IDENTIFICATION OF A RESPONSIVE GENE SET TO EVALUATE THE POTENTIAL IMPACT OF SEISMIC EXPOSURE ON ATLANTIC SALMON (*Salmo salar*) INNER EAR

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Identification of a responsive gene set to evaluate the potential impact of seismic exposure on Atlantic salmon (*Salmo salar*) inner ear

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Abstract

Considerable interest and controversy has arisen over the potential effects of seismic surveys carried out during exploration for oil and gas deposits. Regarding fish, there is a concern that intense sound sources, such as seismic airguns, may injure their auditory system. Salmonid cDNA microarrays, reciprocal suppression subtractive hybridization libraries and quantitative reverse transcription – polymerase chain reaction were used to identify and study a responsive gene set in the inner ear of Atlantic salmon (*Salmo salar*) following seismic airgun exposure. Taken together results point to seismic noise exposure altering salmon ear transcripts that likely play important roles in a variety of processes, including sensory perception of sound. Also, initial results demonstrate that genomics has the potential to greatly enhance our understanding of the impact of seismic airguns on gene and molecular pathways involved in hearing, and provide valuable molecular biomarkers that can act as an early warning sensor to acoustic stress.

Keywords: seismic, fish, ear, microarray, expressed sequence tags, genomics

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	*Meaning*
SSH	suppression subtractive hybridization
QPCR	quantitative reverse transcription – polymerase chain reaction
SPL	sound pressure level
µPa	micro Pascal
RMS	root mean square
SEL	sound exposure level
EDS	energy density spectrum
dB	decibel
TTS	temporary threshold shift
PTS	permanent threshold shift
cGRASP	consortium for Genomics Research on All Salmonids Project
Poly(A)+RNA	mRNA
OD	optical density
RQ	relative quantity
MFC	mean fold change
GOI	gene of interest
EST	expressed sequence tag
NCBI	National Center for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
Ct value	cycle threshold
contig	contiguous sequence
GO	gene ontology

1.0 Introduction

Over the past two decades there has been a growing concern over the potential effects of anthropogenic noise in the aquatic environment (Popper 2003; Popper *et al.*, 2004). The most frequently cited sources relevant to aquatic organisms are seismic exploration, shipping, sonar and pile driving (Popper 2003). Concerns range from effects on animal behavior and the ability to communicate (due to increased masking levels) to more substantial temporary or permanent effects such as physiological changes (stress responses), hearing loss, and structural and cellular tissue damage (to auditory and non-auditory tissues), all of which may directly or indirectly lead to immediate or delayed mortality (Popper and Hastings 2009). The extent of biological damage caused by noise exposure depends on the intensity and duration; continuous or repeated exposure to low level noise can be just as detrimental as short-term exposure to loud intense sounds, such as impulse noise (Ward *et al.*, 1981), which is a sudden increase in sound pressure.

The intensity of noise is indicated by sound pressure level (SPL) and is measured in decibel (dB SPL) referenced relative to 1microPascal (μPa) in water, the threshold for human hearing in water. In studies on effects of sound on aquatic organisms, SPL is often measured as peak-to-peak (as in the current study), root-mean-square (RMS), or sound exposure level (SEL), which is the square of the sound pressure level expressed over time (Popper and Hastings, 2009). Other important metrics used to characterize sound include the frequency (Hertz, cycles per second) as well the acoustic particle velocity.

Seismic surveys carried out during exploration for oil and gas deposits have come in for special attention as the high-energy source levels involved have raised concern over their environmental effects and possible effects on commercial fishing operations (McCauley *et al.*, 2000). Seismic surveys involve the deployment of intense sound producing airguns from a survey vessel almost invariably in multiple arrays with airguns being shot simultaneously, such that an array can produce thousands of shots and cover hundreds of square kilometers over a 24 hour period (Payne *et al.*, 2007). Seismic airguns produce a compressed air bubble that collapses

under the pressure of water causing a sharp concussive "explosion" with peak sound levels of individual airguns as high as 250dB relative to (re) 1µPa at a range of 1 meter from the source (Popper *et. al.,* 2005). By comparison, 186dB re 1µPa is about the sound level at which a human listener will feel pain (Popper 2003). Most of the energy released from seismic airguns is at a frequency of 20-150Hz, but can reach levels up to 1000Hz (Hirst and Rodhouse, 2000), which is within the auditory range of most aquatic species (Hastings and Popper, 2005), and so, has the potential to affect the hearing ability of marine mammals as well as most fish species (Popper 2003).

The range of potential effects in fish from intense sound sources, including seismic airguns, includes behavioral and physiological changes, as well as tissue damage (including sensitive sensory organs such as the inner ear) which can lead to immediate or delayed mortality. Available evidence demonstrating immediate mortality in relation to seismic exposure has been limited to fish larvae and eggs (Payne *et al.*, 2004) only observed within a few meters of the source. Effects of seismic exploration on fishing success have also been noted in a few studies indicating potential for temporary or permanent displacement of some fish populations (Skalski *et al.*, 1992; Engas *et al.*, 1996; Engas and Løkkeborg, 2002) which may result in animals leaving feeding or reproduction grounds. Startle responses have also been observed in a few fish species in relation to seismic airgun sounds (Wardle *et al.*, 2001), even to received sounds as low as 160dB (Skalski *et al*, 1992).

There is also evidence of physiological changes in aquatic animals that may be temporary or permanent in relation to seismic sound. An increase in background noise or exposure to a sudden increase in sound pressure (*i.e.* impulse noise) can cause effects on the non-auditory aspects of an animal's physiology, such as an increase in stress levels which can be measured by the concentration of blood variables indicative of an individual's hormonal or neuro-endocrine status (*e.g.*, Hattingh and Petty, 1992). The physiological response to acoustic stress induced by airgun blasts has been investigated in fish (Santulli *et al.*, 1999; Andrews *et al.*, 2007) as well as shellfish (Andrews *et al.*, 2007; Payne *et al.*, 2007). If exposure to sound results in

increased stress levels in fish, even if the fish do not die as a direct result of the sound exposure, they may be more susceptible to predation or other environmental effects than non-stressed fish (Hastings and Popper, 2005).

In addition to non-auditory physiological changes in relation to seismic sound exposure, there is a major concern that intense sound sources have an impact on the auditory receptor cells (*e.g.* hair cells) or structures, (*e.g.* saccule, lagena) involved in fish hearing which may affect communication, detection of predators and prey, and learning about their environments (Hastings and Popper, 2005). Anything in the environment that alters the ability of an organism to detect and/or analyze its auditory scene can potentially have a detrimental impact on the life of an animal as well as reproduction and survival of the species (Popper and Hastings, 2009). Sounds that are well above those to which an animal is normally exposed are known to cause temporary changes in hearing capabilities in fish [temporary threshold shift (TTS)] (Popper and Clarke, 1976; Scholik and Yan, 2001), while exposure to intense sounds or chronic exposure to lower sound levels can produce damage to sensory cells in fish inner ear, which may lead to permanent hearing loss [permanent threshold shift (PTT)] (Enger, 1981; Hastings *et al.*, 1996; McCauley *et al.*, 2003).

