Find a Gene Project (BGGN 213)

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May 15, 2019

Question 1: Name of the protein

Name: Sonic Hedgehog protein isoform 1 preprotein (SHH)

Accession: NP_000184.1

Species: Homo sapiens

Function: Critical role in development of the nervous system, lungs, teeth, and may also have regenerative functions.

Question 2: BLAST search

Method: TBLASTN search against Xenopus ESTs

Database: Expressed sequence tags (est)

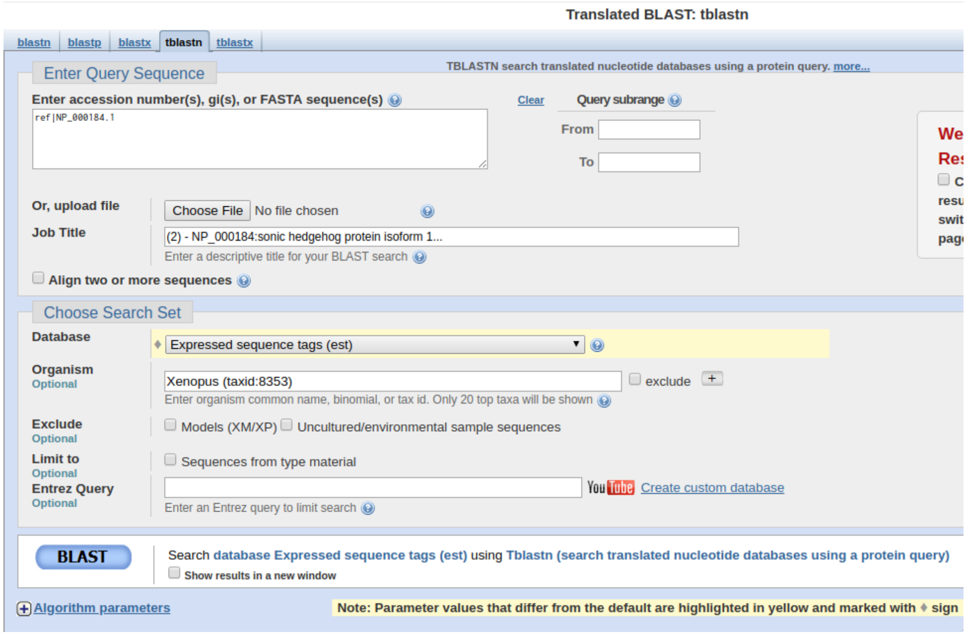
Organism: Xenopus (Taxid: 8353)

Alignment details

Chosen match: Accession BX704643.1, a 874bp clone from Xenopus *tropicalis*.

```
>BX704643.1 BX704643 XGC-tadpole Xenopus tropicalis cDNA
clone TTpA011g13 5', mRNA sequence

cDNA clone TTpA011g13, mRNA sequence.
Length: 874

Score           Expect   Method                         Identities
387 bits(994)   3e-132   Compositional matrix adjust.   197/285(69%)

Positives      Gaps          Frame
220/285(77%)   23/285(8%)    +2


Query  68    RNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG  127
             RNS+RFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG
Sbjct  41    RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG  220

Query  128   WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYYESKAHIHCSVKA  187
             WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVY+ESKAHIHCSVKA
Sbjct  221   WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA  400
```

```
Query  188  ENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLYSDFLTFLDRDDG  247
            ENSVAAKSGGCFPGSA V +E GGTK V++L PGDRVL++D QG L+YSDFL F+D++
Sbjct  401  ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD  580

Query  248  AKKVFYVIETREPRERLLLTAAHLLFVAPHNDSATXXXXXXXXXXXXXXXXXXXRALFAS  307
             KK++YVI+T + R R  +TAAHLLFVA  N + +                   +++FAS
Sbjct  581  VKKLYYVIQTSQTRIR--MTAAHLLFVAQSNGTGS------------------FKSVFAS  700

Query  308  RVRPGQRVYVVAERDGDRRLLPAAVHSVTLSEEAAGAYAPLTAQG  352
             VRPG  +Y    R  D  L  A V  V L EE  G + P    G
Sbjct  701  NVRPGDVIYSADRR--DMTLREAMVEKVDL-EEDIGGFCPRNCPG  826
```



Translated BLAST: tblastn

Enter Query Sequence

TBLASTN search translated nucleotide databases using a protein query. more...

Enter accession number(s), gi(s), or FASTA sequence(s): ref|NP_000184.1

Query subrange — From: — To:

Or, upload file: Choose File No file chosen

Job Title: (2) - NP_000184:sonic hedgehog protein isoform 1...

