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December 3, 2018

Q1.

Protein: Hepatocyte Nuclear Factor 6 (HNF6)

Species: Homo sapiens

Accession number: NP_004489

Function known: A transcription factor in the Cut homeobox family. Expression of HNF6 is enriched in the liver, which it stimulates transcription of liver-specific genes and antagonizes glucocorticoid-stimulated gene expression. May influence glucose metabolism, cell cycle regulation, and may be associated with cancer.

Q2.

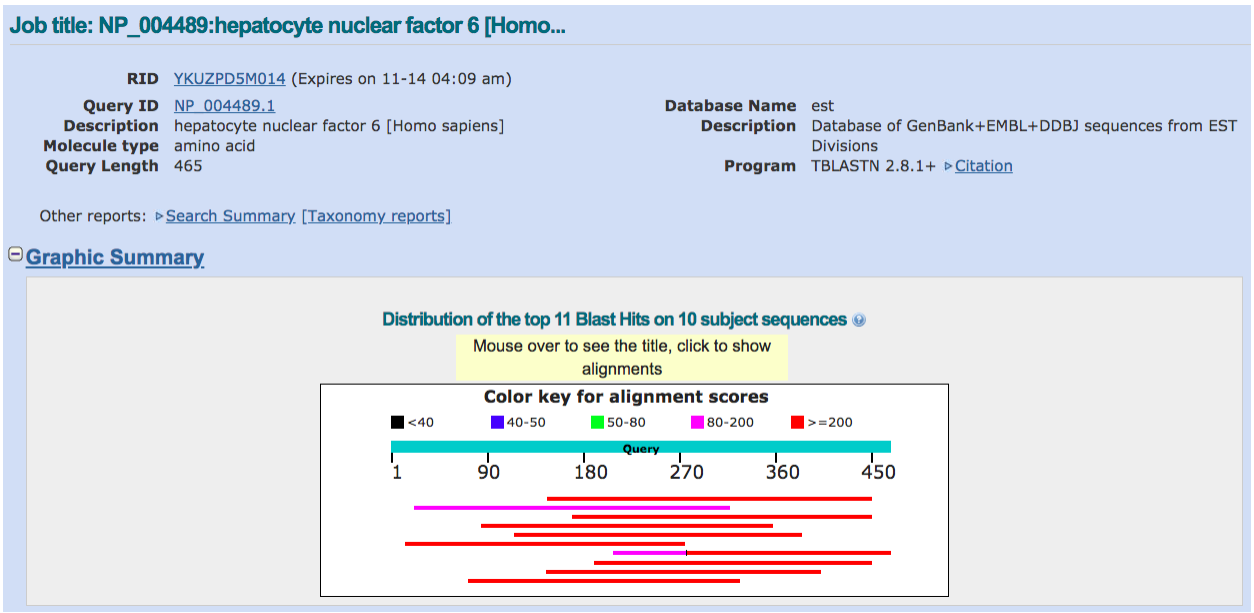
Method: TBLASTN (2.8.1)

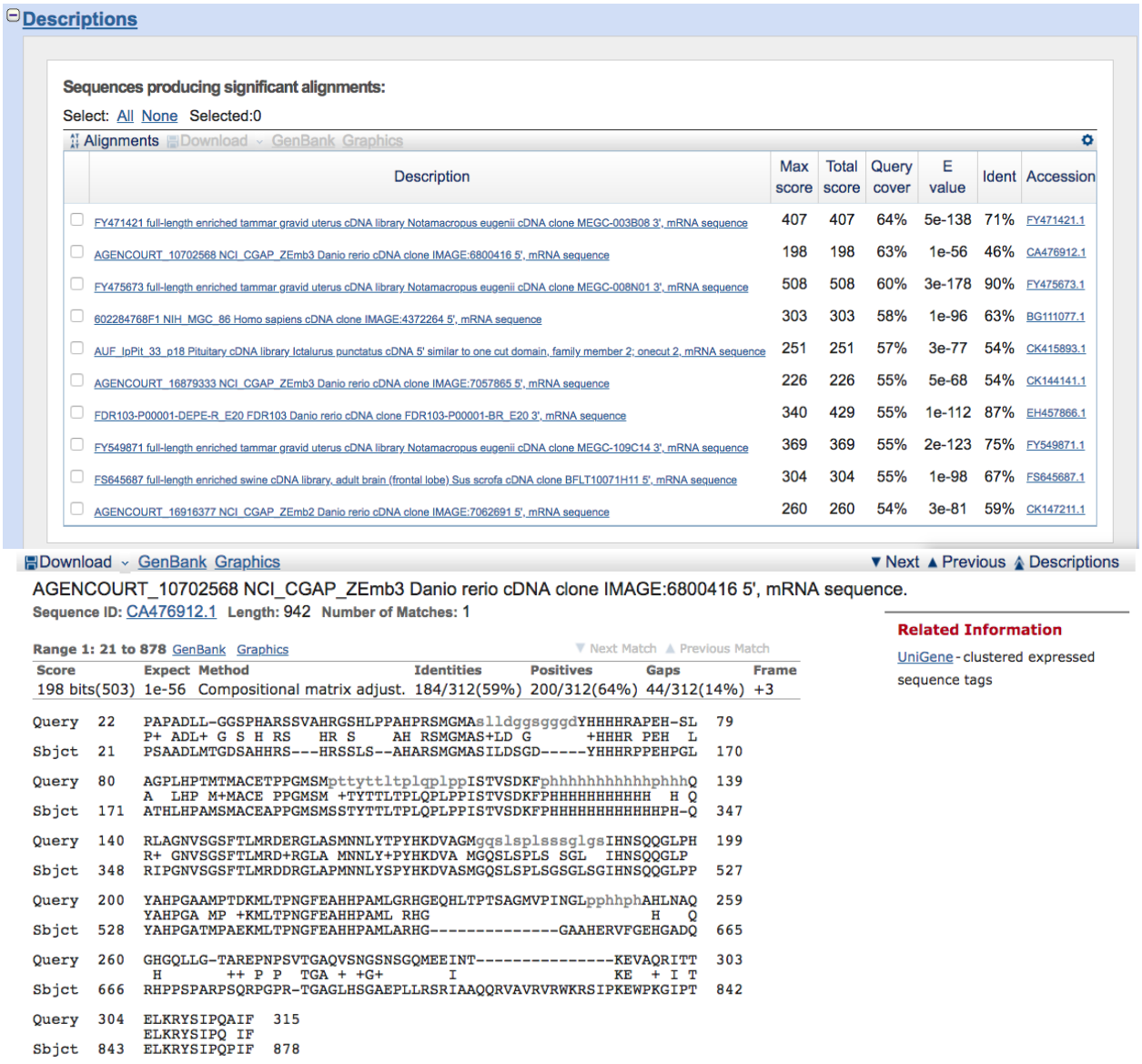
Database: Expressed sequence tags (est)