Only a few studies to date directly address the effects of airgun noise on fish auditory systems. McCauley *et al.* (2003) reported delayed anatomical damage to auditory sensory cells in caged fish following exposure to multiple emissions from a single 20 cubic-inch airgun; however, the effect on hearing was not measured. Popper *et al.* (2005) observed changes in fish hearing sensitivity (TTS) in two out of three species exposed to five shots from a 730 cubic-inch airgun array in a river seismic survey, while Song *et al.* (2008) showed no evidence for anatomical damage to sensory hair cells in the same study. In a marine seismic survey in Western Australia, Hastings *et al.* (2008) observed no effect on hearing in four species of tropical reef fish following exposure to emissions from a 2,055 cubic-inch seismic airgun array. Comparisons between studies on the effects of seismic noise (and other anthropogenic noise) on fish are complicated by differences in the characteristics in the sound signal (due to different sound sources and water

depth), distance from the source, species-specific diffences in sensitivity, and the limited amount of data available; more comprehensive research is needed.

Current methodologies and strategies in biomonitoring (*e.g.* use of histopathology and immunohistochemistry) are likely to be effective means of detecting a wide range of pathological conditions in relation to intense noise exposure; however, there is a need for a complementary suite of molecular biomarkers which will provide information on the molecular pathways and biological processes altered in response to acoustic over-stimulation, as well as allow the detection of subtle changes that are adaptive or compensatory to exposure. These markers, which may be obtained through gene expression profiling, can therefore act as sensitive, early-warning sensors (Carvan *et al.*, 2008; Falciani *et al.*, 2008).

Within the past decade, development of high-throughput genomic research tools has revolutionized research in areas such as toxicology, aquaculture, fish health and ecology. In particular, microarrays are important genomics research tools as they can be used to reveal the relative expression of thousands of genes simultaneously, thereby allowing the rapid identification of molecular pathways altered in response to environmental stressors. Microarray technology has emerged as a key tool for understanding developmental processes and basic physiology and more recently has become a standard tool in the field of ecotoxicology (Denslow *et al.*, 2007; Carvan *et al.*, 2008). In addition to the use of existing genomic tools, such as microarrays, the development of new resources such as targeted suppression subtractive hybridization (SSH) cDNA libraries can provide a more complete understanding of the molecular processes involved in response to environmental stressors through novel gene discovery (Rise *et al.*, 2007). Genomics research strategies have been used to determine how environmental stressors, such as immunogenic stimuli (*e.g.* pathogens, viral and bacterial antigens), temperature stress and a variety of contaminants of environmental concern impact molecular pathways and biological processes in fish (*e.g.* Rise *et al.* 2004; Rise *et al.*, 2008; Feng *et al.*, 2009; Hori *et al.*, 2010; Rise *et al.*, 2010). If we consider noise to be a 'non-chemical' environmental stressor, the same

technologies can be applied to provide valuable information on molecular mechanisms involved in acoustic stress.

Acoustic over-stimulation has been shown to produce anatomical, biochemical and physiological changes in the inner ear of mammalian systems (*reviewed in* LePrell *et al.*, 2007 and Ohlemiller KK, 2008) as well as anatomical changes in the inner ear of fish (McCauley *et al.*, 2003; Smith *et al.*, 2006; Popper *et al.*, 2007; Song *et al.*, 2008; Shuck and Smith, 2009), however, the changes in gene expression that underlie these biological changes are poorly understood. Subtractive cDNA libraries and differential display (a method based on reverse transcriptase - PCR) have been used to identify genes altered in response to acoustic stimulation in the inner ear of mammals and birds (*e.g.* Gong *et al.*, 1996; Lomax *et al.*, 2000; Lomax *et al.*, 2001; Abe *et al.*, 2003), as well as for gene expression profiling in the inner ear of mammals and birds (Cho *et al.*, 2001, Klockars *et al.*, 2002; *reviewed in* Hildebrand *et al.*, 2007) and zebrafish (*Danio rerio*) (Coimbra *et al.*, 2002; McDermott Jr *et al.*, 2007). Microarray technology has also been used to address the impact of noise induced stress in ear of mammalian and bird species (Lomax *et al.*, 2001; Taggart *et al.*, 2001; Cho *et al.*, 2004; Morris *et al.*, 2005; Kirkegaard *et al.*, 2006; Sun *et al.*, 2008) and more recently has been examined in zebrafish (Schuck *et al.*, 2009; meeting abstract). Using microarray technology and validation by quantitative reverse transcription-PCR (QPCR), Schuck *et al.* (2009) demonstrated differential regulation of transcripts encoding genes known to be important in biological processes such as proliferation and differentiation in zebrafish inner ear following acoustic over-exposure to a pure tone. To date, a global gene expression approach to investigate the potential impact of seismic sound exposure on molecular changes in fish inner ear has not been done. In the current study, genomics research technologies [salmonid cDNA microarray hybridizations complemented by suppression subtractive hybridization (SSH) cDNA library construction and characterization; and validation of selected microarray- and SSH-identified transcripts by QPCR] were used to investigate the effects of seismic sounds on Atlantic salmon (*Salmo salar*) inner ear. Microarray technology in combination with SSH cDNA library construction is an effective approach for studying changes in

gene expression in fish inner ear in response to acoustic stimuli, since microarray analyses allow high-throughput expression studies of known (*i.e.* previously identified) genes while SSH cDNA library development allows for novel targeted gene discovery in fish inner ear in response to seismic noise exposure.

The Atlantic salmon is a prominent model for studies involving environmental toxicology and physiology of stress responses (Rise *et al.*, 2007). Salmonid microarrays contain up to thousands of previously identified salmonid transcripts (expressed gene sequences). The microarray platform used in this study was a 16,006 gene (16K) microarray developed by the consortium for Genomic Research on All Salmon Project (cGRASP) which contains 13,421 Atlantic salmon (*Salmo salar*), 2,576 rainbow trout (*Oncorhynchus mykiss*), 4 chinook salmon (*Oncorhynchus tshawytscha*), 3 rainbow smelt (*Osmerus mordax*) and 2 lake whitefish (*Coregonus clupeaformis*) cDNAs (von Schalburg *et al.*, 2005). The 16K cGRASP microarray has been used in studies of cross-species hybridizations (von Schalburg *et al.*, 2005), growth hormone transgenesis (Rise *et al.*, 2006), immune-relevant gene expression responses to pathogens (Morrison *et al.*, 2006; Workenhe *et al.*, 2009), vaccines (Purcell *et al.*, 2006) and toxicogenomic responses to environmental contaminants (Finne *et al.*, 2007). Identification of a responsive gene set in Atlantic salmon inner ear in response to acoustic stress using the 16K cGRASP microarray platform will provide valuable molecular biomarkers to evaluate the potential impact of seismic exposure on fish.