Enter a descriptive title for your BLAST search

☐ Align two or more sequences

Choose Search Set

Database: Expressed sequence tags (est)

Organism (Optional): Xenopus (taxid:8353) ☐ exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude (Optional): ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to (Optional): ☐ Sequences from type material

Entrez Query (Optional):

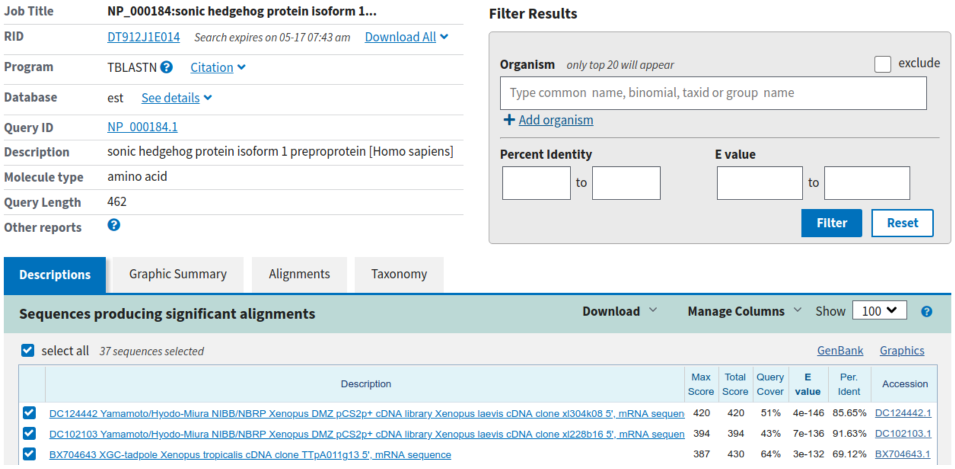
Enter an Entrez query to limit search

Search database Expressed sequence tags (est) using Tblastn (search translated nucleotide databases using a protein query)

☐ Show results in a new window

Note: Parameter values that differ from the default are highlighted in yellow and marked with ♦ sign

Figure 1: BLAST parameters



Job Title	NP_000184:sonic hedgehog protein isoform 1...
RID	DT912J1E014 *Search expires on 05-17 07:43 am* Download All
Program	TBLASTN Citation
Database	est See details
Query ID	NP_000184.1
Description	sonic hedgehog protein isoform 1 preproprotein [Homo sapiens]
Molecule type	amino acid
Query Length	462
Other reports	

Filter Results

Organism *only top 20 will appear* ☐ exclude

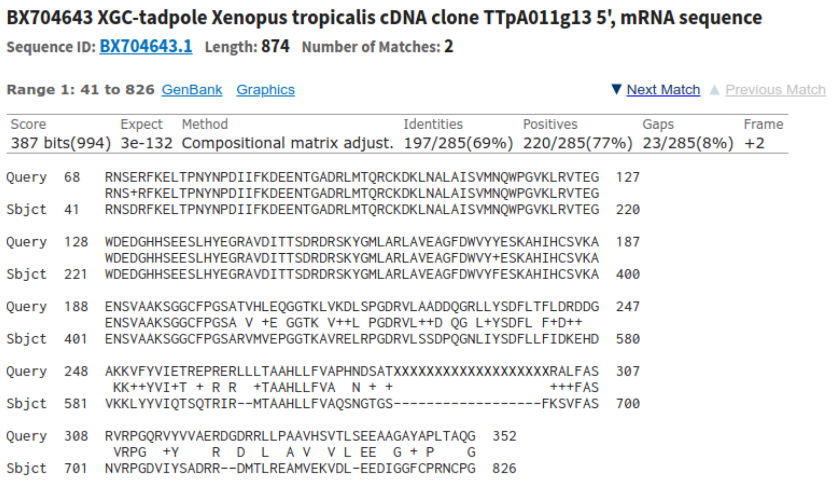
Type common name, binomial, taxid or group name

Sequences producing significant alignments

☑ select all *37 sequences selected*

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	DC124442 Yamamoto/Hyodo-Miura NIBB/NBRP Xenopus DMZ pCS2p+ cDNA library Xenopus laevis cDNA clone xl304k08 5', mRNA sequen	420	420	51%	4e-146	85.65%	DC124442.1
☑	DC102103 Yamamoto/Hyodo-Miura NIBB/NBRP Xenopus DMZ pCS2p+ cDNA library Xenopus laevis cDNA clone xl228b16 5', mRNA sequen	394	394	43%	7e-136	91.63%	DC102103.1
☑	BX704643 XGC-tadpole Xenopus tropicalis cDNA clone TTpA011g13 5', mRNA sequence	387	430	64%	3e-132	69.12%	BX704643.1