Limit organism: All species

Alignment of choice: BLOSUM62

Chosen match: Assession CA476912.1, a 942 bp clone from *Danio rerio*.





Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	FY471421 full-length enriched tammar gravid uterus cDNA library Notamacropus eugenii cDNA clone MEGC-003B08 3', mRNA sequence	407	407	64%	5e-138	71%	FY471421.1
☐	AGENCOURT_10702568 NCI_CGAP_ZEmb3 Danio rerio cDNA clone IMAGE:6800416 5', mRNA sequence	198	198	63%	1e-56	46%	CA476912.1
☐	FY475673 full-length enriched tammar gravid uterus cDNA library Notamacropus eugenii cDNA clone MEGC-008N01 3', mRNA sequence	508	508	60%	3e-178	90%	FY475673.1
☐	602284768F1 NIH_MGC_86 Homo sapiens cDNA clone IMAGE:4372264 5', mRNA sequence	303	303	58%	1e-96	63%	BG111077.1
☐	AUF_IpPit_33_p18 Pituitary cDNA library Ictalurus punctatus cDNA 5' similar to one cut domain, family member 2; onecut 2, mRNA sequence	251	251	57%	3e-77	54%	CK415893.1
☐	AGENCOURT_16879333 NCI_CGAP_ZEmb3 Danio rerio cDNA clone IMAGE:7057865 5', mRNA sequence	226	226	55%	5e-68	54%	CK144141.1
☐	FDR103-P00001-DEPE-R_E20 FDR103 Danio rerio cDNA clone FDR103-P00001-BR_E20 3', mRNA sequence	340	429	55%	1e-112	87%	EH457866.1
☐	FY549871 full-length enriched tammar gravid uterus cDNA library Notamacropus eugenii cDNA clone MEGC-109C14 3', mRNA sequence	369	369	55%	2e-123	75%	FY549871.1
☐	FS645687 full-length enriched swine cDNA library, adult brain (frontal lobe) Sus scrofa cDNA clone BFLT10071H11 5', mRNA sequence	304	304	55%	1e-98	67%	FS645687.1
☐	AGENCOURT_16916377 NCI_CGAP_ZEmb2 Danio rerio cDNA clone IMAGE:7062691 5', mRNA sequence	260	260	54%	3e-81	59%	CK147211.1

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AGENCOURT_10702568 NCI_CGAP_ZEmb3 Danio rerio cDNA clone IMAGE:6800416 5', mRNA sequence.

Sequence ID: CA476912.1 Length: 942 Number of Matches: 1

Related Information

UniGene - clustered expressed sequence tags

Range 1: 21 to 878 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps	Frame
198 bits(503)	1e-56	Compositional matrix adjust.	184/312(59%)	200/312(64%)	44/312(14%)	+3

```
Query  22   PAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMAslldggsgggdYHHHHRAPEH-SL  79
            P+ ADL+ G S H RS    HR S    AH RSMGMAS+LD G      +HHHR PEH  L
Sbjct  21   PSAADLMTGDSAHHRS---HRSSLS--AHARSMGMASILDSGD-----YHHHRPPEHPGL  170

Query  80   AGPLHPTMTMACETPPGMSMpttyttltplqplppISTVSDKFphhhhhhhhhhhphhhQ  139
            A  LHP M+MACE PPGMSM +TYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHH  H Q
Sbjct  171  ATHLHPAMSMACEAPPGMSMSSTYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHHHPH-Q  347

Query  140  RLAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMgqslsplsssglgsIHNSQQGLPH  199
            R+ GNVSGSFTLMRD+RGLA MNNLY+PYHKDVA MGQSLSPLS SGL  IHNSQQGLP
Sbjct  348  RIPGNVSGSFTLMRDDRGLAPMNNLYSPYHKDVASMGQSLSPLSGSGLSGIHNSQQGLPP  527

Query  200  YAHPGAAMPTDKMLTPNGFEAHHPAMLGRHGEQHLTPTSAGMVPINGLpphhphAHLNAQ  259
            YAHPGA MP +KMLTPNGFEAHHPAML RHG                  H   Q
Sbjct  528  YAHPGATMPAEKMLTPNGFEAHHPAMLARHG--------------GAAHERVFGEHGADQ  665

Query  260  GHGQLLG-TAREPNPSVTGAQVSNGSNSGQMEEINT--------------KEVAQRITT  303
             H       ++ P P  TGA + +G+       I                KE  + I T
Sbjct  666  RHPPSPARPSQRPGPR-TGAGLHSGAEPLLRSRIAAQQRVAVRVRWKRSIPKEWPKGIPT  842

Query  304  ELKRYSIPQAIF  315
            ELKRYSIPQ IF
Sbjct  843  ELKRYSIPQPIF  878
```

Q3.