2.0 Materials and Methods

2.1 Experimental animals

Juvenile Atlantic salmon (*Salmo salar*) smolt were obtained from North Water Products Ltd., Daniel's Harbour, NL and held at ambient seawater temperature, in a flow-through system supplied with air at the Northwest Atlantic Fisheries Centre, St. John's, NL. Due to on-going renovations of the main aquarium facility at the Northwest Atlantic Fisheries Centre, fish were held in a temporary facility at the Northwest Atlantic Fisheries Centre, which was under a

constant daylight regime. Fish were fed three times per week commercial 2mm pellets from EWOS, New Brunswick. Fish (average weight 148g, SE 5.5) were approximately 1 year old at the time of exposure.

2.2 Exposures

Fish were transferred to the newly renovated aquarium facility and divided into two $1m^3$ cages, one each for control (non-exposed) and exposed groups, in a 15,000L aquarium at ambient seawater temperature (0.2°C) and acclimated for two weeks. Each cage was placed the same distance from the water intake, and airstones were placed next to each cage. Fish were fasted for two days prior to exposure. Sixteen control, non-exposed, fish from one cage were sampled prior to seismic activity. Immediately following sampling of control fish, 17 fish in the remaining cage were placed 2m from a $10in^3$ Texas Instruments airgun. Seismic airgun exposures were conducted by Fugro-Jacques Ltd, St. John's, NL and sound metrics were recorded by Oceans Ltd, St. John's, NL. Fish were subjected to 50 exposures, 1 exposure every 10 seconds, at an average SPL of ~204 dB peak-to-peak re 1µPa; considered to be a worse case scenario within a few hundred meters of a survey vessel. Received levels were measured with a Reson Model TC 4014 hydrophone and particle velocity was calculated using the pressure gradient method (Fahy, 1977). Sound metrics are reported in Table 2.1. Hydrophone specifications were as follows: usable frequency range: 15Hz to 480 kHz; horizontal directivity pattern: omni directional; vertical directivity pattern: 270 deg +/- 2dB at 100 kHz; receiving sensitivity: -186dB +/- 3dB; operating depth: 900m. Seventeen seismic exposed fish were sampled 16 h following exposure. Water quality measurements were taken before control fish were sampled and again before seismic airgun exposed fish were sampled to ensure no change in water quality between samplings.

Since there was only one aquarium suitable (large enough) for seismic airgun exposures, it was decided that all fish (control and exposed groups) should be acclimated in the same

Table 2.1 Sound metrics for seismic air gun exposure

Metric	*Mean*	*SE*	*Maximum*	*Minimum*	*Units*
SPL Peak to Peak	204.1	0.1	204.7	203.1	dB re 1µPa
SPL Peak	199.2	0.1	199.9	197.0	dB re 1µPa
SPL RMS	173.5	0.1	174.4	172.0	dB re 1µPa
EDS Max Peak	140.4	0.5	145.3	132.4	dB re 1µPa2/Hz
EDS Peak Frequency	13.6	0.04	13.7	13.2	Hz
Particle Velocity	136.0	0.4	140.1	125.4	dB re 1nm/sec

Metrics were measured 2 meters from a $10in^3$ Texas Instruments air gun using a Reson Model TC 4014 hydrophone. Fish received 50 airgun blasts at a rate of 1 every 10 seconds.

aquarium with the control group sampled just prior to seismic activity. The rationale for this experimental design was to reduce as much as possible the potential for stress responses elicited by handling (dip-netting) and transfer between different aquaria. Separate identical cages were used for control and exposed groups instead of sampling both groups from both cages. This was done to avoid the potential for stress responses in the remaining fish from the seismic exposed group from the attemped dip-net capture of previously sampled fish from the control group. Additionally, the only aquarium available that was suitable for seismic airgun exposures was not designed to have a regulated photoperiod. While not ideal, for this reason the fish were held under a constant daylight regime.

2.3 Sampling

Fish were collected by dip-net and euthanized by severing the spinal cord. Blood samples were taken using a 200µl heparinized capillary tubes (Fisher Scientific, Cat. No. 22-362-566) and placed immediately on ice. Blood samples were centrifuged at 2000 x *g* for 4 minutes. Plasma was removed, placed in fresh RNAse and DNAse-free 1.5ml microcentrifuge tubes and frozen at -80°C. Fish lengths and weights were recorded (Table 2.2).

Right and left inner ear from each salmon was removed using aseptic techniques, placed immediately in RNase-free 2ml tubes, and then flash frozen in liquid nitrogen. Tissue samples were transferred to the Ocean Sciences Centre, Memorial University of Newfoundland, in a Wharton-Taylor dry shipper chilled with liquid nitrogen. Tissues were held at -80°C in an ultra-low freezer until RNA isolation. All sampling utensils were previously baked at 220°C for 6 hours and cleaned with RNase Away (Invitrogen, Burlington, ON) between samples. Seismic airgun exposures and sampling was carried out in accordance with an Animal Care Protocol (NAFC 2007-02) issued by the Northwest Atlantic Fisheries Centre's Animal Care Committee.

Table 2.2 Summary of Atlantic salmon length and weight

Control Fish			*Exposed Fish*		
Fish ID	*Weight (g)*	*Length (cm)*	*Fish ID*	*Weight (g)*	*Length (cm)*
C1	187	28.0	E1	114	24.0
C2	170	28.0	E2	121	24.5
C3	159	27.0	E3	187	27.5
C4	160	28.5	E4	169	28.0
C5	115	24.0	E5	163	28.0
C6	185	27.0	E6	131	24.5
C7	203	29.0	E7	140	25.5
C8	138	24.5	E8	126	25.0
C9	147	26.0	E9	200	28.0
C10	106	24.0	E10	120	23.5
C11	119	24.0	E11	173	27.5
C12	176	27.5	E12	112	24.0
C13	105	23.0	E13	192	28.5
C14	133	25.0	E14	140	24.5
C15	136	25.5	E15	172	26.5
C16	162	26.5	E16	142	25.5
			E17	74	22.0
Mean	150	26.1	Mean	146	25.7

Fish weight (g) and length (to the nearest 0.5cm) was recorded at time of sacrifice. There was no statistically significant difference in weights and lengths between control and exposed groups, measured using student t-test $P<0.05$.

