

Tools and databases included in the GRASP gene annotation pipeline
Alignment Tools.5 BLAST 2.2.17 [50] BLAT v.32x1 [51] SSAHA2 1.0.4 [52] Gene Prediction Tools Genscan [53] TMHMM 2.0c [54] Other Repeatmasker 3.1.9 [55] Databases NCBI non-redundant (nr) Database [56] Salmo Salar EST Database [49] TC1 Database Salmo Repeat Mask Library; repbase_fish.sal_Lib8 [49] UniRef Database; Release 13.4 [57] NCBI CDD Conserved Domains Database [58] Ensembl Organism Genome Protein Database, Release 49 [34]: Danio rerio, Zv7 Gasterosteus aculeatus, BROADS1 Oryzias latipes, MEDAKA1 Takifugu rubripes, FUGU4 Tetraodon nigroviridis, TETRAODON7

Primers used for BAC clone screening				
Gene/PCR product	Forward Primer	Reverse Primer	Reference	
<i>LWS- S180r</i>	Fw100; GATCCCTTTGAAGGACCAAACT	RevA; CATCCTAGATACTTCCTTCTGGG	Hoffman et al., 2007; Ward et al., 2008	
<i>LWS- S180/P180</i>	Fw100; GATCCCTTTGAAGGACCAAACT	Rev5; CATGACTACAACCATCCTGG	Hoffman et al., 2007; Ward et al., 2008	
<i>SWS2A</i>	SWS2A-F; ATCCCAGAAGGCCTGCAGTGC	SWS2A.B-R; CGACCACCATCCTGGTCACCT	this study	
<i>SWS2B</i>	SWS2B-F; GTCAGCCTTTGTCTCTTGC	SWS2A.B-R; CGACCACCATCCTGGTCACCT	this study	
Primers used for PCR screening of adult <i>X. helleri</i> cDNA				
Gene/PCR product	Forward Primer	Reverse Primer	Results	Notes
<i>S180r</i>	Xh.S180r-ex1a; CTTGCTCCCTGGAAGAACAAT	RevA; CATCCTAGATACTTCCTTCTGGG	Amplified from Male and Female	Clone and sequenced using T3 and T7 primers
<i>S180r</i> and 5'UTR	Xh.S180r-UTR5; ATCAAGCAGCTCAGATCGTCT	Xh.S180r-R; GGACACTTCAGAGCCGTCAT	Not amplified from either sex	
<i>S180-1</i>	Xh.S180-1-UTR5; CCTCGGGAGGTTACAAGTGA	Xh.S180-1-UTR3; TGCCAGAATGCTATTAGGTG	Amplified from Male and Female	Clone and sequenced using T3 and T7 primers
<i>S180-2</i>	Xh.S180-2-UTR5; AAGGTTTGACAGCCGAGAGA	Xh.S180-2-UTR3; CTTCTATAACAGAGAATGAAGG	Amplified from Male and Female	Clone and sequenced using T3 and T7 primers
<i>P180</i>	Xh.P180-UTR5; CCCAGCAAACTCTCAAGGT	Xh.P180-UTR3; CAGTCCTGGCGCTAATAACAAAC	Amplified from Male and Female	Clone and sequenced using T3 and T7 primers
<i>SWS2A-(1st Round)</i>	Xh.SWS2A-UTR5; GTTTCACGGCTCAGAAAGGA	Xh.SWS2A-UTR3; TTTGCTGTTGTTGGATTGTGA	Not amplified from either sex	
<i>SWS2A- (2nd round)</i>	SWS2A-F; ATCCCAGAAGGCCTGCAGTGC	SWS2-R; CGACCACCATCCTGGTCACCT	Product amplified using these primers in 2nd round of nested PCR	Sequenced directly from purified PCR product using PCR primers
<i>SWS2B</i>	Xh.SWS2B-UTR5; CCATCCCAATGAAGATGAGG	Xh.SWS2B-UTR3; CAGAATGCAATTCTAGAATG	Amplified from Male and Female	Sequenced directly from purified PCR product using PCR primers
<i>RH2-1</i>	RH2-1-F; CACAAGGAACTAGA-GATGGC	RH2-1-R; CATGATTAAGACACATGGTCCT	Amplified from Male and Female	Sequenced directly from purified PCR product using PCR primers
<i>RH2-2 (1st round)</i>	RH2-2-F; CACAGCCTGAGAAGATGGAG	RH2-2-R; CTGTAATCATTAAAGCTGCAGTG	Amplified from Male and Female	
<i>RH2-2 (2nd round)</i>	Xh.RH2-2-F; TATGGGACCCTTAGGCTGTG	Xh.RH2-2-R; GGTAGCTGAGAAGGCAGCTC	Amplified from Male and Female	Sequenced directly from purified PCR product using PCR primers
<i>SWS1 (1st round)</i>	SWS1-F; GGGAACTCAGGGTAAAGATGG	SWS1-R; AACATTATGAAGCTGTGGACAC	Amplified from Male and Female	
<i>SWS1 (2nd round)</i>	Xh.SWS1-F; TTCACCTGCATGCACTCTTC	Xh.SWS1-R; GGTGACAAGCCGGTAGTGTT	Amplified from Male and Female	Sequenced directly from purified PCR product using PCR primers
<i>RH1</i>	RH1-F; AACCACAAGCCGCAACCATG	RH1-R; TGAAGCTTGTGCCTGTTGCTC	Amplified from Male and Female	Sequenced directly from purified PCR product using PCR primers
<i>GPHN</i>	geph-F4; TGGCCTACAAGATAGTGCCAGA	geph-UTR3; TATGTGGACATGCACCGTGA	Amplified from Male and Female	Sequenced directly from purified PCR product using PCR primers
<i>GPHN-S180r fusion 1</i>	geph-R; ATGATTTCACTGCCAGGAC	Xh.S180r-R; GGACACTTCAGAGCCGTCAT	Not amplified from either sex	
<i>GPHN-S180r fusion 2</i>	Xh.S180r-ex1-F; GCAGAAGATTGGGGAAAACA	geph-F4; TGGCCTACAAGATAGTGCCAGA	Not amplified from either sex	

Sequences used in Multipipmaker analysis

Species	Chr/Scaff.	GenBank Acc #	Sequence Start	Sequence End	Ensembl Assembly Version
Medaka	Chromosome 5	N/A	27,010,810 bp	27,015,469 bp	HdrR
<i>Pundamilia pundamilia</i>	N/A	AB448597	N/A	N/A	N/A
Fugu	Scaffold_79	N/A	746,180 bp	748,856 bp	FUGU 4.0
Tetraodon	Chromosome 11	N/A	10,120,482 bp	10,122,886 bp	TETRAODON 8.0
Stickleback	groupXVII	N/A	10,619,138 bp	10,627,091 bp	BROAD S1
Zebrafish	Chromosome 11	N/A	25,240,722 bp	25,242,976 bp	Zv8