Chosen sequence:

```
>D. rerio protein (sequence taken from BLAST result)
PSAADLMTGDSAHHRSHRSSLSAHARSMGMASILDSGDYHHHRPPEHPGLATHLHPAMSMACEAPPGMSM
SSTYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHHHHPHQRIPGNVSGSFTLMRDDRGLAPMNNLYSPYHK
DVASMGQSLSPLSGSGLSGIHNSQQGLPPYAHPGATMPAEKMLTPNGFEAHHPAMLARHGGAAHERVFGE
HGADQRHPPSPARPSQRPGPRTGAGLHSGAEPLLRSRIAAQQRVAVRVRWKRSIPKEWPKGIPTELKRYS
IPQPIF
```

Name: Danio transcription factor

Species: *Danio rerio*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei; Ostariophysi; Cypriniformes; Cyprinidae; Danio.

Q4.

Method: BLASTP 2.8.1

Database: Non-redundant protein sequences (nr)

Sequences producing significant alignments:

Select: All None Selected:0

Alignments | Download | GenPept | Graphics | Distance tree of results | Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
hepatocyte nuclear factor 6 isoform X1 [Danio rerio]	414	414	99%	4e-141	72%	XP_005173695.1
hepatocyte nuclear factor 6 [Danio rerio]	414	414	99%	5e-141	72%	NP_956867.1
PREDICTED: hepatocyte nuclear factor 6-like isoform X1 [Sinocyclocheilus rhinocerous]	383	383	100%	1e-128	72%	XP_016416804.1
PREDICTED: hepatocyte nuclear factor 6-like isoform X2 [Sinocyclocheilus rhinocerous]	382	382	100%	1e-128	72%	XP_016416805.1
PREDICTED: hepatocyte nuclear factor 6-like isoform X1 [Sinocyclocheilus anshuiensis]	380	380	100%	7e-128	72%	XP_016326525.1
PREDICTED: hepatocyte nuclear factor 6-like isoform X2 [Sinocyclocheilus anshuiensis]	380	380	100%	9e-128	72%	XP_016326526.1
PREDICTED: LOW QUALITY PROTEIN: hepatocyte nuclear factor 6-like [Cyprinus carpio]	374	374	100%	1e-125	74%	XP_018949348.1
onecut-1 isoform a [Danio rerio]	374	374	99%	2e-125	72%	AAW02845.1
onecut-1 isoform b [Danio rerio]	374	374	99%	4e-125	72%	AAW02846.1
hepatocyte nuclear factor 6-like isoform X2 [Carassius auratus]	371	371	100%	3e-124	72%	XP_026143615.1
hepatocyte nuclear factor 6-like isoform X1 [Carassius auratus]	371	371	100%	4e-124	72%	XP_026143613.1
PREDICTED: hepatocyte nuclear factor 6-like [Cyprinus carpio]	370	370	99%	1e-123	76%	XP_018949346.1

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hepatocyte nuclear factor 6 isoform X1 [Danio rerio]

Sequence ID: XP_005173695.1 **Length:** 456 **Number of Matches:** 1

See 1 more title(s)

Range 1: 23 to 306 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
414 bits(1064)	4e-141	Compositional matrix adjust.	225/311(72%)	232/311(74%)	53/311(17%)

```
Query  2    SAADLMTGDSAHHRSHRSSLSAHARSMGMASILDSGDYHHHRPPEHPGLATHLHPAMSMA  61
            SAADLMTGDSAHHRSHRSSLSAHARSMGMASILDSGDYHHHRPPEHPGLATHLHPAMSMA
Sbjct  23   SAADLMTGDSAHHRSHRSSLSAHARSMGMASILDSGDYHHHRPPEHPGLATHLHPAMSMA  82

Query  62   CEAPPGMSMSSTYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHHHHPHQRIPGNVSGSFTL  121
            CEAPPGMSMSSTYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHHHHPHQRIPGNVSGSFTL
Sbjct  83   CEAPPGMSMSSTYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHHHHPHQRIPGNVSGSFTL  142

Query  122  MRDDRGLAPMNNLYSPYHKDVASMGQSLSPLSGSGLSGIHNSQQGLPPYAHPGATMPAEK  181
            MRDDRGLAPMNNLYSPYHKDVASMGQSLSPLSGSGLSGIHNSQQGLPPYAHPGATMPAEK
Sbjct  143  MRDDRGLAPMNNLYSPYHKDVASMGQSLSPLSGSGLSGIHNSQQGLPPYAHPGATMPAEK  202

Query  182  MLTPNGFEAHHPAMLARHG----------------------------GAAHERVFGEHGADQ  215
            MLTPNGFEAHHPAMLARHG                              H +V G    +Q
Sbjct  203  MLTPNGFEAHHPAMLARHGEQHMSASSASMVPINVIHHHPHAHLNAQGHGQVLGS-TREQ  261

Query  216  RHPPSPARPSQRPGPRTGAGLHSGAEPLLRSRIAAQQRVAVRVRWKRSIPKEWPKGIPTE  275
             H       S  PG +   G  SG    + ++  AQ+                   I TE
Sbjct  262  NH-------SSVPGSQLNNGSSSGQMEEVNTKEVAQR-------------------ITTE  295

Query  276  LKRYSIPQPIF  286
            LKRYSIPQ IF
Sbjct  296  LKRYSIPQAIF  306
```