2.4 Cortisol determination

Plasma cortisol levels were determined using a mammalian enzyme-linked immunosorbent assay (ELISA) kit (NEOGEN CORP, Lexington, KY, U.S.A; Cat. No. 402710) with the manufacturer's instructions. Briefly, plasma samples were thawed on ice then diluted 1:20 in 1X extraction buffer. Then 50 µl of diluted plasma samples and standards (concentration range from 0-10 ng/ml) were added in duplicate to a 96-well microtiter plate followed by 50 µl of enzyme conjugate (except for blank wells). The microtiter plate was sealed and incubated at 37°C with gentle rocking, then washed three times with supplied wash buffer to remove any unbound enzyme conjugate, standard or plasma sample. One-hundred-fifty µl of K-Blue substrate (supplied with the kit) was added to each of the wells followed by incubation at 37°C with gentle rocking. The absorbance was read at 650 nm using a Synergy HT microplate reader (BioTek, Winooski, VT, USA). Percent binding was determined for standards and plotted against the concentration in Sigma-Stat 3.10 (Systat Software Inc., San Jose, CA, USA) to generate a 4-parameter regression. The 4-parameter equation x = (((((104.6+0.1537)/(C4+0.1537))-1)^(1/0.845))*0.3447) was used in Microsoft Excel 2002 (Microsoft Corporation, Redmond, WA, USA) to determine concentration of unknown plasma samples from seismic exposed and control fish. A Student's t-test was performed in Microsoft Excel 2002 to determine statistical difference between groups ($P<0.05$).

2.5 RNA isolation

Total RNA was isolated from individual salmon ears by manual homogenization in TRIzol Reagent (Invitrogen, Burlington, ON), centrifugation through QIAshredders (Qiagen Inc, Mississauga, ON) following manufacturer's instructions, phase separation by addition of chloroform, removal of aqueous phase and precipitation of RNA by addition of isopropanol, pellet washed with 75% ethanol, dried and resuspended in 50 µl nuclease-free water (Invitrogen). RNA quality and quantity (concentration) were assessed by 1.5% agarose gel electrophoresis (to assess genomic DNA contamination and RNA degradation) and NanoDrop (ND-1000; Thermo

Fisher Scientific, Wilmington, DE, USA) spectrophotometry (optical density (OD) measurements to determine RNA concentration and contamination by proteins (OD 260nm/280nm), salts and solvents (OD 260nm/230nm)). Individual total RNA samples (5-17 μg, based on NanoDrop concentration data) were treated with RNase-free DNase set (Qiagen Inc., Mississauga, ON) at room temperature for 10 min to remove any residual genomic DNA, then column purified using RNeasy MinElute Cleanup Kit (Qiagen Inc., Mississauga, ON), following manufacturer's instructions, and eluted with 12 μl nuclease-free water (Invitrogen, Burlington, ON). RNA quality and quantity (concentration) were again assessed by 1.5% agarose gel electrophoresis and NanoDrop spectrophotometry. NanoDrop results are reported in Table 2.3. All samples used in the preparation of Poly(A)+ RNA pools were from high quality total RNA (OD 260nm/280nm > 1.8; sharp 18S/28S ribosomal RNA bands).

2.6 Poly(A)$^+$ RNA preparation

Column purified total RNA pools were prepared from the left and right ears of 12 seismic exposed and 12 control individuals, (individuals providing ≥5.0 μg column purified total RNA were selected for each pool). Each sample contributed 4.0 μg column purified total RNA to seismic and control pools. Poly(A)$^+$ RNA (mRNA) was isolated from 48.0 μg total RNA pools using MicroPoly (A) Purist Small Scale mRNA Purification Kit (Ambion Inc., Austin TX; Cat. No. 1919) following manufacturer's instructions. Poly(A)$^+$ RNA yield from the left control and seismic exposed ear was 0.8 μg (1.7%) and 0.9 μg (1.9%), respectively, and from the right control and seismic exposed ear was 0.8 μg (1.7%) for each pool.

2.7 Microarray hybridization

Microarray experiments were designed to comply with Minimum Information About a Microarray Experiment (MIAME) guidelines (Brazma *et al.* 2001). Salmonid cDNA microarrays [GRASP16K batch number GG003 (slide numbers GG003-011, GG003-012, GG003-013 and GG003-014)] were purchased from Dr. B.F. Koop (consortium for Genomic Research on All

Table 2.3 Summary of RNA quality/quantity

	Right ear (microarray study)				*Left ear (SSH cDNA library construction)*			
Fish ID	*ear weight (mg)*	*Purified total RNA (ug)*	*RNA 260/280*	*[RNA] (ng/ul)*	*ear weight (mg)*	*Purified total RNA (ug)*	*RNA 260/280*	*[RNA] (ng/ul)*
C1					32	9.0	2.17	1003.3
C2	32	5.0	2.19	558.7				
C3	42	7.3	2.18	809.7	36	6.3	2.18	703.6
C4	31	5.8	2.20	648.0	35	5.2	2.20	580.7
C5	31	6.1	2.18	673.3	35	6.6	2.19	734.6
C6	41	7.2	2.20	796.5	46	8.0	2.19	890.9
C7	40	7.7	2.19	856.2				
C8								
C9					33	6.6	2.14	733.1
C10	26	7.2	2.13	803.9	34	5.0	2.16	552.5
C11	32	7.4	2.12	821.2	38	5.2	2.13	576.1
C12	39	6.2	2.11	688.2				
C13	33	5.4	2.11	604.3	29	6.9	2.12	798.1
C14	36	6.6	2.12	762.5	38	5.5	2.13	643.0
C15	44	6.5	2.12	753.5	50	7.5	2.13	871.3
C16					46	6.5	2.13	752.1
E1	39	6.5	2.18	726.3	41	7.0	2.20	778.1
E2	32	6.9	2.16	771.9				
E3	39	7.5	2.18	832.9	39	8.1	2.18	898.4
E4	49	7.8	2.19	861.3	41	8.2	2.17	907.2
E5	41	8.4	2.18	928.5	37	7.3	2.20	811.7
E6	33	5.3	2.18	590.3	39	6.0	2.18	666.5
E7					50	6.6	2.21	734.7
E8	35	6.0	2.13	664.3	42	6.0	2.20	662.9
E9					29	7.9	2.13	877.3
E10	48	10.0	2.14	1111.1	34	6.8	2.12	755.2
E11	43	7.4	2.12	822.1				
E12	47	8.1	2.12	936.5				
E13	46	6.4	2.15	746.3	53	10.9	2.12	1268.6
E14					39	6.5	2.15	725.9
E15	48	7.4	2.13	826.5	40	7.8	2.12	904.8
E16								
E17								

Individual data are listed for ear weight and quality/quantity measured for total RNA. RNA quality/quantity data are reported for column purified, DNase treated, total RNA. Data for the 12 individuals used to create pools for mRNA isolation are reported, with a 'blank space' indicating individuals that did not contribute to the pool. The right inner ear was used in the microarray study while the left inner ear was used to construct SSH cDNA libraries.

