

Title: DNA mutation analysis in brown bullhead fish from  
Presque Isle Bay

Authors: Steven Mauro, Ph.D.  
Sarah Ewing, Ph.D.

Affiliation: Gannon University

Time Period: May 1, 2014-January 31, 2016

## Executive Summary

Genome sequence comparison of tumor and non-tumor samples obtained from brown bullhead fish from Presque Isle State Park was undertaken to identify regions of DNA mutations in tumor samples. DNA primer sets were constructed to amplify and examine eight of these regions in greater detail, all of which successfully amplified the region targeted. Three genome areas were targeted for sequence analysis in a greater subset of tumor and non-tumor fish samples. The sequence analysis of these samples indicated identical sequence similarity between the tumor and non-tumor samples for all genome areas targeted. The results indicate a non-uniform environmental DNA mutation profile explaining tumor formation in brown bullheads.

## Report

Introduction- In 1991, Presque Isle Bay was designated as the 43<sup>rd</sup> Great Lakes Area of Concern (AOC) by the U.S. Department of State. To be listed as an AOC, one or more of 14 defined beneficial uses of a Great Lakes water resource must be impaired. For Presque Isle Bay, two impairments were listed; the presence of fish tumors or other deformities and restrictions on dredging (Rafferty et. al., 2009).

The presence of fish tumors or other deformities focused on liver and skin tumor incidences of the bioindicator fish *Ameiurus nebulosus*, more commonly known as the brown bullhead. Restrictions on dredging was listed due to high concentrations of heavy metals in the sediment. Improved sediment conditions led to the removal of dredging as a restriction impairment in 2007 (Boughton, 2006). Most recently, in February of 2013, the restriction of fish tumors or other deformities was also removed, and Presque Isle Bay was taken off the list of Great Lakes AOC.

While no longer listed as an AOC, high levels of skin tumors on brown bullheads still remain and the cause of these tumors remain unknown. Moreover, the mutagen source and characterization of DNA mutations that have caused these tumors remains poorly understood (Rutter, 2010). Our previous work has allowed us to obtain complete genome sequence information from tumor and non-tumor tissue samples from fish in Presque Isle Bay, presenting an opportunity for genetic analysis to better understand the molecular makeup of these fish. More specifically, three specific objectives were proposed:

- 1.) Identify unique DNA regions that are mutated in tumors found in brown bullhead fish collected from Presque Isle Bay to begin to better understand the genetic and/or environmental origin of tumor formation.
- 2.) Design molecular probes that target identified mutated regions of brown bullhead DNA for rapid tracking and identification of these mutations in brown bullhead fish.
- 3.) Employ the use of the developed tumor molecular tracking technology to identify incidences of DNA mutation in a larger subset of brown bullhead fish from Presque Isle Bay to test the scalability of our findings.

The completion of these three objectives has demonstrated the utility of genome sequencing to develop molecular tools for tracking DNA regions believed to be of importance in this fish

species. This work has provided insight into the type of mutagen and pattern of distribution of DNA mutation giving rise to tumors for brown bullheads.

## Methodology

### *Genome comparison of tumor and non-tumor fish samples*

Genomes of a tumor and non-tumor DNA brown bullhead fish sample obtained from Presque Isle Bay that had been previously sequenced were separated into smaller scaffolds and aligned using freely available software on the NCBI website. The percent similarity for each scaffold was determined and the area of DNA mutation in each area of DNA sequence dissimilarity was noted. Gene homology searches also performed using the NCBI software freely available was used to identify areas of DNA mutation that were likely to be part of the gene regulatory circuitry. Eight of these areas were targeted for DNA primer development.

### *DNA primer development targeting mutated areas*

DNA primers were developed using Eurofins MWG Operon software. The same company was used to order primers. PCR conditions for each primer set were optimized (95°C for 2 min., 40 cycles of 95°C for 30 sec., 61°C for 30 sec., and 72°C for 90 sec., followed by an extension stage of 72°C for 10 min.) and successful amplification of DNA was determined by gel electrophoresis, using a DNA ladder to confirm the correct size of the amplicon for each of the primer sets used.

### *DNA mutational analysis of targeted areas in a larger subset of brown bullhead fish samples*

Three of the primer sets were used to amplify an additional eighteen tumor or non-tumor fish sample obtained from Presque Isle or Long Point Bay. The amplified DNA was subject to gel electrophoresis, extracted using a Qiagen kit, and sent for sequencing at Eurofins MWG Operon. Snapgene software was used to align the returned sequences for comparison.

## Results

### *Objective #1- Identification of regions that are mutated in a lesion/tumor sample from brown bullhead*

In total, >400 sequence clusters were compared between DNA obtained from a tumor vs. non-tumor tissue of a brown bullhead. The sequence clusters ranged anywhere from <50 base pairs in length to > 1,500 base pairs in length. The comparisons are shown in Table 1.

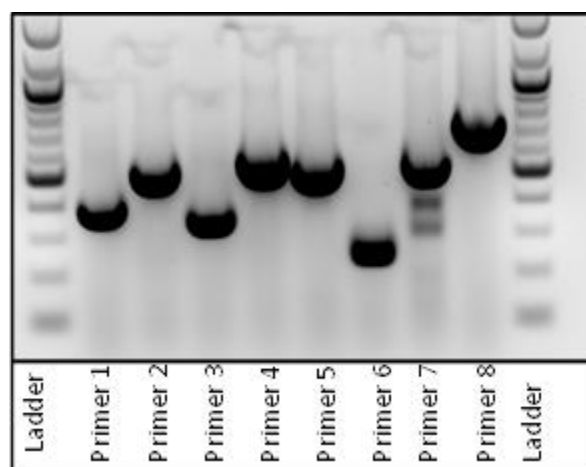
As can be seen from the Table, nearly all of the sequences that were compared contained some type of DNA sequence difference between the tumor and non-tumor sample, represented by the percent similarity for each of the samples compared to one another, providing ample opportunity for identification of target areas for DNA primer development.

*Objective #2- Design molecular probes that target identified mutated regions of brown bullhead DNA for rapid tracking and identification of these mutations in brown bullhead fish.*

In an effort to narrow down the choices of areas to target for DNA primer development, we focused on regions that contained gene sequences that have been implicated in uncontrollable cell growth. The DNA primer sequences designed and areas targeted are provided below.