Figure 2: BLAST search results



BX704643 XGC-tadpole Xenopus tropicalis cDNA clone TTpA011g13 5', mRNA sequence

Sequence ID: BX704643.1 Length: 874 Number of Matches: 2

Range 1: 41 to 826 GenBank Graphics

Score	Expect	Method	Identities	Positives	Gaps	Frame
387 bits(994)	3e-132	Compositional matrix adjust.	197/285(69%)	220/285(77%)	23/285(8%)	+2

```
Query  68   RNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG  127
            RNS+RFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG
Sbjct  41   RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG  220

Query  128  WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYYESKAHIHCSVKA  187
            WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVY+ESKAHIHCSVKA
Sbjct  221  WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA  400

Query  188  ENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLYSDFLTFLDRDDG  247
            ENSVAAKSGGCFPGSA V +E GGTK V++L PGDRVL++D QG L+YSDFL F+D++
Sbjct  401  ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD  580

Query  248  AKKVFYVIETREPRERLLLTAAHLLFVAPHNDSATXXXXXXXXXXXXXXXXXXXRALFAS  307
             KK++YVI+T + R R  +TAAHLLFVA  N + +                   +++FAS
Sbjct  581  VKKLYYVIQTSQTRIR--MTAAHLLFVAQSNGTGS------------------FKSVFAS  700

Query  308  RVRPGQRVYVVAERDGDRRLLPAAVHSVTLSEEAAGAYAPLTAQG  352
             VRPG  +Y    R  D  L  A V  V L EE  G + P    G
Sbjct  701  NVRPGDVIYSADRR--DMTLREAMVEKVDL-EEDIGGFCPRNCPG  826
```

Figure 3: Alignment details

Question 3: Novel Protein Information

Chosen Sequence:

```
>Xenopus tropicalis protein (from BLAST result)
RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG
WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA
ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD
VKKLYYVIQTSQTRIRMTAAHLLFVAQSNGTGSFKSVFASNVRPGDVIYSADRRDMTLRE
AMVEKVDLEEDIGGFCPRNCPG
```

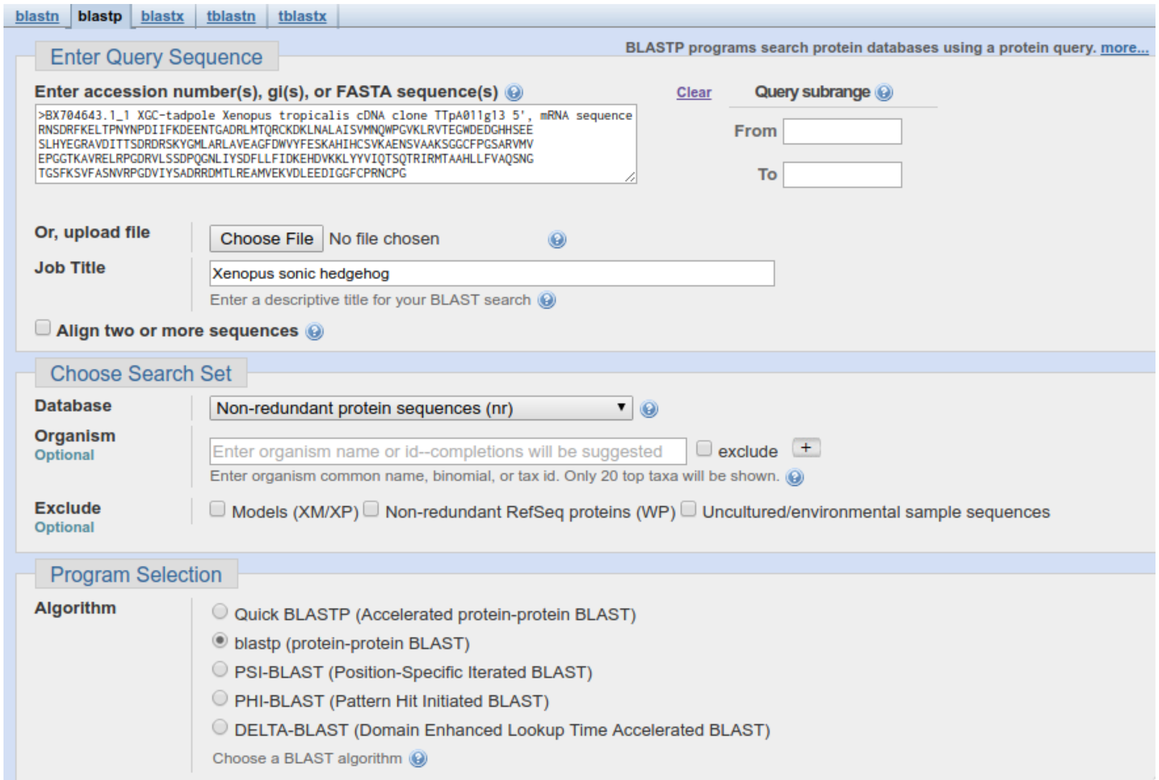
Name: *Xenopus* sonic hedgehog

Species: Xenopus (Silurana) tropicalis (Common name: western clawed frog, Taxid: 8364)

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Amphibia; Batrachia; Anura; Pipoidea; Pipidae; Xenopodinae; Xenopus; Silurana

Question 4: Proof that gene/protein are novel

A BLASTP search against the NR database yielded a top hit to a protein from *Xenopus tropicalis* with 100% coverage and 97.71% identity. The alignment details are given in the screenshots below.



