Find a Gene Project (BGGN 213)

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Question 1: Name of the protein

Name: Sonic Hedgehog protein isoform 1 preprotein (SHH)

Accession: NP_000184.1 Species: Homo sapiens

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 RNS+RFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG	127
Sbjct	41	RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG	220
Query	128	WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYYESKAHIHCSVKA WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVY+ESKAHIHCSVKA	187
Sbjct	221	WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYFESKAHIHCSVKA	400
Query	188	ENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLYSDFLTFLDRDDG ENSVAAKSGGCFPGSA V +E GGTK V++L PGDRVL++D QG L+YSDFL F+D++	247
Sbjct	401	${\tt ENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIYSDFLLFIDKEHD}$	580

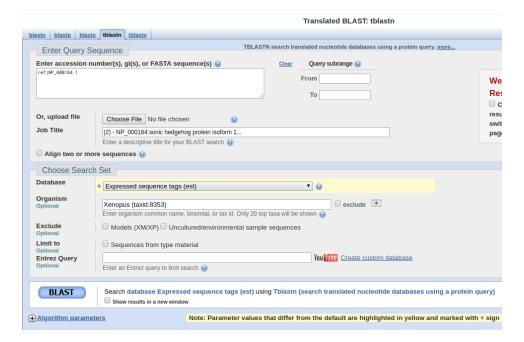


Figure 1: BLAST parameters

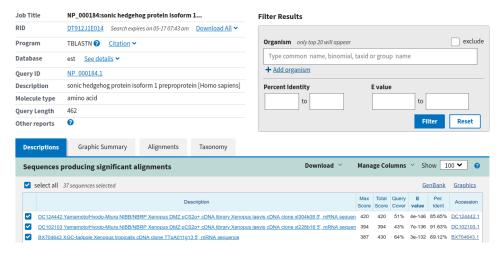


Figure 2: BLAST search results

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

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

Range	1: 41	L to 826 GenBank Graphics	▼ <u>N</u>	ext Match A P	revious Matcl
Score 387 h	its/99	Expect Method Identities Positives 4) 3e-132 Compositional matrix adjust. 197/285(69%) 220/285(7)		Gaps 23/285(8%)	Frame +2
307 5	100(33	4) 30 132 COMPOSICIONAL MACHINI CAGASTI 137/203(0370) 220/203(7)	, ,,,	23/203(070)	12
Query	68	RNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG RNS+RFKELTPNYNPDIIFKDEENTGADRLMTORCKDKLNALAISVMNOWPGVKLRVTEG	127		
Sbjct	41	RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEG	220		
Query	128	WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYYESKAHIHCSVKA WDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVY+ESKAHIHCSVKA	187		
Sbjct	221		400		
Query	188	ENSVAAKSGGCFPGSATVHLEQGGTKLVKDLSPGDRVLAADDQGRLLYSDFLTFLDRDDG ENSVAAKSGGCFPGSA V +E GGTK V++L PGDRVL++D OG L+YSDFL F+D++	247		
Sbjct	401		580		
Query	248	AKKVFYVIETREPRERLLTAAHLLFVAPHNDSATXXXXXXXXXXXXXXXXXXXXXXXXALFAS KK++YVI+T + R R +TAAHLLFVA N + + + +++FAS	307		
Sbjct	581	VKKLYYVIQTSQTRIRMTAAHLLFVAQSNGTGSFKSVFAS	700		
Query	308	RVRPGQRVYVVAERDGDRRLLPAAVHSVTLSEEAAGAYAPLTAQG 352 VRPG +Y R D L A V V L EE G + P G			
Sbjct	701	NVRPGDVIYSADRRDMTLREAMVEKVDL-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.

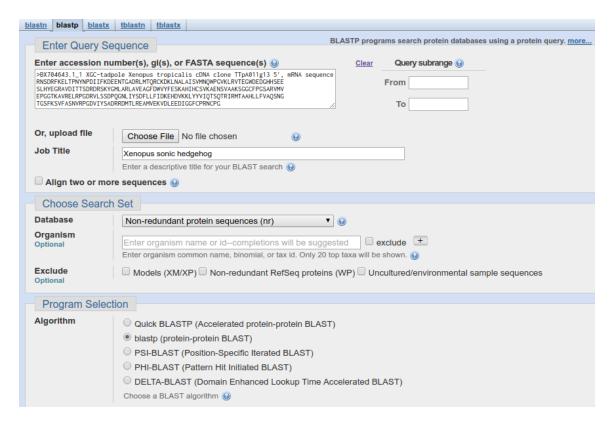


Figure 4: BLASTP parameters

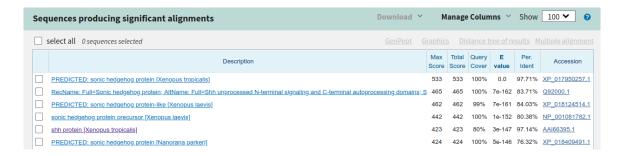


Figure 5: BLASTP results page

▲ Download **∨** GenPept Graphics

PREDICTED: sonic hedgehog protein [Xenopus tropicalis]