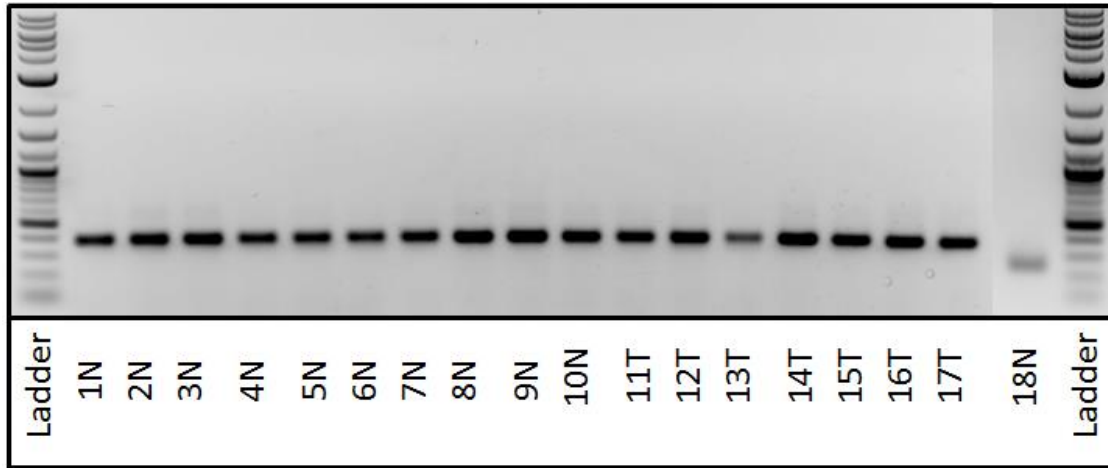
Primer Set Number	Primer Type	Sequence	Putative Sequence Homology		
1	Forward Sequence	TTAGTGCTGGACTGAAAGTCT	<i>Ictalurus punctatus</i>	Channel Catfish	macrophage migration inhibitory factor (MIF)
	Reverse Sequence	CTGCAGGACGTACTACACTGA			
2	Forward Sequence	GCCAGACAGCCGGAAGAAA	<i>Ictalurus punctatus</i>	Channel Catfish	immunoglobulin heavy chain gene locus, partial sequence and
	Reverse Sequence	TGCTGCTGTCCATGAAAACG			
3	Forward Sequence	TCCGCCAGTAAAAACGACGA	<i>Ictalurus punctatus</i>	Channel Catfish	inhibitor of apoptosis protein 1 gene
	Reverse Sequence	TTTCTCCCCCGAAACAACC			
4	Forward Sequence	CCCTTTCACCGGAATCAGAC	<i>Astyanax mexicanus</i>	Mexican Tetra	PREDICTED: tumor necrosis factor receptor superfamily, member 1B (tnfrsf1b), mRNA
	Reverse Sequence	GCGTGAGTGTAGGCTAATCTT			
5	Forward Sequence	GGCAAATAAAACATCTAGCGACTT	<i>Ictalurus punctatus</i>	Channel Catfish	TLR5-1, TLR5-2 (toll-like receptor); T-cell receptor alpha gene
	Reverse Sequence	TGCTCCATGCAATGTTAGAGGTA			
6	Forward Sequence	TTTAGCTTGAGACACCTGGCA	<i>Falco cherrug</i>	Saker Falcon	PREDICTED: caspase activity and apoptosis inhibitor 1 (CAAP1)
	Reverse Sequence	TGTCCCAAATCCCAGAAGACAT			
7	Forward Sequence	CATAGCGCCTAAACCCACAC	<i>Ictalurus punctatus</i>	Channel Catfish	inhibitor of apoptosis protein 1 gene
	Reverse Sequence	CGCCACCTGAACAACGGAAA			
8	Forward Sequence	ACATGCACGCTCATCTGTGA	<i>Ictalurus punctatus</i>	Channel Catfish	TLR20-1 and TLR20-2 genes
	Reverse Sequence	TCAGTGCCAGAACAGTCCAC			

The ability of each primer set to amplify the reference DNA from which the sequences were originally obtained was undertaken using the conditions described in the methods section. The resultant amplified DNA visualized by gel electrophoresis is shown in the Figure here. As can be seen, each PCR reaction produced a DNA band, which was of the predicted size of the amplicon. This demonstrated that the molecular probes designed were successful in targeting the DNA area of interest, thus successfully completing objective #2.



The scalability for using each primer set was tested by amplifying an additional 18 brown bullhead fish samples, which were either tumor or non-tumor in origin. All primer sets were able to amplify all 18 samples, thus demonstrating the ability to use these primer sets to test a larger subset of DNA samples from brown bullhead. An

example of the positive DNA amplification on an agarose gel for all 18 samples using primer set #1 for PCR is shown below as an example. The sample number and designation of whether the sample was obtained from a tumor (T) or normal (N) tissue is indicated.



*Objective #3- Employ the use of the developed tumor molecular tracking technology to identify incidences of DNA mutation in a larger subset of brown bullhead fish from Presque Isle Bay to test the scalability of our findings*

The DNA from the 18 samples were extracted and sequenced for three of the primer sets (primer set #1, #2, and #8) that were developed. In all cases, all nucleotide matched with 100% similarity for all 18 samples analyzed, including both non-tumor and tumor tissues. Hence, no sequence dissimilarities were noted between this larger subset of brown bullhead DNA samples for the regions analyzed. This is summed up in the Table below. A schematic showing the sequence similarity of one of the regions of primer set #2 is also provided in the Appendix C to more specifically show the sequence similarity between samples.

Primer Set	Number of samples sequenced	Number of base pairs sequenced	% DNA similarity between samples
1	18	381	100%
2	18	479	100%
8	18	512	100%

### Conclusions

All of the objectives were met in completion of this project. Based on the results of the objectives, we were able to draw the following specific conclusions:

- 1.) Genomic comparison of a single tumor and non-tumor brown bullhead sample identified many areas of DNA mutation

- 2.) DNA sequence information was able to be utilized to design molecular probes for brown bullheads that target can be used to amplify DNA for a larger subset of brown bullhead samples
- 3.) Targeted areas between a larger subset of samples did not indicate DNA mutations in the areas analyzed between the tumor and non-tumor samples.

While we failed to recognize consistent areas of DNA mutations for areas of tumor samples sequenced, the utility of the procedure to develop molecular target areas has been identified. Further, the lack of uniformity in DNA mutations between samples suggests that the DNA aberrations giving rise to tumors in these fish is not genetic in nature, which would be inherited and identical between one fish sample and the next. While the environmental mutagen has not been determined, the results strongly indicate an environmental cause for the skin deformities noted for brown bullheads in Presque Isle Bay.

#### Additional Research Indicated

DNA sequencing of additional areas in the samples would uncover precisely where the areas of DNA mutation reside between the tumor and non-tumor samples. Since the molecular probes developed indicated non-uniformity in DNA mutation patterns, a more global approach for DNA sequencing would likely provide greater success in sequence mining, an expensive but worthwhile endeavor that would provide valuable insight into the molecular mechanism underlying tumor formation in fish.

In addition to greater molecular characterization, further work that will determine the environmental causative factor(s) is needed. While our data here suggests an environmental basis for fish tumor formation, the exact type of external mutagen has yet to be determined. This information is vital for understanding the root cause of skin tumor formation still plaguing brown bullhead fish in Presque Isle Bay and other areas that are still listed as impaired and areas of concern.