Enter accession number(s), gi(s), or FASTA sequence(s)

```
>BX704643.1_1 XGC-tadpole Xenopus tropicalis cDNA clone TTpA011g13 5', mRNA sequence
RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHSEE
SLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKAENSVAAKSGGCFPGSARVMV
EPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHDVKKLYYVIQTSQTRIRMTAAHLLFVAQSNG
TGSFKSVFASNVRPGDVIYSADRRDMTLREAMVEKVDLEEDIGGFCPRNCPG
```

Or, upload file

Job Title: Xenopus sonic hedgehog

Enter a descriptive title for your BLAST search

☐ **Align two or more sequences**

Database: Non-redundant protein sequences (nr)

Organism Optional: Enter organism name or id--completions will be suggested ☐ exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude Optional: ☐ Models (XM/XP) ☐ Non-redundant RefSeq proteins (WP) ☐ Uncultured/environmental sample sequences

Algorithm

○ Quick BLASTP (Accelerated protein-protein BLAST)

● blastp (protein-protein BLAST)

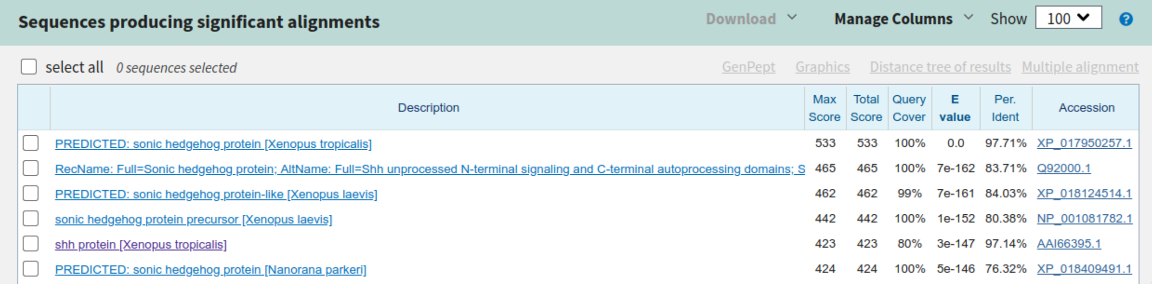
○ PSI-BLAST (Position-Specific Iterated BLAST)

○ PHI-BLAST (Pattern Hit Initiated BLAST)

○ DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

Figure 4: BLASTP parameters

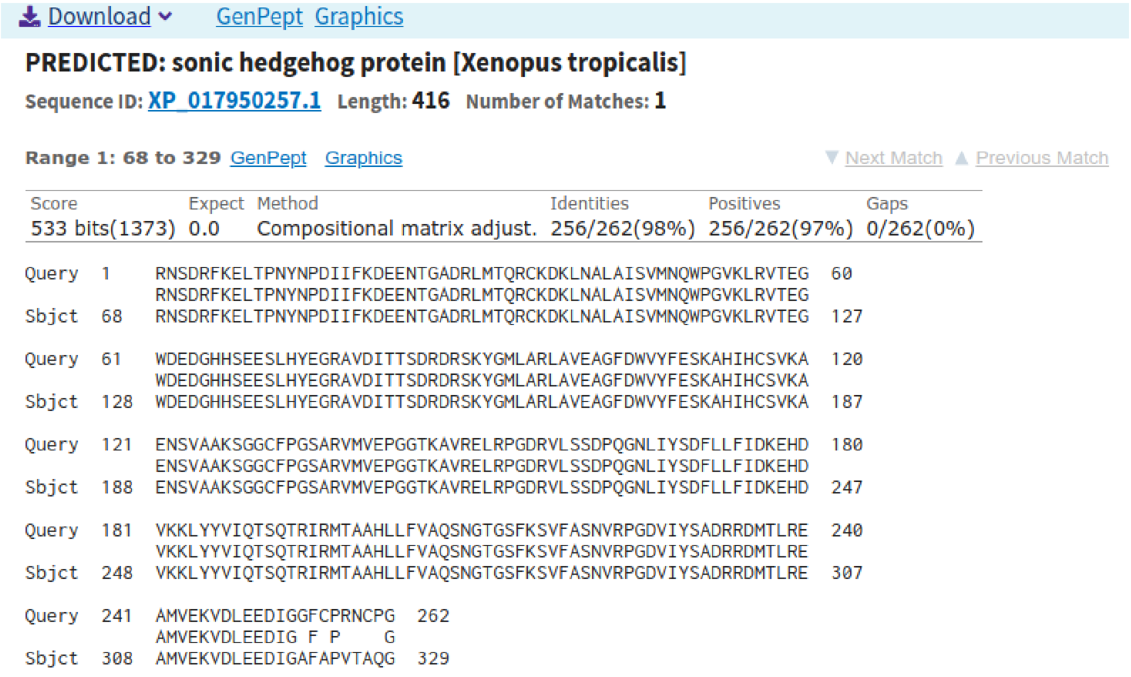


Sequences producing significant alignments

☐ select all *0 sequences selected*

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☐	PREDICTED: sonic hedgehog protein [Xenopus tropicalis]	533	533	100%	0.0	97.71%	XP_017950257.1
☐	RecName: Full=Sonic hedgehog protein; AltName: Full=Shh unprocessed N-terminal signaling and C-terminal autoprocessing domains; S	465	465	100%	7e-162	83.71%	Q92000.1
☐	PREDICTED: sonic hedgehog protein-like [Xenopus laevis]	462	462	99%	7e-161	84.03%	XP_018124514.1
☐	sonic hedgehog protein precursor [Xenopus laevis]	442	442	100%	1e-152	80.38%	NP_001081782.1
☐	shh protein [Xenopus tropicalis]	423	423	80%	3e-147	97.14%	AAI66395.1
☐	PREDICTED: sonic hedgehog protein [Nanorana parkeri]	424	424	100%	5e-146	76.32%	XP_018409491.1