Sequence ID: XP 017950257.1 Length: 416 Number of Matches: 1

Range	1: 68	3 to 329 <u>G</u>	enPept Graphics			,	▼ Next Match
Score		Expect	Method		Identities	Positives	Gaps
533 b	its(13	73) 0.0	Compositional r	matrix adjust.	256/262(98%)	256/262(97	%) 0/262(0%
Query	1		LTPNYNPDIIFKDEE LTPNYNPDIIFKDEE				60
Sbjct	68		LTPNYNPDIIFKDEE				127
Query	61		EESLHYEGRAVDITT EESLHYEGRAVDITT				120
Sbjct	128		EESLHYEGRAVDITT				187
uery	121		GGCFPGSARVMVEPG GGCFPGSARVMVEPG				180
Sbjct	188		GGCFPGSARVMVEPG		•		247
uery	181		QTSQTRIRMTAAHLL OTSOTRIRMTAAHLL				240
Sbjct	248		QTSQTRIRMTAAHLL				307
Query	241	AMVEKVDLE AMVEKVDLE	EEDIGGFCPRNCPG	262			
Sbjct	308	AMVEKVDLE	EEDIGAFAPVTAQG	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 walt1]
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
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake
Human

-MGEMILLRRDLLGGF-ISTLLVPLGLGCGPGRGIGQRRRPQKLTPLAYKQFIPNVSEKT
MGEMRLLRRLLLGTL-VWALLVPSGLTCGPGRGIGKRRQPKKLTPLAYKQFIPNVAEKT
----MSVATGILLLGF-TCSLLIPPGLSCGPGRGIGKRRHPKKLTPLVYKQFIPNVAEKT
-----MLLRRSGLLPLCLGALFLSAGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT
-----MLLRRTGLLPLCLGALFLSAGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT
------MLLLRCLLVL-VSSLLVCSGLACGPGRGFGKRRHPKKLTPLAYKQFIPNVAEKT

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake

LGASGRYEVKITRNSERFQELTPNYNPDIIFKVEENTGADRLMTQRCKDKLNSLAISVMN LGASGRYEGKITRNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN LGASGRYEGKIASNSDRFKELTPNYNPDIVFKDEENTGADRLMTQRCKDKLNALAISVMN -------RNSDRFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN LGASGRYEGKISRNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN LGASGRYEGKITRNSERFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMN Human

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake
Human

QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYF QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYF QWPGVKLRVTEGWDEDGHHLEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY QWPGVKLRVTEGWDEDGHHSEESLHYEGRAVDITTSDRDRSKYGMLARLAVEAGFDWVYY

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake
Human

ESKAHIHCSVKAENSVAVKSGGCFPGSATVTLEQGVRIPVKGLKPGNRVLAVDVEGRLIY ESKAHIHCSVKAENSVAAKSGGCFPASAKVTLEHGVTRPVKDLRPGDRVLAADGQGRLVY ESKAHIHCSVKAENSVAAKSGGCFPAGAEVMVELGGTKAVKDLRPGDRVLSSDAQGNLLY ESKAHIHCSVKAENSVAAKSGGCFPGSARVMVEPGGTKAVRELRPGDRVLSSDPQGNLIY ESKAHIHCSVKAENSVAAKSGGCFPGSAWVNLEEGGTKLVKDLRPGDRVLAADVHGQLFY ESKAHIHCSVKAENSVAAKSGGCFPGSAWVNLEGGGTKLVKDLHPGDRVLAADVHGQLFY

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake
Human

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake
Human

-----RFRSMFASSVRPGHRVLTEDR--EGRGLREATVDRVYL-EEATGAYAPVTAHG
------GFQSIFASRVRPGHRVLTVDQ--EGRGLQEATVTRVYL-EEGAGAYAPVTSHG
------FKSVFASSIQPGDIIYSADP--RAMALNEVKVEKVGL-EEDTGAYAPLTAHG
-----FKSVFASNVRPGDVIYSADR--RDMTLREAMVEKVDL-EEDIGGFCPRNCPG
------PQPIFASRVQPGQQVYVLGQ--GGQTLLQAAVHSVSLQEEASGAYAPLTAQG
------PQPIFASRVQPGQHVYVLAQ--GGQTLLPAAVHSVSLKEEASGAYAPLTAQG
PPSGGALGPRALFASRVRPGQRVYVVAERDGDRRLLPAAVHSVTLSEEAAGAYAPLTAQG

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake
Human

Iberian_newt
Mexican_walking_fish
African_clawed_frog
Xenopus_sonic_hedgehog
Burmese_python
Tiger_snake

---DYSSHSPPAPS-QAEGVHWYSEILYRIGTWVLQADTIHPLGMAAKSS
---DYSPHSPPAPS-QKEGVHWYSEILYHIGTWVLHSDTIHPWGMAAKSS
HHQLDSSPSPQSHH-QPEGIHWYSQLLYQLGTWLLDSNSLHPLGMATKSS
------SPLLFPAAAPEESS-SLAGVHWYSRLLYHIGRWILDSETMHPLGMAS--SPLLFPAAVPEEGA-SLAGVHWYSRLLYYIGRWILGSEMMHPLGMAS---

Question 6: Phylogenetic tree

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

• Tree format: nj

Distance correction: FalseExcludes 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

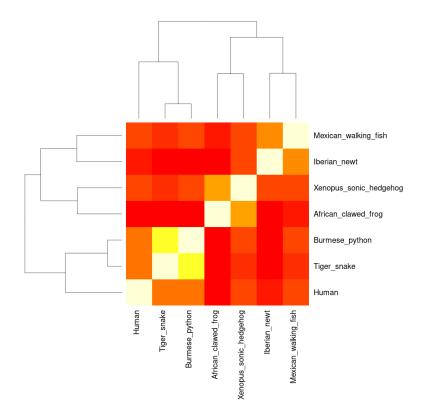


Figure 8: Heatmap derived from the sequence alignments.

Question 8: Protein Database Search