#### Citations

Boughton L. (2006). Delisting the restrictions on dredging beneficial use impairment in the Presque Isle Bay Area of Concern. Pennsylvania Department of Environmental Protection, Office of the Great Lakes. PADEP; TREC Erie, PA. 16505.

Mulugeta S, Hindman R, Olszewski AM, Hoover K, Greene K, Lieberman M, Mauro SA. (2012). Contamination level and location of recreational freshwater influence the ability to predict *Escherichia coli* concentration by qPCR targeting *Bacteroides*. *J. Environ Manage* 103: 95-101.

Rafferty SD, Blazer VS, Pinkney AE, Grazio JL, Obert EC, and Boughton L. (2009). A historical perspective on the “fish tumors or other deformities” beneficial use impairment at Great Lakes Areas of Concern. *J. Great Lakes Res.* 35: 496-506.

Rutter MA (2010). A statistical approach for establishing tumor incidence delisting criteria in areas of concern: A case study. *J. Great Lakes Res.* 36: 646-655.

Smith CJ, Olszewski AM, Mauro SA. (2009). Correlation of shiga toxin gene frequency with commonly used microbial indicators of recreational water quality. *Appl. Environ. Microbiol.* 75: 316-321.

Weinberg RA (2007). *The Biology of Cancer*. Garland Science.

Yoon K, Zhu S, Ewing SJ, Smart RC. (2007). Decreased survival of C/EBP $\beta$ -deficient keratinocytes is due to aberrant regulation of p53 levels and function. *Oncogene* 26: 360-367.

## **Appendix A**

### Staff

Number of individuals: 2

Number of full-time employees (as part of the grant): 0

Number of full-time employees (as part of match): 0

### Students Supported

Number of Undergraduate Students: 1

Number of Graduate Students: 0

Number of Ph.D. Students: 0

Degrees Awarded (please indicate level): 0

### Outreach/Extension

Number of meetings, workshops, or conferences, and number of attendees: 2 conferences, 2 attendees at each conference

Number of public or professional presentations, and number of attendees: 1 public presentation, approximately 20 attendees

## **Appendix B**

### Impact Statement

Relevance: We examined the ability to use molecular techniques to better understand tumor formation in brown bullheads, which is a national problem.

Response: The information provided in this study set the stage for more detailed approaches that will together understand the cause of brown bullhead tumor formation, a critical step needed before remediation can occur.

Results: Recreational water users, individual who fish, and citizens concerned about water quality in general are major stakeholders impacted by the results of these studies and the implications of the findings.

Project partners: Individuals from DEP and DCNR contributed to certain aspects of this study.

### Appendix C- Additional information

#### Genome sequence data and comparison between a bron bullhead tumor and non-tumor sample

Assembly 2 (tumor tissue)	Assembly 1 (non-tumor tissue)	Gene similarity	percent similarity	total # nucleotides matching
719	726	Xenopus (Silurana) tropicalis clone ISB1-162F14, complete sequence	90%	86/96
592	233	NONE		
135	897	Ictalurus punctatus clone SDDE08R serine/threonine-protein kinase Pim-3-like mRNA, partial cds	99%	375/380
303	323	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	98%	137/140
301	787	Ictalurus punctatus inhibitor of apoptosis protein 1 gene, complete cds	91%	159/174
68	368	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	87%	1411/1622
477	658	PREDICTED: Stegastes partitus histone H3 (LOC103367735), mRNA	88%	408/462
398	228	Zebrafish DNA sequence from clone CH211-25001 in linkage group 13, complete sequence	75%	272/364
737	792	Cathorops melanopus zona pellucida glycoprotein mRNA, partial cds	92%	153/166
531	680	NONE		
525	640	Zebrafish DNA sequence from clone DKEY-148E22 in linkage group 5, complete sequence	73%	496/683
476	695	Ictalurus punctatus clone BAC1B immunoglobulin delta heavy chain gene locus, partial sequence	81%	341/420
245	760	NONE		



348	368	Zebrafish DNA sequence from clone BUSM1-18005 in linkage group 7 Contains the gene for a novel protein similar to vertebrate inhibitor of growth family member 4 (ING4), the 5' end of a novel gene and two CpG islands, complete sequence	75%	360/483
116	232	PREDICTED: <i>Astyanax mexicanus</i> protein NLRC3-like (LOC103041940), mRNA	81%	1213/1489
645	42	<i>Ictalurus punctatus</i> TLR20-1 and TLR20-2 genes, complete cds	82%	762/925
293	569	PREDICTED: <i>Chrysemys picta bellii</i> uncharacterized LOC103305841 (LOC103305841), mRNA	73%	1190/1634
258	480	TPA_exp: <i>Rana pipiens</i> Tc1-like transposon transposase gene, complete cds	98%	207/211
490	443	<i>Ictalurus punctatus</i> TLR20-1 and TLR20-2 genes, complete cds	85%	906/1062
459	896	NONE		
697	397	TPA_exp: <i>Rana pipiens</i> Tc1-like transposon transposase gene, complete cds	99%	1614/1619
202	618	<i>Pelteobagrus fulvidraco</i> clone 428 microsatellite sequence	89%	430/483
587	463	<i>Clarias batrachus</i> catalase (cat) mRNA, partial cds	91%	120/132
219	559	NONE		
386	19	<i>Ictalurus punctatus</i> warm temperature acclimation protein 65 KDa-1 (Wap65-1) gene, complete cds	92%	166/181
595	911	<i>Cyprinus carpio</i> clone 775252 microsatellite sequence	92%	44/48
534	123	<i>Salmo salar</i> clone BAC CHORI214-439H13 genomic sequence	96%	463/484
402	556	NONE		
331	260	Zebrafish DNA sequence from clone DKEY-23A13 in linkage group 7, complete sequence	85%	315/372
597	853	<i>Ictalurus punctatus</i> clone 032007 T-cell receptor delta (TRD) gene, partial sequence	84%	176/209
186	476	<i>Cyprinus carpio</i> clone 549222 microsatellite sequence	83%	68/82
90	76	Zebrafish DNA sequence from clone DKEYP-82B4 in linkage group 4, complete sequence	75%	2863/3832