Salmonids Project, University of Victoria, BC, Canada; http://web.uvic.ca/grasp/microarray/array.html). Microarray fabrication and quality control has been previously described (von Schalburg *et al.*, 2005). The array contains 13,421 Atlantic salmon and 2,576 rainbow trout cDNAs (von Schalburg *et.* al., 2005). Microarray experimental design involved comparison of salmon right inner ear pooled mRNA templates from seismic exposed and control fish (n=12 individuals per pool), and each individual fish contributed an equal quantity of high-quality mRNA to the pool (Figure 2.1). Individuals (n=12) were chosen based on sufficient quantity of mRNA to contribute equally to the pool of mRNA, and quality as assessed by NanoDrop spectrophotometry and agarose gel electrophoresis. Since the microarray experiment used pooled mRNA samples, it did not provide information on biological variability of expression of informative genes. However, individual fish total RNA samples contributing to the pools were archived at -80°C and used as templates in the quantitative reverse transcription-PCR (QPCR) validation of a selection of microarray-identified genes. The QPCR data revealed biological variability of expression levels for transcripts validated in this manner.

16K cGRASP microarrays contain 5, 280 bp green fluorescent protein (GFP) cDNA spots printed in each of the 48 sub-grids: one in each the top left, bottom left, and bottom right corners, and two diagonal spots in the top right corner,to assist in gridding. A GFP-spike was used in this study due to uncertainty of the number of transcripts from inner ear tissue that would hybridize to the arrays. GFP plasmid was received from Dr. B.F. Koop (consortium for Genomic Research on All Salmonids Project, University of Victoria, BC, Canada) and a GFP template (280 bp product) was prepared by J. Hall (Memorial University of Newfoundland, Ocean Sciences Centre). GFP template was labeled with Cy5 using a Random Primed DNA Labeling Kit and instructions (Roche Diagnostics, Cat. No. 11 004 760 001). The labeling procedure was performed under low light due to the sensitivity of the Cy5 fluor. Briefly, GFP template (50 ng) was added to nuclease-free water (Invitrogen) to a final volume of 20 µl, heated to 95°C for 10 min then snap cooled on ice for 1 min. The labeling reaction was carried out in 2 µl of 0.5 mM each of dATP, dGTP and dTTP (0.025 mM), 4 µl of Reaction Mixture containing hexanucleotide primers (vial 6 Random Primed

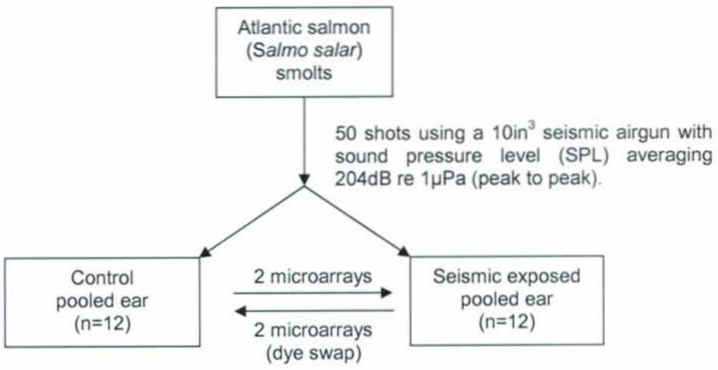


Figure 2.1 Overview of microarray experimental design. Arrows between samples indicate microarrays with the base of each arrow on the Cy3-labeled target and the arrowhead on the Cy5-labeled target. Numbers of technical replicate microarray slides are indicated next to arrows.

DNA Labeling Kit, Roche Diagnostics), 10 µl of 1.0 mM Cy5 labeled dCTP (0.25 mM) (Perkin Elmer, Cat. No. NEL557) and 2 µl of 2U/µl Klenow enzyme (0.1U/µl) (vial 7 Random Primed DNA Labeling Kit, Roche Diagnostics), incubated at 37°C for 45 min then stopped with 4 µl of 0.2 M EDTA. (0.02M) Cy5-labeled GFP was column purified using QiaQuick PCR Purification Kit following manufacturer's instructions (Qiagen Inc, Mississauga, ON) and was later used in the cDNA hybridization step.

Microarrays were prepared for hybridization by washing twice with 0.1% SDS for 5 minutes each, twice in nuclease-free water (Invitrogen) for 5 minutes each and once in nuclease-free water (Invitrogen) for 3 minutes, with gentle agitation in 50 ml sterile conical tubes, followed by a 3 minute dip in 95°C nuclease-free water (Invitrogen), and dried by centrifugation (2000 rpm, 5 min, in a 50 ml sterile conical tube with a Kimwipe stuffed into the bottom). Arrays were placed in a slide box and left in a hybridization oven at 48°C until ready for use.

Microarray hybridizations were performed using the 3DNA Array 900 Detection Kit and instructions (Genisphere Inc., Hatfield, PA). The Array 900 instruction manual, which includes an explanation of the chemistry involved in the Array 900 labeling system, is available online at http://www.genisphere.com/pdf/Array900_10-02-06.pdf. Briefly, 50 ng mRNA from right inner ear of seismic exposed or control fish (n=12 individuals per pool) were reverse transcribed (4 reactions for each treatment) using oligo d(T) primers with unique 5-prime sequence overhangs for the Cy3 and Cy5 labeling reactions. To two of the seismic exposed ear mRNA pools and two of the control ear mRNA pools, 5 pmole reverse transcription (RT) primer with 3DNA capture sequence specific for Cy3 Capture Reagent was added. To the remaining two mRNA samples from seismic exposed and control non-exposed ear, 5 pmole RT primer with 3DNA capture sequence specific for Cy5 Capture Reagent was added. Nuclease-free water (Invitrogen) was added to each RNA-RT primer mix to make 6 µl total volume. Each RNA-RT primer mix was heated to 80°C for 5 minutes in a thermal cycler (Tetrad, BioRad, Hercules, CA) with a heated lid and immediately transferred to ice. Each RNA-RT primer mix was reverse transcribed in 50 mM Tris-HCl, pH 8.3; 70 mM KCl; 3 mM $MgCl_2$, 10 mM DDT, 0.5 mM each of dATP, dCTP, dGTP and

dTTP, 0.5 µl (10 Units) Superase-In RNase inhibitor (vial 4 Genisphere Array 900 Kit) and 100 Units Superscript II (Invitrogen, Burlington, ON). Reactions were incubated at 42°C for 2.5 hours in a hybridization oven. Following cDNA synthesis each reaction was stopped by addition of 2 µl of 0.5 M NaOH/50 mM EDTA then incubated at 65°C for 10 minutes in a thermal cycler (Tetrad, BioRad, Hercules, CA) to denature DNA/RNA hybrids and RNA. To neutralize the reaction, 2.4 µl of 1M Tris-HCl, pH 7.5 was added, giving a total volume of 14.9 µl. Each of the two cDNA targets was prepared for hybridization by mixing 14.9 µl of Cy3 labeled seismic exposed ear cDNA with 14.9 µl of Cy5 labeled control non-exposed ear cDNA (technical replicates). Each of the two dye swaps was prepared by mixing 14.9 µl of Cy5 labeled seismic exposed ear cDNA with 14.9 µl of Cy3 labeled control non-exposed ear cDNA (see Figure 2.1 for an overview on the 4 separate pooled targets that were prepared). To each cDNA hybridization mix, 2 µl of LNA dT blocker (vial 9 Genisphere Array 900 kit), 3 µl of GFP spike and 35 µl of 2X formamide-based hybridization buffer (vial 7 Genisphere Array 900 Kit) were added and the reaction mixtures were incubated at 80°C for 10 minutes in a thermal cycler (Tetrad, BioRad, Hercules, CA) with a heated lid. The cDNA hybridization mixtures were placed in a 48°C hybridization oven until applied to the arrays.