Figure 5: BLASTP results page



PREDICTED: sonic hedgehog protein [Xenopus tropicalis]

Sequence ID: XP_017950257.1 Length: 416 Number of Matches: 1

Range 1: 68 to 329 GenPept Graphics

Score	Expect	Method	Identities	Positives	Gaps
533 bits(1373)	0.0	Compositional matrix adjust.	256/262(98%)	256/262(97%)	0/262(0%)

```
Query  1    RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG  60
            RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG
Sbjct  68   RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG  127

Query  61   WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA  120
            WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA
Sbjct  128  WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA  187

Query  121  ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD  180
            ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD
Sbjct  188  ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD  247

Query  181  VKKLYYVIQTSQTRIRMTAAHLLFVAQSNGTGSFKSVFASNVRPGDVIYSADRRDMTLRE  240
            VKKLYYVIQTSQTRIRMTAAHLLFVAQSNGTGSFKSVFASNVRPGDVIYSADRRDMTLRE
Sbjct  248  VKKLYYVIQTSQTRIRMTAAHLLFVAQSNGTGSFKSVFASNVRPGDVIYSADRRDMTLRE  307

Query  241  AMVEKVDLEEDIGGFCPRNCPG  262
            AMVEKVDLEEDIG F P    G
Sbjct  308  AMVEKVDLEEDIGAFAPVTAQG  329
```