Related Information

Gene - associated gene details

New Genome Data Viewer - aligned genomic context

Identical Proteins - Identical proteins to XP_005173695.1

Q5.

Relabeled sequences:

```
>Novel_zebrafish
PSAADLMTGDSAHHRSHRSSLSAHARSMGMASILDSGDYHHHRPPEHPGLATHLHPAMSMACEAPPGMSM
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DVASMGQSLSPLSGSGLSGIHNSQQGLPPYAHPGATMPAEKMLTPNGFEAHHPAMLARHGGAAHERVFGE
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HGADQRHPPSPARPSQRPGPRTGAGLHSGAEPLLRSRIAAQQRVAVRVRWKRSIPKEWPKGIPTELKRYS
IPQPIF

>Original_human
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LAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMGQSLSPLSSSGLGSIHNSQQGLPHYAHPGAAMPTD
KMLTPNGFEAHHPAMLGRHGEQHLTPTSAGMVPINGLPPHHPHAHLNAQGHGQLLGTAREPNPSVTGAQV
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WKWLQEPEFQRMSALRLAACKRKEQEHGKDRGNTPKKPRLVFTDVQRRTLHAIFKENKRPSKELQITISQ
QLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTKA

>Sumatran_orangutan
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HHRAPEHSLAGPLHPTMTMACETPPGMSMPTTYTTLTPLQPLPPISTVSDKFPHHHHHHHHHHHPHHHQR
LAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMGQSLSPLSSSGLGSIHNSQQGLPHYAHPGAAMPTD
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SNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLCRSQGTLSDLLRNPKPWSKLKSGRETFRRM
WKWLQEPEFQRMSALRLAACKRKEQEHGKDRGNTPKKPRLVFTDVQRRTLHAIFKENKRPSKELQITISQ
QLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTKA

>Gibbon
MNAQLTMEAIGELHGVSHEPVPAPADLLGGSPHARSSVAHRGSHLPPAHPRSMGMASLLDGGSGGGDYHH
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LAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMGQSLSPLSSSGLGSIHNSQQGLPHYAHPGAAMPTD
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WKWLQEPEFQRMSALRLAACKRKEQEHGKDRGNTPKKPRLVFTDVQRRTLHAIFKENKRPSKELQITISQ
QLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTKA

>Cheetah
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>Chimp
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LAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMGQSLSPLSSSGLGSIHNSQQGLPHYAHPGTTMPTD
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>Cat
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>Grizzly_bear
MNAQLTMEAIGELHGVSHEPVPAPADLLGGSPHARSSVAHRGSHLPPAHPRSMGMASLLDGGGGGGDYHH
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LAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMGQSLSPLSTSGLGGIHNSQQGLPHYAHPGAAMPTD
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QLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTKA

>Dog
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LAGNVSGSFTLMRDERGLASMNNLYTPYHKDVAGMGQSLSPLSSSGLSGIHNSQQGLPHYAHPGAAMPTD
KMLTPNGFEAHHPAMLGRHGEQHLTPTSAGMVPINGLPPHHPHAHLNAQGHGQLLGTAREPNPSVTGAQV
SNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLCRSQGTLSDLLRNPKPWSKLKSGRETFRRM
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>Killer_whale
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>Tropical_clawed_frog
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SFTLMRDDRGLASMNNLYSPYHKEVTGMGQSLSPLSGSGLGSIHGAQQGPPHYAHPSAAMPTEKMLTPNG
FEAHHPAMLTRHGEQHLTPPSAGMVPINGIPHHPHAHLNAQSHGQILASTRDQNPPSVTGSQINNGSNSG
QMEEINTKEVAQRITTELKRYSIPQAIFAQRVLCRSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEP
EFQRMSALRLAALVPADPVFQHSGQLPADSLVKIGYPSQSTQSNHMSCKRKEQEHGKDRGNTPKKPRLVF
TDVQRRTLHAIFKENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGNSGSGNTSSSSSTC
TKA

>Sea_squirt
MPVSLGQSVTTSPSAKSASILNQQHPGIATDFITMATAASGELNGFHHLHHHHHPSEQYYRHEHYHHHFH
HPNFDGYPNYNDRDTPIAGDMQKNNLHNFASKSMSLEGEKLDENCNKSPNYLPPIGDALLRRDNRSDASK
NNAKEEDESGCSKFVMQETDNSLTELQKSSAVSEHEKKEEVQLKTNDAPEDFSVKTEQSELYQFHARNFS
IFTPSSQRGTPDEGMNLIPVETTDHTSIDSYFRSDATNANPNSNPIDSVPSSVDGPSYATLTPLQPLPSI
SSVSDKYMPTNETSYATLTNQELTDCSSYSKMGGMGHSLPPLSNRMILNGLAAQTRGGMQSQAAIDAVNQ
AAAAAVGLSHYNKPVLSSNIIPPPPPVSNPYDPHVFGRIDQCNDMGAGFPGGHMFPHRSTGFVSQYGLQD
LSSSLQVSAPSERRRPTHEDIPADNGKRHSGSDRLGGSGLQPHSSNSASSSRTQQIEEVNTKEVASKITQ
ELKRYSIPQAIFAQRVLCRSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSSLRLAACKRK
EDEKSYENSVNSPKKPRLVFTDLQRRTLHAIFKESKRPSKEMQIQISQQLGLEVTTVSNFFMNARRRSLD
KWQDDESGYNSKENSRSNNPSSDHHLSASPNHQQQQQQQQQQQQQQQQQQAYQQHTQDSRLSYAPGESLLS
PLCGSPSGHLHFPPPHHLHHHNLHQQQQNTMLSASHLTSSGLVHPYQSQHQLLGSDVTGLVNPR
```