Microarray hybridizations were performed under low light due to the light sensitivity of the fluorescent Cy3 and Cy5 fluors. The cDNA targets (60 µl) were hybridized to the pre-warmed arrays by application of 22x60mm HybriSlip coverslips (Grace Biolabs, Bend, OR, USA), in microarray hybridization chambers (Corning, Corning, NY, USA) in a 50°C water bath for 16 hours in the dark. Coverslips were floated off the arrays in 49°C, 2XSSC, 0.2% SDS prepared in nuclease-free water (Invitrogen). Arrays were then washed once in 49°C, 2X SSC, 0.2% SDS for 15 minutes, then at room temperature 2X SSC for 15 min and finally 0.2X SSC for 15 minutes then dried by centrifugation (2000 rpm for 5minutes) at room temperature. Arrays were incubated at 48°C until application of 3DNA capture reagent. The 3DNA capture reagents were thawed in the dark for 20 min, vortexed well to break up aggregates, placed at 50°C for 10 minutes and vortexed well before use. Formamide buffer (vial 7 Genisphere Array 900 kit) was also pre-warmed to 50°C and gently mixed prior to use. The 3DNA capture hybridization mixture was

prepared by addition of 25 µl nuclease-free water (Invitrogen) and 2.5 µl of each of the Cy3 and Cy5 capture reagents (vial 1 Genisphere Array 900 Kit), to 30 µl warmed formamide buffer (vial 7 Genisphere Array 900 Kit). The mixtures were kept in a 48°C hybridization oven until use. 3DNA capture hybridization mixture (57 µl) was added to each array and left to hybridize at 50°C for 4 hours in the dark. Coverslips were floated off in 49°C, 2X SSC, 0.2% SDS buffer. Arrays were washed in 49°C, 2X SSC, 0.2% SDS buffer for 15 minutes, then at room temperature in 2X SSC buffer for 15 minutes, then 0.2X SSC buffer for 15 minutes and dried by centrifugation (2000 rpm for 5 minutes) at room temperature. Slides were placed in a dark slide case until scanning.

2.8 Data extraction and analyses

Fluorescent images of hybridized arrays were acquired immediately using a ScanArray GX Plus Microarray Scanner (Perkin Elmer, Wellesley, MA, USA) at 10 µm resolution using ScanArray Express software (Perkin Elmer, Wellesley, MA, USA). The Cy3 and Cy5 cyanine fluors were excited at 543 nm and 633 nm, respectively, using 90% laser power and photomultiplier tube (PMT) settings set at PMT 75 for Cy5 and PMT 78 for Cy3. Fluorescent intensity data (*i.e.* numerical data) were extracted from TIFF image data using ImaGene 5.5 software (BioDiscovery, El Segundo, CA, USA). Quality statistics for salmonid features are compiled in Table 2.4.

Extracted data was imported into GeneSpring GX version 7.3.1 (Silicon Genetics, Agilent Technologies, Palo Alto, CA, USA) for data transformation (background correction, and setting background values <0.01 to 0.01), normalization (Lowess), and analysis (formation and comparison of fold change gene lists through generation of scatterplots and Venn diagrams). A scatterplot showing the background corrected Lowess normalized (BCLN) data from one microarray (slide number GG003-014 with ear from seismic exposed fish labeled Cy-5 and ear from control non-exposed fish labeled Cy-3) is shown in Figure 2.2. Transcripts greater than 1.75-fold up-regulated in seismic exposed ear compared to control ear (S/C) and transcripts greater than 1.75-fold down-regulated in seismic exposed ear compared to control ear (C/S) are in red

Table 2.4 Summary of quality statistics for microarray raw data for individual slides

Slide ID	*Signal*		*Background*		*Signal/ background*		*Mean background + 3 SD*		*# Spots passing threshold*		*% Spots passing threshold*	
	Cy5	*Cy3*	*Cy5*	*Cy3*	*Cy5*	*Cy3*	*Cy5*	*Cy3*	*Cy5*	*Cy3*	*Cy5*	*Cy3*
GG003_011	1270	1021	289	10	4.4	101	476	93	10411	15194	65%	95%
GG003_012	1872	1309	340	21	5.5	63	828	188	6069	14583	38%	91%
GG003_013	1814	1245	352	29	5.2	43	1031	401	4397	10427	27%	65%
GG003_014	1560	1110	337	22	4.6	51	697	195	6763	13906	42%	87%
Mean	1629	1171	330	21	4.9	65	758	219	6910	13528	43%	84%
SE	138	65	14	4	0.3	13	116	65	1268	1066		

Raw data presented are for salmonid spots from each slide in the microarray study. The 'Mean raw background + 3SD' was used to set thresholds to exclude lower quality data. The number and percentage of the spots passing set thresholds are reported.

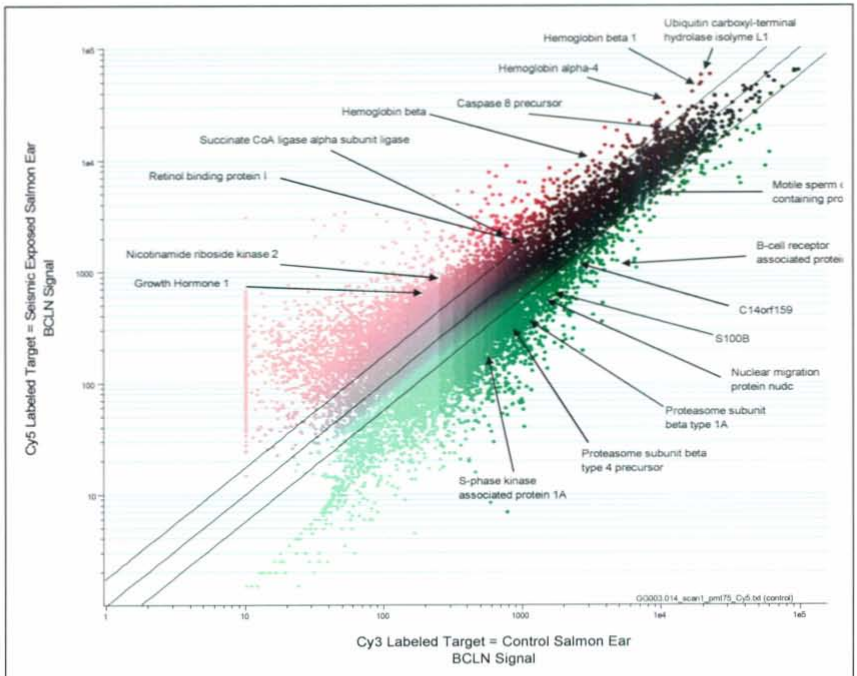


Figure 2.2 Scatterplot of genes differentially regulated greater than 1.75-fold from 1 slide in microarray study