Figure 6: Alignment for top result

Question 5: Multiple sequence alignment

Relabeled sequences for alignment

```
>Xenopus_sonic_hedgehog (from BLAST result)
RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG
WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA
ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD
VKKLYYVIQTSQTRIRMTAAHLLFVAQSNGTGSFKSVFASNVRPGDVIYSADRRDMTLRE
AMVEKVDLEEDIGGFCPRNCPG

>Human |Sonic hedgehog protein (NP_000184.1) [Homo sapiens]
MLLLARCLLLVLVSSLLVCSGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKTLGASGRYEGKISRNS
ERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHSEESLH
YEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYYESKAHIHCSVKAENSVAAKSGGCFPGSATVHLEQG
GTKLVKDLSPGDRVLAADDQGRLLYSDFLTFLDRDDGAKKVFYVIETREPRERLLLTAAHLLFVAPHNDS
ATGEPEASSGSGPPSGGALGPRALFASRVRPGQRVYVVAERDGDRRLLPAAVHSVTLSEEAAGAYAPLTA
QGTILINRVLASCYAVIEEHSWAHRAFAPFRLAHALLAALAPARTDRGGDSGGGDRGGGGGRVALTAPGA
ADAPGAGATAGIHWYSQLLYQIGTWLLDSEALHPLGMAVKSS

>Mexican_walking_fish |sonic hedgehog [Ambystoma mexicanum] ARX70249.1
MGEMRLLLRRLLLGTLVWALLVPSGLTCGPGRGIGKRRQPKKLTPLAYKQFIPNVAEKTLGASGRYEGKI
TRNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHSE
ESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKAENSVAAKSGGCFPASAKVT
LEHGVTRPVKDLRPGDRVLAADGQGRLVYS DFLMFLDKEEAVTKVFYVIETERPRQRLRLTAAHLLFAA
RHPANSSSSTGFQSIFASRVRPGHRVLTVDQEGRGLQEATVTRVYLEEGAGAYAPVTSHGTVVIDKVLAS
CYAVIEEHSWAHWAFAPLRLGYGILSIFSPQDYSPHSPPAPSQKEGVHWYSEILYHIGTWVLHSDTIHPW
GMAAKSS
```

```
>Iberian_newt |sonic hedgehog-related protein [Pleurodeles waltl]
MGEMILLRRDLLGGFISTLLVPLGLGCGPGRGIGQRRRPQKLTPLAYKQFIPNVSEKTLGASGRYEVKITRNSERFQELT
PNYNPDIIFKVEENTGADRLMTQRCKDKLNSLAISVMNQWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSK
YGMLARLAVEAGFDWVYFESKAHIHCSVKAENSVAVKSGGCFPGSATVTLEQGVRIPVKGLKPGNRVLAVDVEGRLIYSD
FLLFMDEEETARKVFYVIETSLPRERLRLTAAHLLFVAQEHPGNASAGNFRSKFGHRRFRSMFASSVRPGHRVLTEDREG
RGLREATVDRVYLEEATGAYAPVTAHGTVVIDRVLASCYAVIEEHSWAHWAFAPLRVGFGILSFFSPQDYSSHSPPAPSQ
AEGVHWYSEILYRIGTWVLQADTIHPLGMAAKSS

>African_clawed_frog |sonic hedgehog protein-like|XP_018124514.1 [Xenopus laevis]
MSVATGILLLGFTCSLLIPPGLSCGPGRGIGKRRHPKKLTPLVYKQFIPNVAEKTLGASGRYEGKIASNSDRFKELTPNY
NPDIVFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHLEESLHYEGRAVDITTSDRDRSKYGM
LARLAVEAGFDWVYYESKAHIHCSVKAENSVAAKSGGCFPAGAEVMVELGGTKAVKDLRPGDRVLSSDAQGNLLYSDFLL
FIDQQHDVKKLFYVIETSHPRRRIRLTAAHLLFVAQVNGSRSFKSVFASSIQPGDIIYSADPRAMALNEVKVEKVGLEED
TGAYAPLTAHGTLVIDQVLSSCYALIEEHTWAHLAFAPLRFGLSLSPYLFPKDSSPSPQSHHQLDSSPSPQSHHQPEGIH
WYSQLLYQLGTWLLDSNSLHPLGMATKSS

>Burmese_python |sonic hedgehog protein|XP_007433256.1 [Python bivittatus]
MLLRRRSGLLPLCLGALFLSAGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKTLGASGRYEGKISRNSERFKELTPN
YNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYG
MLARLAVEAGFDWVYYESKAHIHCSVKAENSVAAKSGGCFPGSAWVNLEEGGTKLVKDLRPGDRVLAADVHGQLFYSEFL
AFLDREDPPVHKLFYVIETQRPQTRLLLTAAHLLFVAPPQNHSHSQPQPIFASRVQPGQQVYVLGQGGQTLLQAAVHSVS
LQEEASGAYAPLTAQGTILINQVLASCYAVIEEHSWAHWAFAPFRMAHAVLAMLDPEGLSSPLLFPAAAPEESSSLAGVH
WYSRLLYHIGRWILDSETMHPLGMAS

>Tiger_snake |sonic hedgehog protein |XP_026536709.1 [Notechis scutatus]
MLLLRRTGLLPLCLGALFLSAGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKTLGASGRYEGKITRNSERFKELTPN
YNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYG
MLARLAVEAGFDWVYYESKAHIHCSVKAENSVAAKSGGCFPGSAWVNLEGGGTKLVKDLHPGDRVLAADVHGQLFYSEFL
AFLDREEPPVHKLFYVIETQTPQARLLLTAAHLLFVAPPHNHSHSQPQPIFASRVQPGQHVYVLAQGGQTLLPAAVHSVS
LKEEASGAYAPLTAQGTILINQVLASCYAVIEEHSWAHWAFAPFRIAHAALAVLNLEGLSSPLLFPAAVPEEGASLAGVH
WYSRLLYYIGRWILGSEMMHPLGMAS
```

Alignment

Using local MUSCLE (v3.8) program with bio3d `seqaln()`. The fasta output was converted to CLUSTALW format using `seaview`.

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

Iberian_newt            -MGEMILLRRDLLGGF-ISTLLVPLGLGCGPGRGIGQRRRPQKLTPLAYKQFIPNVSEKT
Mexican_walking_fish    MGEMRLLLRRLLLGTL-VWALLVPSGLTCGPGRGIGKRRQPKKLTPLAYKQFIPNVAEKT
African_clawed_frog     ----MSVATGILLLGF-TCSLLIPPGLSCGPGRGIGKRRHPKKLTPLVYKQFIPNVAEKT
Xenopus_sonic_hedgehog  ------------------------------------------------------------
Burmese_python          ----MLLRRRSGLLPLCLGALFLSAGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT
Tiger_snake             ----MLLLRRTGLLPLCLGALFLSAGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT
Human                   ----MLLLARCLLLVL-VSSLLVCSGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT

Iberian_newt            LGASGRYEVKITRNSERFQELTPNYNPDIIFKVEENTGADRLMTQRCKDKLNSLAISVMN
Mexican_walking_fish    LGASGRYEGKITRNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN
African_clawed_frog     LGASGRYEGKIASNSDRFKELTPNYNPDIVFKDEENTGADRLMTQRCKDKLNALAISVMN
Xenopus_sonic_hedgehog  ------------RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN
Burmese_python          LGASGRYEGKISRNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN
Tiger_snake             LGASGRYEGKITRNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN
Human                   LGASGRYEGKISRNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN
```

```
Iberian_newt           QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYF
Mexican_walking_fish   QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYF
African_clawed_frog    QWPGVKLRVTEGWDEDGHHLEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY
Xenopus_sonic_hedgehog QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYF
Burmese_python         QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY
Tiger_snake            QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY
Human                  QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY


