Online resource 1a. Alignment of cytoplasmic Ig-like domains of *Xenopus* B7H6

Cytoplasmic Ig-like domain-1 (Ig)

```
AAI66200     LKKEPPKLSPITGAEHLVHMNEATLSCQISGFRPKPIRITLCLQRKGGEVMEIYSWDSEAQTDH----SIKRKELSVPIEL
AAH84632     Y..VA................R.....L.............W.K.N...E...D.....T..GSSAPV.HH.E.QRVV.VP

AAI66200     EREGLVGNGE-TNLPPTQRALRVEMVPLIKTSKKHISNCQCTIRITPDIEEDDGAELTVRVTHSALRGPISVSCRLKVNGV
AAH84632     .....MS..NG.....A..P.Q.....V.TSKSQRL.......H...N...................L..........G..
```

Cytoplasmic domain-2 (C1)

```
AAI66200     PPTISDIVPPLRILNEEPVTLTCPINGFKPKPLSVTWLKVDSGGRETELLSWDRGSTNIRDP
AAH84632     K.KLAK.IS..H..HG..L......Y...............QR..........Q.T...N..

AAI66200     KYSHQMLEN--EHGEHS-----NAFISVLAMKPTVKEDHGAKYICRTYHYATGSQADKTMEMLVS
AAH84632     ....-LA.DER.K...EEEDDI.Y.V.N.TL......Y...Q.V...H..T.EHT.E.R.K.Q.L
```

Cytoplasmic domain-3 (C1)

```
AAI66200     AVPELGPIQKSPEILYVGEEMTLSCRIHSFHPEPLKVRWYKGDELLESSTD
BX732750                                       .G.C........A....
AAH84632     S....D..ENA..V....D..K..........RT.D.S...EN...S.YN.

AAI66200     QAHRGASHLLDCTSRATYRPSVRDIGKSFRCEVSHESLQHPKCVSWELKHL
BX732750     ......N............................................
AAH84632     PILHNN.G.FHF...T..T.C......T.......PT..N.........Y.
```

Cytoplasmic domain-4 (Ig)

```
AAI66200     VSEPRVSAIKCHPEAPECNQPATLSCAIENYYPKDLKIRWYRGLEVISSHN
BX732750     I.............V...................V.T.Q.....K...ID.
AAH84632     I..........D..V.G...TT....D..KF..........W.....K.EG

AAI66200     DTETKEDPPGSGLFSKMTEIHLTPTHSDHGTEFHMEIIHNMKITKKTFCLQFKG
BX732750     .PKAA.EDQE.....RTS..QII..IN......YLK.T.G..NFE.N.PMKLR.
AAH84632     .P.GAKED.E.....RT...QII..IN...KQ.L......F..Y...-SMKC..
```

Cytoplasmic domain-5 (C1)

```
AAI66200     FPEVKDITCNKYPPKYGEALTLSCEVIGCSARDITAEWREGNNPIRGQMRAEPQI
BX732750     L.D..E.................................V.....KRE.E.KT..
AAH84632     -------------------------------------------------------

AAI66200     DRDSVTFLLTLTPTAEHYGKLLTCLIKHKDLPQPIKKNISLKLP
BX732750     ....I............H...F.............R.I.C...L
AAH84632     ------------.....S...F.......E.....E........
```

Online resource 1b. Deduced amino acid sequence of B7HXen.