Alignment using EBI's MUSCLE:

```
CLUSTAL multiple sequence alignment by MUSCLE (3.8)


Sea_squirt                ----------MPVSLGQSVTTSPSAKSASILNQQH-PGIATDFITMATAA
Novel_zebrafish           ----------PSAADLMTGDSAHHRS---HRSSL--SAHARSMGMASIL
Tropical_clawed_frog      MNAQLTMDAIGDLHGISHESVPGTADLM-GSSPHHRGSVTHRSNHL-SAHPRSMGMASIL
Killer_whale              MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Chimp                     MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Dog                       MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Grizzly_bear              MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Cat                       MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Original_human            MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Sumatran_orangutan        MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Gibbon                    MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
Cheetah                   MNAQLTMEAIGELHGVSHEPVPAPADLL-GGSPHARSSVAHRGSHLPPAHPRSMGMASLL
                                             * . .     *.  ..    . .      .  : **:

Sea_squirt                SGELNGFHHLHH----HHHPSEQYYRHEHYHHHFHHPNFDGYPNYNDRDTPIAGDMQKNN
Novel_zebrafish           DSG-----DYH-----HHRPPE-------------HPGLATH------------------
Tropical_clawed_frog      DGG-----DYHHHHHHHHRPPD-------------H-ALTGP------------------
Killer_whale              DGG-SGSGDYHH----HHRAPE-------------H-SLAGP------------------
Chimp                     DGG-SGGGDYHH----HHRAPE-------------H-SLAGP------------------
Dog                       DGG-GGGGDYHH----HHRAPE-------------H-SLAGP------------------
Grizzly_bear              DGG-GGGGDYHH----HHRAPE-------------H-SLAGP------------------
Cat                       DGG-GGGGDYHH----HHRAPE-------------H-SLAGP------------------
Original_human            DGG-SGGGDYHH----HHRAPE-------------H-SLAGP------------------
Sumatran_orangutan        DGG-SGGGDYHH----HHRAPE-------------H-SLAGP------------------
Gibbon                    DGG-SGGGDYHH----HHRAPE-------------H-SLAGP------------------
Cheetah                   DGG-GGGGDYHH----HHRAPE-------------H-SLAGP------------------
                          ..        *     **...:             *  :

Sea_squirt                LHNFASKSMSLEGEKLDENCNKSPNYLPPIGDALLRRDNRSDASKNNAKEEDESGCSKFV
Novel_zebrafish           LH----PAMSMA-------CEAPPG---------------------------------MS
Tropical_clawed_frog      LH----PTMTMA-------CDTPPG---------------------------------MS
Killer_whale              LH----PTMTMA-------CETPPG---------------------------------MS
Chimp                     LH----PTMTMA-------CETPPG---------------------------------MS
Dog                       LH----PTMTMA-------CETPPG---------------------------------MS
Grizzly_bear              LH----PTMTMA-------CETPPG---------------------------------MS
Cat                       LH----PTMTMA-------CETPPG---------------------------------MS
Original_human            LH----PTMTMA-------CETPPG---------------------------------MS
Sumatran_orangutan        LH----PTMTMA-------CETPPG---------------------------------MS
Gibbon                    LH----PTMTMA-------CETPPG---------------------------------MS
Cheetah                   LH----PTMTMA-------CETPPG---------------------------------MS
                          **     :*::        *: .*.                                  :

Sea_squirt                MQETDNSLTELQKSSAVSEHEKKEEVQLKTNDAPEDFSVKTEQSELYQFHARNFSIFTPS
Novel_zebrafish           MSSTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHH-
Tropical_clawed_frog      MSSTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHH------
Killer_whale              MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Chimp                     MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Dog                       MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Grizzly_bear              MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Cat                       MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Original_human            MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
```

```
Sumatran_orangutan      MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Gibbon                  MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
Cheetah                 MPTTYTTLTPLQPLPPISTVSDK----------------------FPHHHHHHHHHHHP
                        *  * .:** **  ..:*  ..*                       : .* .:.

Sea_squirt              SQRGTPDEGMNLIPVETTDHTSIDSYFRSDATNANPNSNPIDSVPSSVDGPSYATLTPLQ
Novel_zebrafish         ------------------------------------HPHQRIPGNVSG----SFTLMR
Tropical_clawed_frog    ------------------------------------HPHQRIPGNVSG----SFTLMR
Killer_whale            ------------------------------------HHHQRLAGNVSG----SFTLMR
Chimp                   ------------------------------------HHHQRLAGNVSG----SFTLMR
Dog                     ------------------------------------HHHQRLAGNVSG----SFTLMR
Grizzly_bear            ------------------------------------HHHQRLAGNVSG----SFTLMR
Cat                     ------------------------------------HHHQRLAGNVSG----SFTLMR
Original_human          ------------------------------------HHHQRLAGNVSG----SFTLMR
Sumatran_orangutan      ------------------------------------HHHQRLAGNVSG----SFTLMR
Gibbon                  ------------------------------------HHHQRLAGNVSG----SFTLMR
Cheetah                 ------------------------------------HHHQRLAGNVSG----SFTLMR
                                                            :  : :...*.*    ::* :.

Sea_squirt              PLPSISSVSDKYMPTNETSYATLTNQELTDCSSYSKMGGMGHSLPPLSNRMILNGLAAQT
Novel_zebrafish         DDRGLAPMNNLYSP-----------------YHKDVASMGQSLSPLSG----SGL----
Tropical_clawed_frog    DDRGLASMNNLYSP-----------------YHKEVTGMGQSLSPLSG----SGL----
Killer_whale            DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSG----SGL----
Chimp                   DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
Dog                     DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
Grizzly_bear            DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLST----SGL----
Cat                     DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
Original_human          DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
Sumatran_orangutan      DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
Gibbon                  DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
Cheetah                 DERGLASMNNLYTP-----------------YHKDVAGMGQSLSPLSS----SGL----
                           .::.:.: * *                   :..: .**:**.***     .**

Sea_squirt              RGGMQSQAAIDAVNQAAAAAVGLSHYNKP--VLSSNIIPPPPPVSNPYDPHVFGRIDQCN
Novel_zebrafish         -SGIHN-----------SQQGLPPYAHPGATMPAEKMLTPNGF-EAHHPAMLAR-----
Tropical_clawed_frog    -GSIHG-----------AQQGPPHYAHPSAAMPTEKMLTPNGF-EAHHPAMLTR-----
Killer_whale            -GGIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Chimp                   -GSIHN-----------SQQGLPHYAHPGTTMPTDKMLTPNGF-EAHHPAMLGR-----
Dog                     -SGIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Grizzly_bear            -GGIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Cat                     -GGIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Original_human          -GSIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Sumatran_orangutan      -GSIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Gibbon                  -GSIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
Cheetah                 -GSIHN-----------SQQGLPHYAHPGAAMPTDKMLTPNGF-EAHHPAMLGR-----
                         ..::.           :  * . * :*  .:.:: : .*  . :.: * :: *

Sea_squirt              DMGAGFPGGHMFPHRSTGFVSQYGLQDLSSSLQVSAPSERRRPTHEDIPADNGKRHSGSD
Novel_zebrafish         ------HGG----AAHERVFGEHGA-----------DQRHPPS----------------
Tropical_clawed_frog    ------HGEQHLTPPSAGMVPINGI-----------PHHPHAHLNA--------QSHG
Killer_whale            ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Chimp                   ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Dog                     ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Grizzly_bear            ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
```