631	790	Pig DNA sequence from clone CH242-16009 on chromosome 7, complete sequence	99%	89/90
242	299	Zebrafish DNA sequence from clone DKEY-48K17 in linkage group 2, complete sequence	77%	2701/3501
254	391	PREDICTED: <i>Astyanax mexicanus</i> tumor necrosis factor receptor superfamily, member 1B (tnfrsf1b), mRNA	98%	47/48
420	607	<i>Ictalurus punctatus</i> TLR20-1 and TLR20-2 genes, complete cds	93%	1970/2112
10	89	<i>Noturus flavipinnis</i> clone NFC1a microsatellite sequence	86%	240/278
354	796	Zebrafish DNA sequence from clone CH211-10M5 in linkage group 25, complete sequence	80%	260/327
650	618	<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	94%	768/815
197	461	NONE		
493	721	Zebrafish DNA sequence from clone DKEY-70B24 in linkage group 8, complete sequence	73%	240/331
480	873	PREDICTED: <i>Astyanax mexicanus</i> high density lipoprotein binding protein (hdlbp), transcript variant X2, mRNA	83%	789/945
272	731	NONE		
620	681	<i>Ictalurus punctatus</i> strain Stuttgart Xbal element 6, complete sequence	74%	211/284
127	273	NONE		
216	476	<i>Ictalurus punctatus</i> inhibitor of apoptosis protein 1 gene, complete cds	95%	208/220
275	384	NONE		
761	430	NONE		
317	475	NONE		
142	310	NONE		
196	384	PREDICTED: <i>Danio rerio</i> paraneoplastic antigen Ma1 homolog (LOC101885171), mRNA	100%	30/30
703	859	NONE		
309	323	Allotetraploid red crucian carp x <i>Cyprinus carpio</i> clone BAC AT150F8.1, complete sequence	72%	309/428
19	526	Zebrafish DNA sequence from clone CH211-218K8, complete sequence	96%	68/71

7	647	PREDICTED: <i>Cynoglossus semilaevis</i> uncharacterized protein K02A2.6-like (LOC103394707), mRNA	78%	2416/3105
43	348	Zebrafish DNA sequence from clone CH211-212J22 in linkage group 23, complete sequence	100%	37/37
623	609	<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	84%	638/762
25	760	Zebrafish DNA sequence from clone CH73-160I11 in linkage group 18, complete sequence	80%	100/125
327	546	<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	91%	526/580
706	624	Allotetraploid red crucian carp x <i>Cyprinus carpio</i> clone BAC AT150F8.1, complete sequence	71%	304/427
364	731	<i>Ictalurus punctatus</i> warm temperature acclimation protein 65 KDa-1 (Wap65-1) gene, complete cds	88%	161/183
617	24	<i>Salmo salar</i> clone CH214-714P22 MHC Class I (Sasa-UBA) gene, Sasa-UBA*0601 allele, partial cds; and proteasome subunit beta type 8 (PSMB8a), proteasome subunit beta type 10 (PSMB10a), PSMB9a-like (PSMB9a-like), proteasome subunit beta type 9 (PSMB9), transporter 2 ATP binding cassette (TAP2a), bromodomain containing 2 (BRD2), collagen Type XI Alpha2 (COL11A2), retinoid X receptor beta (RXRB), solute carrier 39 (zinc transporter) member 7 (SLC39A7), and reverse transcriptase-like protein genes, complete cds	95%	1041/1095
181	137	<i>Carassius auratus</i> red var. piwi-like 1 mRNA, complete cds	87%	48/55
513	563	<i>Ictalurus punctatus</i> TLR20-1 and TLR20-2 genes, complete cds	82%	510/620
368	723	<i>Oncorhynchus mykiss</i> SYPG1 (SYPG1), PHF1 (PHF1), and RGL2 (RGL2) genes, complete cds; DNasell pseudogene, complete sequence; LGN-like, PBX2 (PBX2), NOTCH-like, TAP1 (TAP1), and BRD2 (BRD2) genes, complete cds; and MHCII-alpha and Raftlin-like pseudogenes, complete sequence	95%	55/58

598	47	Dicentrarchus labrax chromosome sequence corresponding to linkage group 1, bottom part, complete sequence	100%	30/30
743	945	Ictalurus punctatus transferrin gene, complete cds	90%	1693/1882
114	607	Ictalurus punctatus clone BAC1B immunoglobulin delta heavy chain gene locus, partial sequence	92%	168/182
323	83	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	98%	778/797
509	624	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	88%	186/211
422	877	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	91%	3612/3948
234	119	Zebrafish DNA sequence from clone CH73-160111 in linkage group 18, complete sequence	78%	105/134
377	726	Zebrafish DNA sequence from clone CH211-198G14, complete sequence	95%	75/79
766	320	Ictalurus punctatus mitochondrion, complete genome	84%	3296/3928
214		NONE		
81	155	NONE		
128	323	Allotetraploid red crucian carp x Cyprinus carpio clone BAC AT150F8.1, complete sequence	71%	375/531
713	921	Schizothorax prenanti heat shock protein Hsp70 (Hsp70) mRNA, complete cds	86%	1671/1941
108	344	Zebrafish DNA sequence from clone DKEY-5413 in linkage group 4, complete sequence	94%	68/72
52	144	Zebrafish DNA sequence from clone CH211-27E6 in linkage group 12, complete sequence	97%	74/76
627	607	NONE		
642	645	Clarias batrachus catalase (cat) mRNA, partial cds	92%	121/132
215	596	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	82%	893/1085
740	813	NONE		
702	933	Pelteobagrus fulvidraco beta-actin gene, promoter region and complete cds	84%	584/699
751	754	Xenopus (Silurana) tropicalis clone CH216-146M19, complete sequence	84%	1159/1382

74	443	Zebrafish DNA sequence from clone CH211-105J21 in linkage group 6, complete sequence	81%	151/186
115	144	Zebrafish DNA sequence from clone DKEYP-4C4, complete sequence	96%	76/79
586	943	Ictalurus punctatus leukocyte immune-type receptor 3 (LOC100304647), mRNA	98%	244/249
13	207	Ictalurus punctatus clone BAC1B immunoglobulin delta heavy chain gene locus, partial sequence	85%	209/247
76	169	Zebrafish DNA sequence from clone DKEY-19D22, complete sequence	95%	77/81
147	596	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	75%	246/328
646	709	Ichia angusta strain DL-1 mitochondrion, complete genome	100%	29/29
296	701	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	94%	188/199
738	929	NONE		
640	411	Hoplias malabaricus (clone Hop4) satellite DNA sequence	91%	118/130
616	772	Zebrafish DNA sequence from clone CH73-50A19 in linkage group 1, complete sequence	83%	69/83
199	591	Zebrafish DNA sequence from clone RP71-87M8 in linkage group 6, complete sequence	69%	697/1003
607	859	PREDICTED: Falco peregrinus caspase activity and apoptosis inhibitor 1 (CAAP1), mRNA	100%	30/30
31	232	PREDICTED: Astyanax mexicanus NACHT, LRR and PYD domains-containing protein 12-like (LOC103042819), mRNA	80%	1368/1702
569	305	Ictalurus punctatus clone 032O07 T-cell receptor delta (TRD) gene, partial sequence	80%	218/273
107	513	PREDICTED: Danio rerio si:ch211-149a19.3 (si:ch211-149a19.3), transcript variant X5, mRNA	80%	1328/1656
167	462	Zebrafish DNA sequence from clone CH211-71K14 in linkage group 1, complete sequence	75%	273/366
549	538	Salmo salar IgH locus A genomic sequence	94%	858/908