Scatterplot of microarray slide GG003_014 showing Cy5 background corrected Lowess normalized signal / Cy3 background corrected Lowess normalized signal ratio for genes greater than 1.75-fold up-regulated (red spots) and genes greater than 1.75-fold down-regulated (green spots) in seismic exposed salmon ear relative to control salmon ear. Relative expression is represented by increasing color intensity of spots for Cy5 (red) and Cy3 (green). Randomly selected genes differentially regulated greater than 1.75-fold in response to seismic noise exposure are shown to demonstrate how scatterplots are used to create gene lists (see Materials and Methods).

and green, respectively, in the scatterplot shown. Gene lists were prepared from transcripts with greater than 1.75-fold difference in expression in BCLN signal between the two treatments for each microarray, and Venn diagrams were used to identify reproducibly informative transcripts on at least three of the four microarrays in the study (including at least one dye swap). Gene lists were compiled (containing information about BCLN signal intensities in each channel, quality flags and Cy5/Cy3 ratios) were copied and pasted into Excel for each slide. Thresholds were set for each slide by calculating the mean of the median local background values plus three standard deviations for all spots in the dominant channel using raw data generated from ImaGene. In the "up-regulated in seismic compared to control" gene list, seismic is the "dominant channel" (*i.e.* the channel with the higher transcript expression); in the "down-regulated in seismic compared to control" gene list, control is the "dominant channel". Genes differentially regulated greater than 1.75-fold in at least three slides of the study (including at least one dye-swap) with BCLN signal above threshold on all 4 slides were selected as high trust data.

Expressed sequence tags (ESTs) obtained from GenBank accession numbers of reproducibly informative transcripts were submitted to the National Centre for Biotechnology and Information (NCBI) Basic Local Alignment Search Tool (BLAST) server for annotation using the BLASTX (against the non-redundant protein library) and BLASTN (against the non-redundant nucleotide library) databases. The data were sorted first by functional classification (based on gene ontology biological process or molecular function terms of best Blast hit or related sequences (*e.g.* putative human orthologues or putative function), then by decreasing mean fold change for genes up-regulated greater than 1.75-fold and genes down-regulated greater than 1.75-fold on at least 3 of the 4 slides in the study. A list of individual slide data and complete functional annotation for reproducibly informative genes differentially regulated greater than 1.75-fold in at least 3 slides of the study are reported in Supplemental Tables S1 and S2 (Appendices 7.1 and 7.2).

2.9 Quantitative reverse transcription – polymerase chain reaction (QPCR)

Validation of selected genes from the microarray experimental results was conducted using QPCR. Eight genes of interest (GOI) greater than 1.75-fold differentially expressed in seismic noise exposed and control ear (growth hormone I, nicotinamide riboside kinase 2, c-type lectin receptor A, retinol binding protein I cellular, caspase-8 precursor, hemoglobin subunit alpha-4, C14orf159 protein, and proteasome subunit beta type-4 precursor) were subjected to QPCR using two PCR primers (forward and reverse) per GOI, SYBR Green I dye chemistry and 7500 Fast Real-Time PCR System (Applied Biosystems, Foster, CA, USA). At least two QPCR primer pairs were designed for each GOI from EST FASTA files using Primer3 (from the Whitehead Institute for Biomedical Research, http://frodo.wi.mit.edu) and Primer Express software (Applied Biosystems) with the following guidelines: product size 80-200 bp, T_m 60°C ± 1°C , at least two of the six 3' terminal bases G/C. The primer pair chosen for each GOI was determined to have a single peak in the dissociation curve, no primer dimer product in the no-template control and the best amplification efficiency (*i.e.* closest to 100%) of the primer sets tested. Amplification efficiency (Pfaffl, 2001) was calculated for both experimental and control samples, with the reported value being the average of the two. Standard curves were generated using a 5-point 1:4 dilution series starting with cDNA corresponding to 10 ng of input total RNA, with >85.9% efficiency for all primer pairs for relative quantification by the 7500 Fast Real Time PCR System Software Relative Quantification Study Application (Applied Biosystems, Foster City, CA, USA).

All individual RNA samples contributing to pools for seismic exposed and control ear were quantified by QPCR. 60S ribosomal L6 RNA was selected as a normalizer gene, since the MFC was within 1.2-fold on all 4 microarrays and its expression was stable (within ± 1 Ct value) for all individuals tested by QPCR. Total RNA was prepared as previously described using TRIzol reagent and methods (Invitrogen). For each individual, first-strand cDNA was synthesized from 1 µg of DNase treated, column cleaned, high-quality total RNA (defined as having an OD

260nm/280nm ratio > 1.8 and sharp 18S/28S ribosomal bands), using 250 ng of random primers (Invitrogen, Cat. No. 48910-011), 1 µl of dNTPs (10 mM each) (Invitrogen, Cat. No. 10297-018) and nuclease-free water (Invitrogen) to a final volume of 13 µl, then incubated at 65°C for 5 min in a thermal cycler (Tetrad, BioRad, Hercules, CA) and chilled on ice for 2 min. To the 13 µl reaction mixture, 4 µl of 5x First Strand Buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 2 µl of DDT (0.1 M) and 200 Units of Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (supplied together by Invitrogen, Cat. No. 28025-013) were added. Thermal cycling parameters for reverse transcription reactions were as follows: 25°C for 10 min, 37°C for 50 min, and 70°C for 15 min. First strand cDNAs were diluted 10-fold in nuclease-free water (Invitrogen) and used as templates for QPCR. PCR amplification for each individual was performed in duplicate with the Applied Biosystems 7500 Fast Real-Time PCR System in a 13 µl reaction using 2 µl cDNA (corresponding to 10 ng of input total RNA), 50 nM each of forward and reverse primer and 1x Fast SYBR Green Master Mix (Applied Biosystems) and expression levels were normalized to 60S ribosomal L6 RNA. The real-time analysis program consisted of 1 cycle of 95°C for 20 s, 40 cycles of 95°C for 3 s, and 60°C for 30 s, with fluorescence detection at the end of each 60°C step. For each GOI, the individual sample with the lowest expression (highest normalized Ct value) was set as the calibrator. Gene expression data are presented as mean relative quantity (RQ) values (mean Ct value relative to the calibrator) ± standard error. Overall mean-fold change (MFC) was calculated as (average RQ seismic exposed group) / (average RQ control non-exposed group). Sigma-Stat 3.10 (Systat Software Inc., San Jose, CA, USA) was used to determine significant difference in gene expression (with a *P*-value threshold of 0.05) in ear from seismic exposed salmon compared to control non-exposed salmon. GOIs, QPCR primer sequences and amplification efficiencies are shown in Table 2.5.