Leader

```
AAH85212         MVSRLFLLLLLLLRIAI
AAI61104         MGDPGGAAVLLRFLRIQGFLCLFLLPQT
```

Extracellular V domain

```
AAH85212         FSALEMTAPPTHRAEMGSNIIIPCTFRVDEPPVDLKFLAIIWHFQDKEVLNYTNTSLV
AAI61104         GSVLEVIARSPHVAQKGSNTLIPCTFRVNKFPADPKFLAIYWDFNGRRILTYDN--VV

AAH85212         STQNPRLSLNKDTTEDGVASLIISNVTISDGGLYRCRVLYSPKHMYKEVRLDIQ
AAI61104         STTDPRFSLDPSSAPRGEASLSVSNAQISDGGAYSCSVTYSPEQQQKEITVEIQ
```

Extracellular Ig domain

```
AAH85212         APPQITITNKVIIKDKESVLSASITRFYPVDIDIKWLRDGKILAGVILLTPQRNVDGT
AAI61104         APPQITITNRVVTENKESALRASIIGFYPQDLDVTWLRDGAILRGVTMNKPQRDPDGT

AAH85212         YQVNNYMTIVPTEENRKQNFSIWVQHESLSSPLQEDFQLIYG
AAI61104         HSTTSAVTIVPTEGNRNQNFSIRVQHESLSVPLQEDFQLLYA
```

Extracellular C domain

```
AAH85212         AAPSIHITHQLFHLHREQELVCQAWGFYPESIVMNWFLNGSHVETANRKTVNSSAVES
AAI61104         AAPSIHISHGSFYVNEEQTLMCRVWGYYPEDIAVSWFLNGSRVEPSEIKRINSSALEL

AAH85212         IYRILPTDQNWGMEISCEVEHQTLRSPLVEKLLILGN
AAI61104         PYRFLPTAESQGMEISCVVEHRALTEPLVQSLKVELT
```

TM region

```
AAH85212         DGKVKHRAVVLSIAILSVIITFTLISLFLWRKFSSPK
AAI61104         DAQWSNTLIIPIAIVVVALVAVVAVLILKKKKRCCIRK
```

Cytoplasmic domain-1 (Ig)

```
AAH85212         APELLGPIEYSLCGAEEIQYSLNLQNFYPRDINIKWTYGERNEKVNTSSNKFIRTAGE
AAH85212         KTFSVVSECRIPWHQYTMRVIWEHETLAEPQYREVRVT
```

Cytoplasmic domain-2 (C1)

```
AAH85212         DLPWRPVMSQILTPDVYVGTEAEIQCNISGYFPDKLTVTWYKKKENGKEELVNNGGRY
AAH85212         QIPDIQSQYQPDWTLTCTARLLFSPSLTEEHGTQFICRVKHPSLGEEIERRTGTLYVR
```

Cytoplasmic domain-3 (Ig)

```
AAH85212         ARPKVSKFRMAGEWGLSLEAEGFFPRDIGFSWEVVQGNKRDVTSLPCEFSISENTDGT
AAH85212         YKATSTCHCLKDQLNYLHTLRVSVQHEALGAPVCKDITQ
```

Cytoplasmic domain-4 (C1)

```
AAH85212         DFPWRPQVGEIVVPELIELEETQLTCNISNYFPDALTVTWFIKNKESEELTDVTQYIR
AAH85212         YRHDTVSQYQPDQTLTCTTSLTFSPSLGMDLGAEFICRVKHPSLGEEIERRTGTLYVR
```

C-terminus domain?

```
AAH85212         GGPTSLSP*
```

Online resource 1c. Alignment of V-IgSF domain of *Xenopus* B7H6

```
AAI66200         DALGVKTSDVPIKSQRNQEAFIPCTVTDYGAGRVDPKLLSVEWKLKPLNGSEEQVYLYVS 60
1420-1           ..............P..........I...............V.................. 60
707-5            ....ITMGGSLVNAL.AHDV.....I.G.S--EL.LTK...T.T.SNKAATNQL..KFL. 58
707-2            GS.RIEMGAE.V..L..E......S...W.P.DIS.PK.....TQRT.D.R.G....... 60
707-4            GS.R.EMGAE.V..L..E......S...W.P.DIS.PK.....AQ.T....KG....... 60
AAH84632         .T.......F...FL.................EL.V..F.F...M.TM...DK....... 60
AAH82995         E.VE..V..S.V.VLKD.DV.....ISR.SSTEL.LQR...Q.I.----..D-P..I.DG 55

AAI66200         GTPTPTRPGSYIS-RDLITGNVGLHLPRVQFTDEGEYTCTVIYTPNKAVGHSVLQVS 116
1420-1           ..H..........-S.................................W.Q...... 116
707-5            QS.Q.........D.......A....................F..D..M.E...... 115
707-2            ..R..........D....................................Q...... 117
707-4            ..H..........D....................................Q...... 117
AAH84632         ..H..........DSEI.R...A..................F.....V....T.... 117
AAH82995         .SHN.I....QLD-IH..K..A..Y.AQ..VAE........T.DNEEDE.R.S.E.. 111
```

