SCY1-like protein 1, Scyl1 |
| **Organism:** | Mus musculus |
| **Species Group:** | No |
| **Protein Target Classification:** | - Unclassified protein |

The majority of the functional parts of this mouse protein appear to be associated with cellular structure, protein binding and kinase activity. Perhaps this is reasonable to assumed for an external receptor that interacts with the cell walls of other cells (such as

bacteria or virus capsids)

| | |
|---|---|
| **GoComponent** | • GO:0005737 (cytoplasm)<br>• GO:0005793 (endoplasmic reticulum-Golgi intermediate compartment)<br>• GO:0005794 (Golgi apparatus)<br>• GO:0005801 (cis-Golgi network)<br>• GO:0005815 (microtubule organizing center)<br>• GO:0005829 (cytosol)<br>• GO:0005856 (cytoskeleton)<br>• GO:0030126 (COPI vesicle coat) |
| **GoFunction** | • GO:0004672 (protein kinase activity)<br>• GO:0005515 (protein binding)<br>• GO:0005524 (ATP binding) |
| **GoProcess** | • GO:0006468 (protein phosphorylation)<br>• GO:0006890 (retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum)<br>• GO:0016192 (vesicle-mediated transport) |

# The End

Thank you for reviewing my assignment! Hopefully it was informative!

I really appreciate this course and feel like I've learned much from it! Thanks for your efforts in putting it together and teaching us!