5	171	Hoplias malabaricus (clone Hop4) satellite DNA sequence	88%	115/130
224	623	NONE		
565	621	NONE		
533	505	NONE		
126	559	PREDICTED: Poecilia reticulata transmembrane protein 222-like (LOC103472001), mRNA	96%	70/73
438	90	Zebrafish DNA sequence from clone CH211-124H9 in linkage group 5, complete sequence	82%	99/121
472	520	NONE		
754	864	NONE		
624	307	NONE		
689	556	NONE		
88	594	Zebrafish DNA sequence from clone CH73-160111 in linkage group 18, complete sequence	80%	105/131
692	556	NONE		
547	127	NONE		
20	127	Zebrafish DNA sequence from clone CH211-10K11 in linkage group 17, complete sequence	95%	89/94
735	923	NONE		
744	832	Zebrafish DNA sequence from clone CH211-227G21 in linkage group 19, complete sequence	87%	272/314
590	173	Ictalurus punctatus immunoglobulin G light chain LPG15 gene, partial sequence	96%	612/625
749	845	Ictalurus furcatus clone CBZC8684 globoside alpha-13-n-acetylgalactosaminyltransferase 1 (GBGT1) mRNA, complete cds	95%	891/936
580	126	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	85%	902/1061
194	485	NONE		
457	559	Vitis vinifera contig VV78X028997.31, whole genome shotgun sequence	92%	47/51
380	593	Zebrafish DNA sequence from clone CH211-160M17 in linkage group 25, complete sequence	98%	46/47
294	119	NONE		
150	189	NONE		
378	373	PREDICTED: Stegastes partitus gastrula zinc finger protein xLCGF3.1-like (LOC103352961), transcript variant X3,	93%	39/42

		mRNA		
686	434	Salmo salar clone BAC CHORI214-114L13 von Willebrand factor A domain containing 5A (VWA5A) gene, complete cds; gaps in BAC sequence with unknown sequence contribute to premature stops in the CDS (VWA5A-like protein) pseudogene, complete sequence; and MHC class I antigen (Sasa-SAA) gene, Sasa-SAA*0101 allele and v-akt murine thymoma viral oncogene 2-like protein (AKT2) gene, complete cds	94%	1556/1659
63	373	Ictalurus punctatus clone BAC1B immunoglobulin delta heavy chain gene locus, partial sequence	94%	72/77
685	921	Ictalurus punctatus TLR5s, TLR5-1, and TLR5-2 genes, complete cds	76%	406/533
244	591	Sclerotinia sclerotiorum 1980 hypothetical protein (SS1G_09531) partial mRNA	93%	40/43
593	312	Ictalurus punctatus TLR5s, TLR5-1, and TLR5-2 genes, complete cds	87%	279/322
500	874	Salmo salar clone 39N03 TCR-alpha/delta locus, genomic sequence	87%	352/403
694		NONE		
180	335	Zebrafish DNA sequence from clone CH73-16011 in linkage group 18, complete sequence	80%	105/131
664	910	Candida dubliniensis CD36 conserved hypothetical protein (CD36_41340) mRNA, complete cds	95%	37/39
433	454	Ictalurus punctatus inhibitor of apoptosis protein 1 gene, complete cds	91%	139/152
747	948	Zebrafish DNA sequence from clone RP71-23D18 in linkage group 22, complete sequence	76%	384/506
46	374	Ictalurus punctatus strain Kansas XbaI element 1, complete sequence	75%	210/281
720	919	Zebrafish DNA sequence from clone CH73-243C6 in linkage group 2, complete sequence	94%	44/47
708	726	NONE		
443	127	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	88%	214/242

430	123	PREDICTED: <i>Astyanax mexicanus</i> tripartite motif-containing protein 29-like (LOC103029040), transcript variant X1, mRNA	82%	394/478
644	528	Zebrafish DNA sequence from clone CH211-159E17 in linkage group 12, complete sequence	100%	28/28
508	740	NONE		
341	443	<i>Mus musculus</i> BAC clone RP23-39N24 from 15, complete sequence	100%	28/28
64	228	<i>Crotalaria podocarpa</i> clone K50_1_gy retrotransposon Ty3/gypsy-like reverse transcriptase-like gene, partial sequence	100%	28/28
611	556	NONE		
409	68	<i>Ictalurus punctatus</i> TLR20-1 and TLR20-2 genes, complete cds	84%	908/1079
369	506	<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	87%	230/263
653	707	<i>Mus musculus</i> BAC clone RP23-112D14 from 8, complete sequence	100%	28/28
659		NONE		
583	553	NONE		
97	463	<i>Ictalurus punctatus</i> immunoglobulin G light chain LPG16 gene, partial sequence	93%	1526/1648
246	438	<i>Ictalurus punctatus</i> leukocyte immune-type receptor TS32.15 L1.1c mRNA, partial cds	98%	269/275
527	173	<i>Noturus flavipinnis</i> clone NFC1a microsatellite sequence	86%	164/190
366	569	NONE		
701	739	NONE		
676	818	<i>Xenopus (Silurana) tropicalis</i> clone CH216-146M19, complete sequence	83%	1369/1648
629	889	NONE		
579	253	<i>Xenopus (Silurana) tropicalis</i> clone CH216-78G20, complete sequence	80%	418/520
763	712	<i>Ictalurus punctatus</i> mitochondrion, complete genome	88%	2349/2662
231	61	Zebrafish DNA sequence from clone DKEY-95P16 in linkage group 9, complete sequence	80%	127/158
18	171	<i>Mus musculus</i> BAC clone RP24-97K3 from chromosome 8, complete sequence	96%	85/89



119	513	PREDICTED: <i>Astyanax mexicanus</i> NACHT, LRR and PYD domains-containing protein 12-like (LOC103028274), transcript variant X6, mRNA	81%	1218/1504
657	454	NONE		
521		Zebrafish DNA sequence from clone DKEYP-30D6 in linkage group 3, complete sequence	78%	1178/1519
264	373	NONE		
560	780	<i>Salmo salar</i> IgH locus B genomic sequence	89%	58/65
739		<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	79%	200/252
463	561	NONE		
711		NONE		
56	77	<i>Cathorops melanopus zona pellucida</i> glycoprotein mRNA, partial cds	92%	153/166
89	540	PREDICTED: <i>Astyanax mexicanus</i> NACHT, LRR and PYD domains-containing protein 12-like (LOC103030411), mRNA	81%	1398/1726
672	754	<i>Xenopus (Silurana) tropicalis</i> clone CH216-146M19, complete sequence	83%	1370/1642
350	114	<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	95%	629/673
745		NONE		
30	114	Zebrafish DNA sequence from clone CH73-338L23 in linkage group 23, complete sequence	88%	647/736
290	772	NONE		
691	853	NONE		
736	796	<i>Dicentrarchus labrax</i> chromosome sequence corresponding to linkage group 1, top part, complete sequence	84%	85/101
502	243	<i>Ictalurus punctatus</i> strain Kansas XbaI element 3, complete sequence	76%	196/258
510	907	<i>Oncorhynchus mykiss</i> SYPG1 (SYPG1), PHF1 (PHF1), and RGL2 (RGL2) genes, complete cds; DNaseII pseudogene, complete sequence; LGN-like, PBX2 (PBX2), NOTCH-like, TAP1 (TAP1), and BRD2 (BRD2) genes, complete cds; and MHCII-alpha and Raftlin-like pseudogenes, complete sequence	84%	1144/1358
342	440	NONE		