Online resource 1d. Alignment of V-IgSF domain of *Xenopus* B7HXen

```
AAI61104         GSVLEVIARSPHVAQKGSNTLIPCTFRVNKFPADPKFLAIYWDFNGRRILTYDNV--- 55
1657-1           ......T.P.........D....................................--- 55
1146-1           ......T.P.........D........IDP......L.N.K.N...QIR.SCTDSE-- 55
1146-2           VA....S.PLTQISM...D........IDP......L.D.K.H...QIRE..SDKV-- 56
1119-1           .AA..LT.PPT.R.TL.AD.........ETP.V..RH...F.Y.QDKE..NVTTT--- 55
1119-2           .AA.DLTVPPS.R.RV..D.S......LDRRSV...L.T.S.Y.QD.E..RFPGT--- 55
1119-3-1         .AA..LM.PPT.R.TL.AD.........ETP.V..RH.G.F.Y.QDKE.FNVIST--- 55
1119-3-2         -DA.LIGDEE.PT.TL..DVIL....S.GQP-VSLQY...L.T.QNKMLFRL.NK--- 54
1119-4           .AA..LM.PPT.R.TL.AD.........ETP.V..RH.G.F.S.QDKE.FNVIST--- 55
867-1            C.A...TVPPNQSSPM.RDA.L......DNP.MN......L.H.GDKEV.R...K--- 55
867-4            C.A...TVPPDQSSPM.RDA.L......DNP.MN......L.H.GDKEV.R...K--- 55
867-5            C.A.K.SDPPS.K.LL..TASL....SLG.S.I.HSA.S.I.T.RDKE..R.NKG--- 55
867-7            ..A.K.SDPPS.K.LL..T.SL....SLG.S.INHSA.S.I.T.RDKE..R.NKG--- 55
867-9            -.....F.PPTQQTLL...V.L....Q.ENSQIL.NF..VF.YIGDKE.VR.DNKG-- 54
3534-1           .AA..LM.PPT.R.TL.AD.........ETP.V.SRH...F.Y.QDKE..NVIST--- 55
1318-3           ..I.DIS.P..QRVLL.NEAQLR...SLA.P.IN.L...VF.Y.QDME..R...K--- 55
1318-2           .AT.QLA.PPSRQVLV..RAIL..MIS.GNASL.LNY.VVI.F.QNKE..RF..K--- 55
1318-1           .TT.QLA.PPSRQVLV..RAIL..MIS.GNASL.LNY.VVI.F.QNKE..RL..K--- 55
1146-3           .AE...Q.PPA.TVTL..DVTL..S.S.GPTQV.LQY.T.L.Y.QDTE..FFNAH--- 55
1707-2           .AE...Q.PPA.TVTL..DVTL..S.S.GPTQV.LQL...L.Y.QDTE..VFNAHGSI 58
AAH85212         F.A..MT.PPT.R.EM...II.......DEP.V.L.....I.H.QDKEV.N.T.TSL- 57
Hagfish          ICG.K.ESP.EVGFVD.K.VSL..I.T..DHSEPIDLSKLELEWTNGHAILTYKNGKI 58

AAI61104         VSTTDPRFSLDPSSAPRGEASLSVSNAQISDGGAYSCSVTYSPEQQQKEITVEIQ 110
1657-1           ............N....................T..................... 110
1146-1           ..........N.N..LL................T.......GT.W.E...RL... 110
1146-2           E.......F...N...W................T.......GTDW.E.KTIL... 111
1119-1           .GSSN..L..NRETIRA.V....IA.VE.....L.R...L...DRRY..VRLD.. 110
1119-2           .GAPN..L..NKDTTKD.V....LTGVR.....L.K...GHGF.RSE...RLD.. 110