Iberian_newt           ESKAHIHCSVKAENSVAVKSGGCFPGSATVTLEQGVRIPVKGLKPGNRVLAVDVEGRLIY
Mexican_walking_fish   ESKAHIHCSVKAENSVAAKSGGCFPASAKVTLEHGVTRPVKDLRPGDRVLAADGQGRLVY
African_clawed_frog    ESKAHIHCSVKAENSVAAKSGGCFPAGAEVMVELGGTKAVKDLRPGDRVLSSDAQGNLLY
Xenopus_sonic_hedgehog ESKAHIHCSVKAENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIY
Burmese_python         ESKAHIHCSVKAENSVAAKSGGCFPGSAWVNLEEGGTKLVKDLRPGDRVLAADVHGQLFY
Tiger_snake            ESKAHIHCSVKAENSVAAKSGGCFPGSAWVNLEGGGTKLVKDLHPGDRVLAADVHGQLFY
Human                  ESKAHIHCSVKAENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLY


Iberian_newt           SDFLLFMDEEE-TARKVFYVIETSLPRERLRLTAAHLLFVAQEHPGNASAGNFRSKFGHR
Mexican_walking_fish   SDFLMFLDKEE-AVTKVFYVIETERPRQRLRLTAAHLLFAAR-HPANSSSST--------
African_clawed_frog    SDFLLFIDQQH-DVKKLFYVIETSHPRRRIRLTAAHLLFVAQ----VNGSRS--------
Xenopus_sonic_hedgehog SDFLLFIDKEH-DVKKLYYVIQTS--QTRIRMTAAHLLFVAQ----SNGTGS--------
Burmese_python         SEFLAFLDREDPPVHKLFYVIETQRPQTRLLLTAAHLLFVAP--PQNHSHSQ--------
Tiger_snake            SEFLAFLDREEPPVHKLFYVIETQTPQARLLLTAAHLLFVAP--PHNHSHSQ--------
Human                  SDFLTFLDRDD-GAKKVFYVIETREPRERLLLTAAHLLFVAP--HNDSATGEPEASSGSG


Iberian_newt           -------RFRSMFASSVRPGHRVLTEDR--EGRGLREATVDRVYL-EEATGAYAPVTAHG
Mexican_walking_fish   -------GFQSIFASRVRPGHRVLTVDQ--EGRGLQEATVTRVYL-EEGAGAYAPVTSHG
African_clawed_frog    --------FKSVFASSIQPGDIIYSADP--RAMALNEVKVEKVGL-EEDTGAYAPLTAHG
Xenopus_sonic_hedgehog --------FKSVFASNVRPGDVIYSADR--RDMTLREAMVEKVDL-EEDIGGFCPRNCPG
Burmese_python         --------PQPIFASRVQPGQQVYVLGQ--GGQTLLQAAVHSVSLQEEASGAYAPLTAQG
Tiger_snake            --------PQPIFASRVQPGQHVYVLAQ--GGQTLLPAAVHSVSLKEEASGAYAPLTAQG
Human                  PPSGGALGPRALFASRVRPGQRVYVVAERDGDRRLLPAAVHSVTLSEEAAGAYAPLTAQG


Iberian_newt           TVVIDRVLASCYAVIEEHSWAHWAFAPLRVGFGILSFFSPQ-------------------
Mexican_walking_fish   TVVIDKVLASCYAVIEEHSWAHWAFAPLRLGYGILSIFSPQ-------------------
African_clawed_frog    TLVIDQVLSSCYALIEEHTWAHLAFAPLRFGLSLSPYLFPK-----------DSSPSPQS
Xenopus_sonic_hedgehog ------------------------------------------------------------
Burmese_python         TILINQVLASCYAVIEEHSWAHWAFAPFRMAHAVLAMLDPE----------------GLS
Tiger_snake            TILINQVLASCYAVIEEHSWAHWAFAPFRIAHAALAVLNLE----------------GLS
Human                  TILINRVLASCYAVIEEHSWAHRAFAPFRLAHALLAALAPARTDRGGDSGGGDRGGGGR