49	228	Oncorhynchus tshawytscha clone ChinookGH2 growth hormone 2 gene, complete cds; and skeletal muscle sodium channel alpha subunit-like, myosin alkali light chain-like, and microtubule-associated protein Tau-like genes, complete sequence	92%	66/72
44	123	Mus musculus BAC clone RP23-39N24 from 15, complete sequence	100%	28/28
568	712	Zebrafish DNA sequence from clone CH73-51F10 in linkage group 11, complete sequence	75%	210/280
256	563	Ictalurus punctatus inhibitor of apoptosis protein 1 gene, complete cds	92%	216/234
494	873	Ictalurus punctatus inhibitor of apoptosis protein 1 gene, complete cds	90%	212/236
670	596	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	85%	768/900
241	505	PREDICTED: Neolamprologus brichardi sterile alpha motif domain-containing protein 9-like (LOC102789392), mRNA	97%	37/38
699	312	NONE		
4	61	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	89%	220/246
297	150	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	87%	1963/2259
54		Argyrosomus regius isolate Are4c12 U2 snRNA gene, partial sequence; intergenic spacer 1 and U1 snRNA gene, complete sequence; and intergenic spacer 2, partial sequence	95%	158/167
484	529	PREDICTED: Stegastes partitus tripartite motif containing 45 (trim45), mRNA	88%	152/172
295	443	Zebrafish DNA sequence from clone CH211-261D9 in linkage group 8, complete sequence	77%	453/588
750	873	PREDICTED: Takifugu rubripes hyaluronan synthase 1-like (LOC101075370), mRNA	82%	190/232
26	2	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	94%	91/97
393	362	NONE		
218	620	Haplochromis chilotes DNA, containing V2R gene cluster region, clone: 51I23	83%	279/338
157	233	Cyprinus carpio piwi-like 1 mRNA, complete cds	88%	68/77

53	445	Mus musculus chromosome 13, clone RP23-220G21, complete sequence	97%	75/77
563	37	TPA_exp: Rana pipiens Tc1-like transposon transposase gene, complete cds	99%	1619/1621
148	767	Zebrafish DNA sequence from clone CH73-115B4 in linkage group 8, complete sequence	100%	29/29
767	699	Ictalurus punctatus mitochondrion, complete genome	88%	2012/2280
159	394	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	97%	184/190
728	938	Ictalurus punctatus clone BAC1B immunoglobulin delta heavy chain gene locus, partial sequence	81%	131/161
415	920	PREDICTED: Lepisosteus oculatus A-kinase anchor protein 7 isoform gamma-like (LOC102685174), mRNA	93%	373/399
625	166	Ctenopharyngodon idella retinoic acid inducible protein I (RIG-I) gene, complete cds	71%	709/1005
746	754	Xenopus (Silurana) tropicalis clone CH216-3D8, complete sequence	84%	746/892
655	81	Ictalurus punctatus warm temperature acclimation protein 65 KDa-1 (Wap65-1) gene, complete cds	87%	289/333
639	618	NONE		
622	81	NONE		
468	565	PREDICTED: Cynoglossus semilaevis neurensin 1 (nrsn1), mRNA	81%	93/115
143	432	NONE		
345	513	PREDICTED: Lepisosteus oculatus E3 ubiquitin-protein ligase UBR2-like (LOC102697060), mRNA	94%	34/46
359	648	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	87%	527/604
656	824	NONE		
192	310	NONE		
267	397	PREDICTED: Nomascus leucogenys myosin, heavy chain 10, non-muscle (MYH10), mRNA	84%	61/73
448	191	Ictalurus punctatus leukocyte immune-type receptor TS32.15 L1.1a mRNA, partial cds	88%	73/83
731	920	Noturus flavipinnis clone NFC7 microsatellite sequence	87%	146/168
572	685	NONE		

722		NONE		
732	513	PREDICTED: <i>Astyanax mexicanus</i> uncharacterized LOC103032132 (LOC103032132), mRNA	79%	1243/1582
700		NONE		
566	818	<i>Xenopus (Silurana) tropicalis</i> clone CH216-146M19, complete sequence	84%	1375/1645
158	391	PREDICTED: <i>Poecilia reticulata</i> uncharacterized LOC103478689 (LOC103478689), ncRNA	93%	81/87
436		NONE		
748	818	<i>Xenopus (Silurana) tropicalis</i> clone ISB-196B20, complete sequence	83%	266/319
98		NONE		
730	712	NONE		
299	411	NONE		
406	252	Zebrafish DNA sequence from clone DKEY-279J5 in linkage group 13, complete sequence	96%	77/80
675	824	Zebrafish DNA sequence from clone CH211-191K19 in linkage group 7, complete sequence	77%	111/144
3		NONE		
8		<i>Ictalurus punctatus</i> TLR5s, TLR5-1, and TLR5-2 genes, complete cds	89%	99/111
734	942	NONE		
75	821	<i>Microhyla heymonsi</i> voucher CAS:HERP:210748 28S ribosomal RNA gene, partial sequence	97%	376/386
600	691	NONE		
516	792	Zebrafish DNA sequence from clone DKEY-181C10 in linkage group 15, complete sequence	78%	198/254
727	63	NONE		
733	475	NONE		
553	936	NONE		
120	761	<i>Tanganikallabes</i> sp. JW-2012 voucher SAIAB:80011 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	99%	1009/1018
229	506	<i>Ictalurus punctatus</i> immunoglobulin heavy chain gene locus, partial sequence and unknown gene	87%	230/263