1119-3-1         .GSSN..L..NRDTIRA.V....IA.VE.....L.R...L...DRRY..VRLD.. 110
1119-3-2         GKQLS..VTFSDAD.MK.I..VQLH.VSVA.A.I.M.KII....KKE.D..LKV. 109
1119-4           .GSSN..L.INRDTIRA.V....IA.IE.....L.R...L...DMRY..VRLD.. 110
867-1            GKVSS..V.I.ERALLE.N....L..VTV....T.R...I....T.K...RLR.H 110
867-4            GKVSS..V.I.ERALLE.N....L..VTV....T.R...I....T.K...RLR.H 110
867-5            RTLSQA.L...AQAIEE.RV...V..VTV..E.T.T.V.S.NM-K.EQGVKL.VA 110
867-7            RTLSQA.L...AQAIEE.RV...V..VTV..E.T.T.V.S.NM-K.EHVMELQVE 110
867-9            K.ISHS.VTA.EKGFPQ.N..ITL..VA...Q.T.R.LIIH..SRKE.D...KV. 109
3534-1           .GSSN..L..NRDTI.A.V....IA.IE.....L.R...L...NRRY..VRLD.. 110
1318-4           GLSLS..V.FSKEA.NN.DV.V.LA.VS.....I.R.L.I....KME..VLL..F 110
1318-2           CFSSTS.R.FEQQEVDN.NF..AL..VRS..E.V.T.A.V....REDR.IRLIVL 110
1318-1           CFSSTS.R.FEQQEVDN.NF..AL..VRS..E.V.T.A.V....RED..IRLNVL 110
1146-3           GKESE..VTIREED.GK.I...HLAQIRL..A.L.K.L.I.I.RSHT..VQLTVH 110
1707-2           KEESE..VTIREED.GK.I...HLAQIRL..A.L.K.M.I.I.RSHT..VQLTVH 113
AAH85212         ...QN..L..NKDTTEDGV...II..VT.....L.R.R.L...KHMY..VRLD.. 112
Hagfish          ETSSSFKGRVMMNEQNLRNGDA.LILNNVTEVDMLT.I...NG.TRRDT.ILQYK 113
```

Figure legend:

a) Alignment of cytoplasmic Ig-like domains of *Xenopus* B7H6 EST sequences. Cytoplasmic Ig-like domains are determined based on the exon-intron organization from genomic sequences. b) Deduced amino acid sequence of full-length EST sequences for *X. laevis* B7HXen. Only the extracellular domains of AAI61104 (*X. tropicalis*) are shown since there is no obvious cytoplasmic Ig domain in this sequence. Domains are predicted based on the sequence similarity to other sequence and exon assignment from *X. tropicalis* genome. c) The deduced amino acid sequences of V-IgSF domains of B7H6 are retrieved from the *Xenopus* genomic scaffolds and aligned with EST sequences. d) The deduced amino acid sequences of V-IgSF domains of *X. tropicalis* B7HXen are retrieved from scaffolds and aligned with EST sequences. The membrane distal V-IgSF of Hagfish IgSF3 sequence was aligned. Gaps and Identical aa residues are indicated with dashes and dots, respectively. Canonical amino acids characteristic for IgSF domains are highlighted in grey and predicted TM regions are underlined.