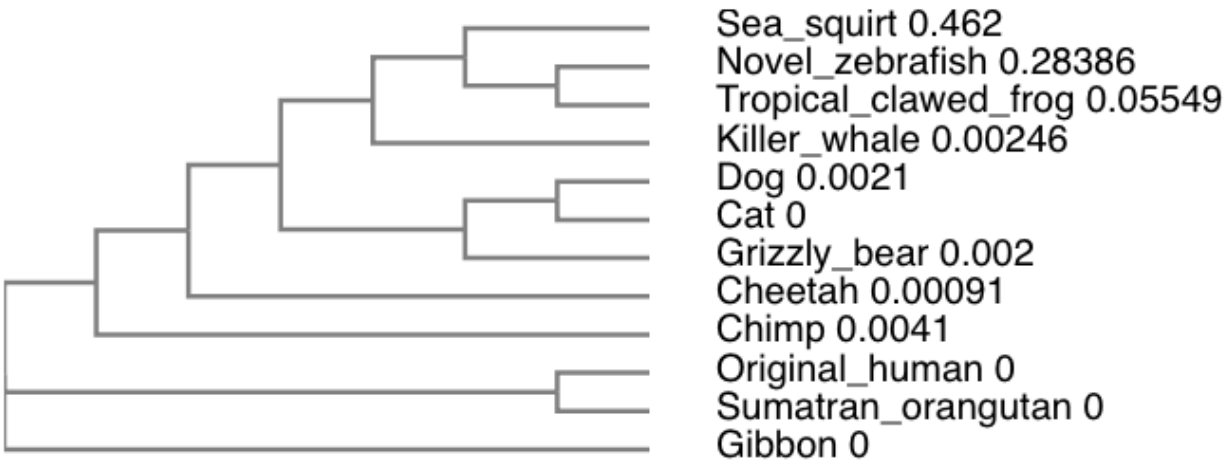
```
Cat                     ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Original_human          ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Sumatran_orangutan      ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Gibbon                  ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
Cheetah                 ------HGEQHLTPTSAGMVPINGL-----------PPHHPHAHLNA--------QGHG
                              *          ..   *              ..  :

Sea_squirt              RLGGSGLQPH-----SSNSASSSRTQQIEEVNTKEVASKITQELKRYSIPQAIFAQRVLC
Novel_zebrafish         ----PARPSQRP---GPRTGAGLHSGAEPLLRSRIAAQ-----------------QRVAV
Tropical_clawed_frog    QILASTRDQNPPSVTGSQINNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Killer_whale            QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Chimp                   QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Dog                     QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Grizzly_bear            QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Cat                     QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Original_human          QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Sumatran_orangutan      QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Gibbon                  QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
Cheetah                 QLLGTAREPN-PSVTGAQVSNGSNSGQMEEINTKEVAQRITTELKRYSIPQAIFAQRVLC
                            .    :     ...   . .:     :.:. .*.                ***

Sea_squirt              RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSSLRLAA------------
Novel_zebrafish         RVR-----------WKR------SIPKEW------------------------------
Tropical_clawed_frog    RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAALVPADPVFQHSG
Killer_whale            RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Chimp                   RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Dog                     RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Grizzly_bear            RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Cat                     RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Original_human          RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Sumatran_orangutan      RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Gibbon                  RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
Cheetah                 RSQGTLSDLLRNPKPWSKLKSGRETFRRMWKWLQEPEFQRMSALRLAA------------
                        * .           *..      :: . *

Sea_squirt              ----------------------CKRKEDEKSYENSVNSPKKPRLVFTDLQRRTL-HAIF
Novel_zebrafish         ------------------------------------------PKGIPTELKRYSIPQPIF
Tropical_clawed_frog    QLPADSLVKIGYPSQSTQSNHMSCKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Killer_whale            ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Chimp                   ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Dog                     ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Grizzly_bear            ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Cat                     ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Original_human          ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Sumatran_orangutan      ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Gibbon                  ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
Cheetah                 ----------------------CKRKEQEHGKDRG-NTPKKPRLVFTDVQRRTL-HAIF
                                                           *. : *:::* :: :.**

Sea_squirt              KESKRPSKEMQIQISQQLGLEVTTVSNFFMNARRRSLDKWQDDESGYNSKENSRSNNPSS
Novel_zebrafish         ------------------------------------------------------------
Tropical_clawed_frog    KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGNSGSGNTSSSSTCTK
Killer_whale            KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Chimp                   KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
```

```
Dog                     KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Grizzly_bear            KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Cat                     KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Original_human          KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Sumatran_orangutan      KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Gibbon                  KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK
Cheetah                 KENKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKWQDEGSSNSGNSSSSSSTCTK


Sea_squirt              DHHLSASPNHQQQQQQQQQQQQQQQQQQAYQQHTQDSRLSYAPGESLLSPLCGSPSGHLHF
Novel_zebrafish         ------------------------------------------------------------
Tropical_clawed_frog    A-----------------------------------------------------------
Killer_whale            A-----------------------------------------------------------
Chimp                   A-----------------------------------------------------------
Dog                     A-----------------------------------------------------------
Grizzly_bear            A-----------------------------------------------------------
Cat                     A-----------------------------------------------------------
Original_human          A-----------------------------------------------------------
Sumatran_orangutan      A-----------------------------------------------------------
Gibbon                  A-----------------------------------------------------------
Cheetah                 A-----------------------------------------------------------


Sea_squirt              PPPHHLHHHNLHQQQQNTMLSASHLTSSGLVHPYQSQHQLLGSDVTGLVNPR
Novel_zebrafish         ----------------------------------------------------
Tropical_clawed_frog    ----------------------------------------------------
Killer_whale            ----------------------------------------------------
Chimp                   ----------------------------------------------------
Dog                     ----------------------------------------------------
Grizzly_bear            ----------------------------------------------------
Cat                     ----------------------------------------------------
Original_human          ----------------------------------------------------
Sumatran_orangutan      ----------------------------------------------------
Gibbon                  ----------------------------------------------------
Cheetah                 ----------------------------------------------------
```