709		Mus musculus BAC clone RP23-146B12 from chromosome 14, complete sequence	97%	34/35
687	362	NONE		
478	731	Ictalurus punctatus warm temperature acclimation protein 65 KDa-1 (Wap65-1) gene, complete cds	94%	101/107
715	618	NONE		
310		NONE		
277	372	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	92%	353/384
717	693	Ictalurus punctatus toll-like receptor 21 (TLR-21) gene, complete cds	80%	207/258
452	243	Ictalurus punctatus strain Kansas Xbal element 3, complete sequence	75%	171/229
752	943	NONE		
1		Ictalurus punctatus 18S small subunit ribosomal RNA gene, complete sequence	99%	714/715
637	480	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	93%	68/73
643		PREDICTED: Poecilia reticulata beta-1,3-galactosyltransferase 1-like (LOC103462987), transcript variant X2, mRNA	80%	110/138
262	229	Zebrafish DNA sequence from clone DKEY-48K17 in linkage group 2, complete sequence	81%	734/902
201	387	PREDICTED: Haplochromis burtoni uncharacterized LOC102301131 (LOC102301131), misc_RNA	94%	97/84
658	397	NONE		
36	872	NONE		
137		Zebrafish DNA sequence from clone CH211-197H24 in linkage group 16, complete sequence	96%	70/73
249	20	Rana catesbeiana clone rcat-evr-508-145 Transposable element Tc1 transposase putative mRNA, complete cds	91%	110/121
469	734	NONE		
105	641	NONE		
570	233	NONE		
286	760	NONE		
575	296	Xenopus laevis U2 snRNA gene	1000%	53/53
725	644	NONE		

511	397	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	81%	872/1075
283		PREDICTED: Astyanax mexicanus zinc-binding protein A33-like (LOC103028823), transcript variant X3, mRNA	87%	293/335
669	642	NONE		
146	658	Salmo gairdnerii H1 histone gene	77%	491/636
421	296	Zebrafish DNA sequence from clone DKEY-48K17 in linkage group 2, complete sequence	78%	1389/1778
741	664	NONE		
156	445	NONE		
206	68	NONE		
14	826	Oplegnathus fasciatus interferon regulatory factor 5 (IRF5) gene, complete cds	83%	136/164
588	373	NONE		
612	394	Oreochromis niloticus MHC class IA antigen UBA1, UBA2, UAA1 genes, partial cds, UAA3 and UAA2 pseudogenes, UAA4, UAA5 and UAA6 pseudogene fragments	93%	41/44
174	471	Zebrafish DNA sequence from clone DKEY-46I8 in linkage group 12, complete sequence	83%	155/187
677	597	Allotetraploid red crucian carp x Cyprinus carpio clone BAC AT150F8.1, complete sequence	72%	242/335
184	932	NONE		
716	262	NONE		
614	631	PREDICTED: Takifugu rubripes general transcription factor II-I repeat domain-containing protein 2-like (LOC101072663), mRNA	83%	996/1204
329	341	Ictalurus punctatus TLR4-1 and TLR4-2 genes, complete cds	77%	413/536
514	880	NONE		
724		NONE		
343	264	Zebrafish DNA sequence from clone CH211-204M6 in linkage group 17, complete sequence	95%	87/92
729		Siniperca chuatsi microsatellite PY1-82 sequence	94%	73/78
332		Ictalurus punctatus immune-type receptor 10 gene, complete cds	97%	445/457
423	443	Ictalurus punctatus cholecystokinin B gene, complete cds	88%	148/168

111		Pan troglodytes BAC clone CH251-388K24 from chromosome 17, complete sequence	93%	77/83
338	818	Zebrafish DNA sequence from clone CH1073-142P16 in linkage group 17, complete sequence	88%	90/102
680	944	NONE		
674		NONE		
35	88	Lates calcarifer clone Lca122 microsatellite sequence	99%	72/73
51	20	Ctenopharyngodon idella clone GCFL-0405E6 MHC class I antigen (Citd-UBA) gene, complete cds; and tapasin (tpsn) gene, partial cds	94%	1583/1635
300	605	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	92%	237/258
522		NONE		
548		PREDICTED: Astyanax mexicanus zinc finger protein 160-like (LOC103038508), mRNA	97%	31/32
212		Zebrafish DNA sequence from clone CH211-189K21 in linkage group 14, complete sequence	82%	1199/1454
647	808	NONE		
573	271	NONE		
678	894	PREDICTED: Astyanax mexicanus protocadherin gamma-A12-like (LOC103021388), partial mRNA	85%	1041/1226
552	296	Xenopus laevis U2 snRNA gene	100%	37/37
336	641	NONE		
304	515	NONE		
630	877	NONE		
550	173	Ictalurus punctatus cytochrome P450 CYP27A18 (CYP27A18) mRNA, partial cds	90%	79/88
577	585	Ictalurus punctatus clone BAC1B immunoglobulin delta heavy chain gene locus, partial sequence	85%	195/230
280	541	PREDICTED: Danio rerio si:ch211-149a19.3 (si:ch211-149a19.3), transcript variant X5, mRNA	83%	134/161
705	592	NONE		
537	946	PREDICTED: Maylandia zebra uncharacterized LOC101482465 (LOC101482465), mRNA	89%	234/263
321	644	NONE		
361	434	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and	97%	1109/1149

		unknown gene		
396	561	Dicentrarchus labrax chromosome sequence corresponding to linkage group 1, bottom part, complete sequence	90%	47/52
594	616	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	90%	293/327
515	733	NONE		
561	772	Ictalurus punctatus TLR4-1 and TLR4-2 genes, complete cds	83%	134/161
487		NONE		
584	134	Ictalurus punctatus immunoglobulin G light chain LPG5 gene, partial sequence	92%	145/158
431	769	Dicentrarchus labrax chromosome sequence corresponding to linkage group 1, bottom part, complete sequence	81%	122/151
390	563	Salmo salar clone BAC CHORI214-439H13 genomic sequence	95%	724/764
526	780	NONE		
546	784	NONE		
615		Xenopus (Silurana) tropicalis clone CH216-132A9, complete sequence	81%	241/296
391		NONE		
413		Dicentrarchus labrax chromosome sequence corresponding to linkage group 1, bottom part, complete sequence	86%	329/382
340	807	Salmo salar clone 39N03 TCR-alpha/delta locus, genomic sequence	91%	244/268
228	350	Mus musculus targeted KO-first, conditional ready, lacZ-tagged mutant allele Zfp213:tm1a(EUCOMM)Hmgu; transgenic	96%	77/80
168		PREDICTED: Pundamilia nyererei TGF-beta-activated kinase 1 and MAP3K7-binding protein 1-like (LOC102211077), transcript variant X3, mRNA	83%	140/169
70	61	Gasterosteus aculeatus clone VMRC26-150D01, complete sequence	87%	1097/1258
11		Mus musculus targeted non-conditional, lacZ-tagged mutant allele Map1lc3b:tm2e(EUCOMM)Wtsi tm1e(EUCOMM)Wtsi; transgenic	95%	71/75
461	544	NONE		