Iberian_newt           ---DYSSHSPPAPS-QAEGVHWYSEILYRIGTWVLQADTIHPLGMAAKSS
Mexican_walking_fish   ---DYSPHSPPAPS-QKEGVHWYSEILYHIGTWVLHSDTIHPWGMAAKSS
African_clawed_frog    HHQLDSSPSPQSHH-QPEGIHWYSQLLYQLGTWLLDSNSLHPLGMATKSS
Xenopus_sonic_hedgehog ------------------------------------------------
Burmese_python         SPLLFPAAAPEESS-SLAGVHWYSRLLYHIGRWILDSETMHPLGMAS---
Tiger_snake            SPLLFPAAVPEEGA-SLAGVHWYSRLLYYIGRWILGSEMMHPLGMAS---
Human                  VALTAPGAADAPGAGATAGIHWYSQLLYQIGTWLLDSEALHPLGMAVKSS
```

Question 6: Phylogenetic tree

I used the simple phylogeny tool from EBI with the following parameters:

- Tree format: `nj`
- Distance correction: `False`
- Excludes Gaps: `False`
- Clustering Methods: `Neighbor-joining`
- Percent identity matrix: `False`

The resulting tree is this.

Figure 7: Phylogenetic tree derived from the sequence alignments in the previous section

Question 7: Heatmap of aligned sequences

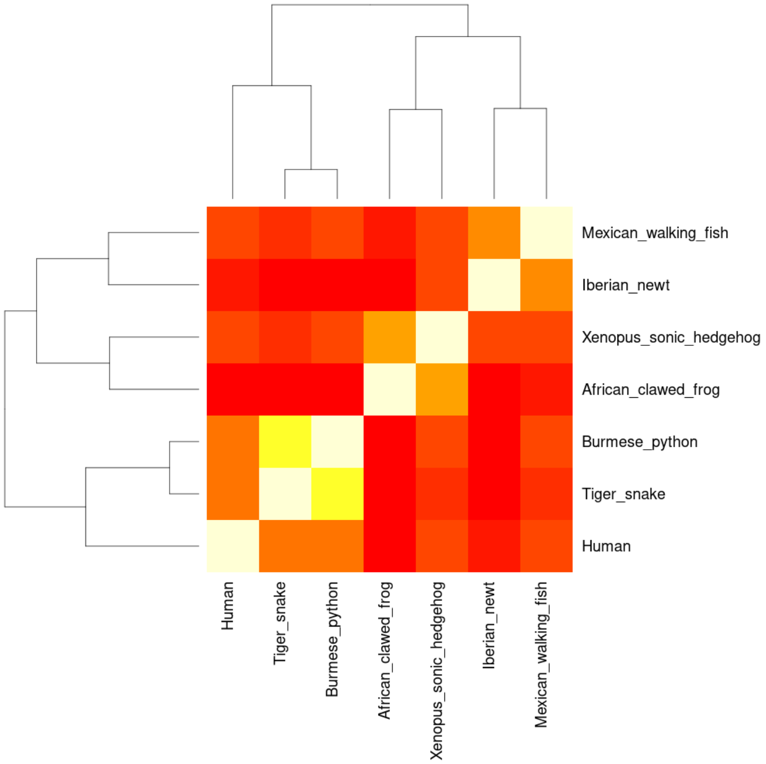


Figure 8: Heatmap derived from the sequence alignments.

Question 8: Protein Database Search

Consensus sequence:

```
>Consensus Sequence 50% threshold
----MLLLRR-LLL-L----LL---GL-CGPGRG-GKRRHPKKLTPLAYKQFIPNVAEKT
LGASGRYEGKI-RNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN
QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY
ESKAHIHCSVKAENSVAAKSGGCFPGSA-V-LE-GGTK-VKDLRPGDRVLAAD-QG-L-Y
SDFL-FLD-E---V-KLFYVIET--PR-RLRLTAAHLLFVA---P-N-S-----------
--------F-S-FASRVRPG--VY--D----G--L-EA-V--V-L-EEA-GAYAPLTA-G
T--I--VLASCYAVIEEHSWAHWAFAP-R-----L--L-P--------------------
---------P--------GVHWYS-LLY-IGTW-L-S---HPLGMA-KSS
```

Table 1: Similar atomic resolution structures from PDB to the consensus sequence generated from alignments requiring at least 50% sequence identity threshold.

ID	Name	Technique	Resolution	Source	Evalue	Identity
6DMY	Sonic Hedgehog protein	Electron microscopy	3.6	Homo sapiens	3.98e-124	98.286
1VHH	SONIC HEDGEHOG	X-ray diffraction	1.7	Mus musculus	1.79e-116	99.383
3K7G	Indian Hedgehog protein	X-ray diffraction	1.5	Homo sapiens	7.96e-113	88.136

Question 9: Molecular figure

Figure 9: VMD rendering of `3K7G` Indian Hedgehog protein from Homo *sapiens*

The Indian Hedgehog protein is very likely to have similar structural motifs as and functions to Xenopus sonic hedgehog, since this protein has 88.14% identity to the consensus sequence. Sequence similarity above 80% is considered very similar.

Question 10: CHEMBL Assays

Performing a CHEMBL search for the Xenopus SHH sequence from Question 3 gave me hits to two SHH genes from different species, Mus *musculus* (CHEMBL5387) and Homo *sapiens* (CHEMBL5602). For the top hit (CHEMBL5387), there are 26 binding assays and 23 Functional assays. There are also 125 ligand efficiency assays.

The study referenced below involved a small molecule that binds Shh in human cells, robotnikinin. Robotnikinin significantly inhibits Shh activity and presents as a "valuable. . . probe of dieases associated with aberrant Shh-pathway activity."

Stanton, B.Z., Peng, L.F., Maloof, N., Nakai, K., Wang, X., Duffner, J.L., Taveras, K.M., Hyman, J.M., Lee, S.W., Koehler, A.N. and Chen, J.K., 2009. A small molecule that binds Hedgehog and blocks its signaling in human cells. *Nature chemical biology*, 5(3), p.154.