Note: The gap overhang in this alignment file was necessary becasue the sea_squirt's sequence has multiple insertions compared to other species' sequences.

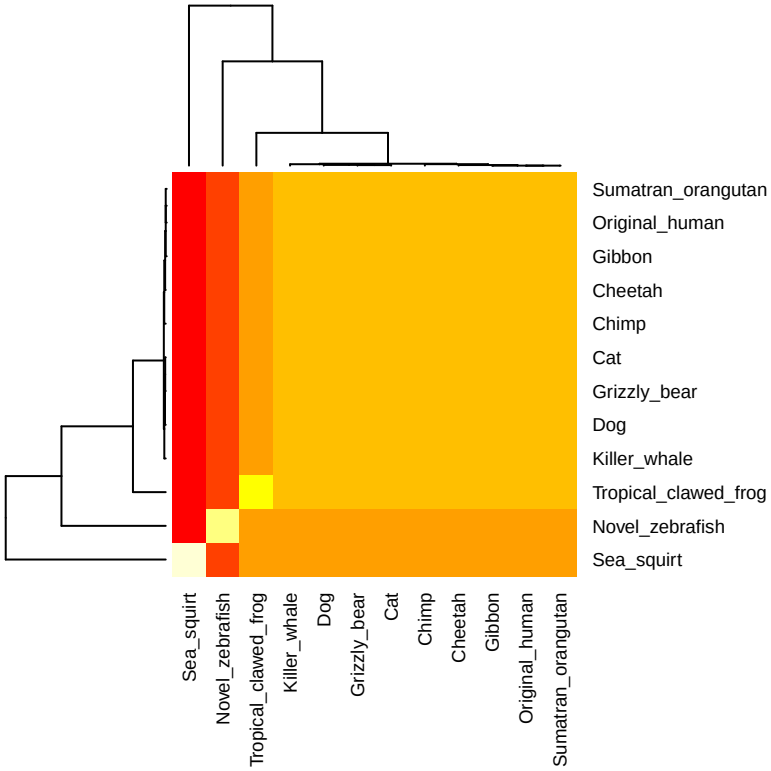
Q6.