545	443	Carassius auratus ubiquitin-like protein 2 mRNA, complete cds	82%	183/223
285	675	PREDICTED: Astyanax mexicanus NACHT, LRR and PYD domains-containing protein 12-like (LOC103036384), mRNA	82%	130/158
357	83	Coregonus clupeaformis malate dehydrogenase, cytoplasmic (MDH) gene, complete cds	97%	304/315
571	57	Zebrafish DNA sequence from clone DKEY-112M2 in linkage group 21, complete sequence	94%	68/73
333	394	Ictalurus punctatus TLR22 gene, complete cds	94%	126/134
710	463	Ictalurus punctatus immunoglobulin light chain F class (IgL) mRNA, clone pf12, partial cds	96%	163/170
557	684	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	91%	1345/1484
324	207	NONE		
312	513	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	90%	89/99
447	473	NONE		
634	591	NONE		
714		PREDICTED: Poecilia reticulata heat-stable enterotoxin receptor-like (LOC103481041), transcript variant X3, mRNA	89%	160/180
306	663	NONE		
466	604	NONE		
517				
363	698			
132	32			
288	837			
375	78	NONE		
649	954	TPA_exp: Rana pipiens Tc1-like transposon transposase gene, complete cds	99%	1217/1219
96	111	Gasterosteus aculeatus clone CH213-101E8, complete sequence	100%	74/74
591				
384				
172	568	PREDICTED: Astyanax mexicanus uncharacterized LOC103032384 (LOC103032384), ncRNA	81%	220/270
449	529	NONE		
235	123			

441				
394				
726	123	Oncorhynchus mykiss clone BAC20-C13 zinc transporter (SLC39A7.B) gene, MHC class I antigen (Onmy-UGA) gene, Onmy-UGA-OSU allele, RING1.B, ribosomal protein S18 (RPS18.B), vacuolar protein sorting-associated protein 52-like protein (VPS52.b), putative adhesion protein (VIG-B319.b), hypothetical protein, and cyclic AMP-dependent transcription factor ATF-6 alpha-like protein (ATF6) genes, complete cds; and TNXB (TNXB.B) gene, partial cds	94%	719/762
171				
238				
292	63	NONE		
543				
417				
621				
6	155	NONE		
149	637	PREDICTED: Lepisosteus oculatus zinc-binding protein A33-like (LOC102698949), mRNA	87%	186/213
102				
460				
606	595	PREDICTED: Astyanax mexicanus polymeric immunoglobulin receptor-like (LOC103035517), partial mRNA	84%	255/304
679				
696	19	NONE		
603	720	Cyprinus carpio clone 440171 microsatellite sequence	79%	768/977
403	435	NONE		
325	787	Zebrafish DNA sequence from clone DKEY-218I12 in linkage group 16, complete sequence	96%	46/48
681	811			
558	748	Chrysemys picta BAC clone CHY3-100H12 from chromosome unknown, complete sequence	84%	1041/1242
141				
213	286			
387	454	Zebrafish DNA sequence from clone CH211-194B17 in linkage group 25, complete sequence	74%	310/419

540	806	NONE		
344				
475				
437	568	NONE		
144	79	NONE		
356	641	NONE		
261	563	Zebrafish DNA sequence from clone DKEY-206F21 in linkage group 23, complete sequence	83%	115/139
542				
82				
23	43	TPA: Danio rerio microRNA dre-mir-430a-10 precursor	83%	58/70
539	593	Zebrafish DNA sequence from clone ZFOS-2742F3 in linkage group 11, complete sequence	78%	135/172
129	417	NONE		
455				
133				
505	596	Oreochromis niloticus KLR8 pseudogene, partial sequence; KLR9 (KLR9) gene, C-type lectin (CLECT2)-like protein pseudogene, and C-type lectin (CLECT2)-like protein gene, complete sequence; KLR10 pseudogene, partial sequence; C-type lectin natural killer cell receptor-like protein gene, complete sequence; and transposon TX1-like ORF2 pseudogene, partial sequence	82%	117/143
602	739	NONE		
205	182	PREDICTED: Astyanax mexicanus activating transcription factor 7-interacting protein 1-like (LOC103025890), transcript variant X2, mRNA	77%	394/899
177	436	Ictalurus punctatus immunoglobulin heavy chain gene locus, partial sequence and unknown gene	83%	175/211
618	252	Zebrafish DNA sequence from clone DKEYP-3B12 in linkage group 4, complete sequence	98%	54/55
162	217	NONE		
688				
382	89			
408				
109				
488	548	NONE		

428	341	Zebrafish DNA sequence from clone CH211-67F13 in linkage group 13, complete sequence	81%	119/147
388	398	Ictalurus punctatus macrophage migration inhibitory factor (MIF) gene, complete cds	84%	265/317
183				
110	127	Zebrafish DNA sequence from clone DKEY-54I3 in linkage group 4, complete sequence	100%	84/84
153	658	Salmo salar clone BAC CHORI214-439H13 genomic sequence	100%	44/44
562				
326				
71	232	PREDICTED: Astyanax mexicanus uncharacterized LOC103034921 (LOC103034921), mRNA	88%	192/218
355	509	NONE		
328	453	Zebrafish DNA sequence from clone CH211-11P18 in linkage group 22, complete sequence	83%	139/168
596	344	Zebrafish DNA sequence from clone DKEY-31A1 in linkage group 4, complete sequence	97%	36/37
759				
319	111	Zebrafish DNA sequence from clone DKEY-265E15 in linkage group 4, complete sequence	96%	79/82
276				
289				
239	818	Ictalurus punctatus TLR20-1 and TLR20-2 genes, complete cds	92%	100/109
451				
80	322	Drosophila melanogaster chromosome 2R	100%	78/78
503	897			
282	763	NONE		
764				
198	432	NONE		
204	664	PREDICTED: Astyanax mexicanus scavenger receptor cysteine-rich type 1 protein M130-like (LOC103031577), mRNA	87%	284/325
233	602	PREDICTED: Stegastes partitus uncharacterized LOC103357563 (LOC103357563), ncRNA	85%	382/449
470				

768	544	Tachysurus fulvidraco clone yc-IgL3-1 immunoglobulin light chain 3 mRNA, partial cds	95%	433/456
760				
374	861	PREDICTED: Astyanax mexicanus NACHT, LRR and PYD domains-containing protein 12-like (LOC103044598), mRNA	85%	64/75
404	837	Zebrafish DNA sequence from clone DKEY-14K21 in linkage group 23, complete sequence	91%	52/57
483	594	NONE		
95	42	NONE		
397				
125	254	NONE		
83	935	NONE		
311	836	Cynoglossus semilaevis clone Newcyse80 microsatellite sequence	77%	483/629
381	785	NONE		
92	293	NONE		

Example of DNA sequence comparison of the 18 samples analyzed with primer set #2

Area sequenced	70	80	90	100	110	120
Reference Sequence	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC GACTCGCAGTAGATGGAGCAAGTTTTGAACAAAAAAGTTCGGTCTGGTCATCTTGTTAG					
Top strand	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
1N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
2N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
3N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
4N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
5N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
6N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
7N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
8N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
9N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
10N	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
11T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
12T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
13T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
14T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
15T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
16T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
17T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					
18T	CTGAGCGTCATCTACCTCGTTCAAACCTTGTTTTTCAAGGCAAGACCAGTAGAACAATC					