Phylogenetic tree using EBI's "Simple Phylogeny" feature.



Q7.

Read MUSCLE alignment file into Seaview and saved file as FASTA.



Q8.

Because my consensus sequence contains a lot of gaps, I will choose the sequence with the highest identity in the alignment. To do so, I will first calculate the sum of each row in the identity matrix. Then, I will find the first sequence with the maximum sum.

```
sums <- rowSums(identity)
which.max(sums)
```

```
## Original_human
##              9
```

Thus, in the main protein structure database, I will use the Original_human sequence to search for the most similar atomic resolution structure to my aligned sequences.

```
human <- read.fasta("human.fasta")
hits <- blast.pdb(human, database = "pdb")
```

```
##  Searching ... please wait (updates every 5 seconds) RID = 0CP700WD01R
##  ...
##  Reporting 67 hits
```

```
# head(hits, 3)
```

We see that the top 3 unique hits are **2D5V_A**, **1S7E_A**, and **1WH6_A**. I will save these to a new dataframe and add my own annotations: structure ID, method used to solve the structure, resolution, and source organism.

```
top <- hits$hit.tbl[1:3,]
anno <- pdb.annotate(top$pdb.id)
```

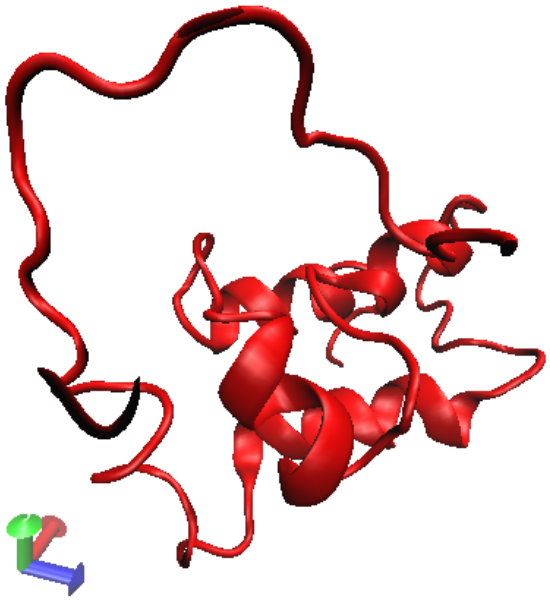
```
## Warning in pdb.annotate(top$pdb.id): ids should be standard 4 character
## PDB-IDs: trying first 4 characters...
```

```
top_anno <- merge(top, anno, by.x = "pdb.id", by.y = "row.names")

# Only take relevant columns
relevant_colnames <- c("pdb.id", "experimentalTechnique", "resolution", "source", "evalue", "identity")
x <- match( relevant_colnames, colnames(top_anno) )
top_anno_relevant <- top_anno[,x]

# Last thing, split PDB identifier on the underscore
ids <- top_anno_relevant$pdb.id
# Split PDB identifier on the underscore
ids_split <- strsplit(ids, "_")
# [[ is synonymous to $. They both select an element from a list
top_anno_relevant$pdb.id <- lapply(ids_split, "[[", 1)
top_anno_relevant
```

```
##   pdb.id experimentalTechnique resolution            source    evalue
## 1   1S7E          SOLUTION NMR       <NA>      Mus musculus 5.70e-106
## 2   1WH6          SOLUTION NMR       <NA>      Homo sapiens  3.02e-13
## 3   2D5V     X-RAY DIFFRACTION        2.0 Rattus norvegicus 1.78e-112
##   identity
## 1  100.000
## 2   46.341
## 3  100.000
```

Q9.

Here, I will generate a molecular figure of the first identified PDB structure, 1S7E, using VMD. After downloading the pdb file from Protein Data Bank, load the molecule into VMD.



The sequence similarity is 100% for 1S7E. Thus, this structure from *Mus musculus* is very likely to be similar to my "novel" *Danio rerio* protein.

Q10.

Searched ChEMBEL with my novel sequence. Found 1 Binding Assay and 0 Functional Assay for CHEMBL2176818 (*Mus musculus*, which was the closest because no *Danio rerio* data was available) but 0 Ligand Efficiency Data.

Binding Assay: https://www.ebi.ac.uk/chembl/assay/inspect/CHEMBL2186578

Pioglitazone and rosiglitazone are two diabetes drugs that share a common functional core: glitazone. Two variants of the glitazone scaffold, pioglitazone and rosiglitazone, are tested to identify off-target binding events in the rat heart. The purpose of these tests is to explain recently reported cardiovascular risk associated with these drugs.

Results suggest that glitazone has affinity for dehydrogenases. Both drugs bind ion channels and modulators, with implications in congestive heart failure, arrhythmia, and peripheral edema. Additional proteins involved in glucose homeostasis, synaptic transduction, and mitochondrial energy production were detected and potentially contribute to drug efficacy and cardiotoxicity.

Hoffmann BR, El-Mansy MF, Sem DS, Greene AS. Chemical proteomics-based analysis of off-target binding profiles for rosiglitazone and pioglitazone: clues for assessing potential for cardiotoxicity. *J. Med. Chem.* (2012)55:8260-8271. doi: 10.1021/jm301204r.