2.10 SSH cDNA library construction

Reciprocal suppression subtractive hybridization (SSH) cDNA library construction from Poly(A)$^+$ RNA pools generated from left inner ear of seismic exposed and control salmon

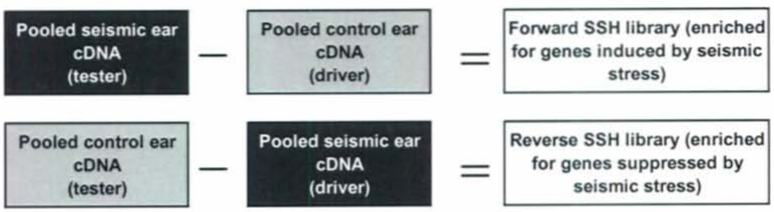
Table 2.5 Primers used in quantitative reverse transcription – polymerase chain reaction (QPCR)

EST	*Gene name of best BLASTX or BLASTN Hit*	*QPCR primer sequences*	*Amplicon length (bp)*	*Efficiency (%)*
CA045755	Growth hormone I	F 5'GAAACCGAACCCTGGAGACA3' R 5'CGCCAAGTGCAGGAAGTCA3'	94	91.6
CB492469	Nicotinamide riboside kinase 2	F 5'ATAGCGTTGGTTCAGTACGTCGAT3' R 5'GGAGTCTGACCACAGGGATGA3'	100	89.3
CA056108	C type lectin receptor A	F 5'CAGAAGGTGCCAGATTTGCT3' R 5'TGGGACAGGAGAGGTTAAGG3'	146	108.5
CA058654	Retinol-binding protein I, cellular	F 5'ATCGTTCCCAGTGGTTTCAG3' R 5'GAGGGAAGAGGATGGACACA3'	118	97.1
CA060239	Caspase-8 precursor	F 5'CCCAGTATCGGACATCCATC3' R 5'GGCCACTGTTGAAGACCACT3'	167	85.9
CB515375	Hemoglobin subunit alpha-4	F 5'CTTCTTCACTGGAGCGGAAC3' R 5'AACGGACAAGACGCAGCTAT3'	195	96.7
CB517114	C14orf159 protein	F 5'AGCAACGGCATGGTATTTTC3' R 5'CCTTTCACCCCACACACTCT3'	151	102.3
CB512698	Proteasome subunit beta type-4 precursor	F 5'CAGGAAGCTCTCGCCATTGT3' R 5'GTCACAGCTACAGCCCCAAAG3'	128	97.5
contig 29*	Cytosolic non-specific dipeptidase	F 5'GGCGTTACCCTACCGTTACC3' R 5'GGACCTGTCGGATGGAGAAC3'	111	101.0
CA060895	60S Ribosomal L6	F 5'AAGCAGGAGACCACTGGAGA3' R 5'ATGTGGCCCGTAAACTGAAG3'	164	91.4

* In addition to the above microarray-selected transcripts, contig 29 (cytosolic non-specific dipeptidase) was selected from the forward SSH cDNA library for validation by QPCR.

(0.53 µg per pool) was performed using the CLONTECH PCR_Select cDNA Subtraction Kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions with the first hybridization performed at 68°C for ~8 h and the second hybridization at 68°C for ~16 h. Seismic exposed fish were the tester in the forward subtraction (*i.e.* enriched for ear transcripts that were up-regulated by seismic sound exposure) and the driver in the reverse subtraction (*i.e.* enriched for ear transcripts that were suppressed by seismic sound exposure); control fish were the driver in the forward subtraction and the tester in the reverse subtraction (Figure 2.3). The resulting forward and reverse SSH cDNA libraries were amplified using the Advantage 2 Polymerase Kit (Clontech, Mountain View, CA, USA) following the manufacturer's instructions, and purified using the MinElute PCR Purification Kit following the manufacturer's instructions (QIAGEN, Mississauga, ON). Higher molecular weight sub-libraries were prepared by electrophoresing the PCR products from forward and reverse SSH cDNA libraries on a 1.5% agarose gel with a 1 kb ladder (Invitrogen, Burlington, ON) as a size marker, followed by excision of the cDNA smear greater than 500 bp and gel purification using the QIAquick Gel Extraction Kit following the manufacturer's instructions (Qiagen, Mississauga, ON). PCR products (total and higher molecular weight >500bp) from forward and reverse SSH cDNA libraries were TA-cloned into pGEM-T Easy Vector following the manufacturer's instructions (Promega, Madison, WI, Cat. No. A1360). Briefly, 25 ng of PCR product was added to 5 µl of 2x Rapid Ligation Buffer T4 DNA Ligase (60 mM Tris-HCl (pH 7.8), 20 mM $MgCl_2$, 20 mM DTT, 2 mM ATP, 10% polyethylene glycol), 1 µl (50 ng) of pGEM-T Easy Vector, 1 µl (3 Units) of T4 DNA Ligase and brought to a final volume of 10 µl using nuclease-free water (Invitrogen), then left at room temperature for ~1 hr. The ligation mixtures were purified by addition of nuclease-free water (Invitrogen) up to 100 µl, 10 µl of 4 M ammonium acetate and 275 µl of 100% ethanol, mixed and left to precipitate for 2.5 h at -20°C. The ligation mixtures were centrifuged at 16,000 x *g* for 1 h at 4°C, the supernatants were carefully removed, then 500 µl of 80% ethanol was added to wash the pellets. The ligation mixtures were then centrifuged at 20,000 x g for 10 min at 4°C. The supernatants were carefully removed and the pellets were left to airdry for 5 min. Five µl of nuclease-free water (Invitrogen)

A. mRNA is isolated and reverse transcribed into cDNA.
B. cDNA from treated and non-treated inner ear are subtracted from one another to create 'forward' and 'reverse' SSH libraries.
C. 'Library' is the term used for collections of DNA fragments.



D. cDNA clones are isolated and sequenced generating expressed sequence tags (ESTs).

Figure 2.3 Overview of reciprocal SSH cDNA library construction
Salmon left inner ear mRNA was used to generate pools from seismic exposed salmon (n=12) and control salmon (n=12), with individuals contributing an equivalent amout of mRNA to each pool. The forward SSH library, using a pooled seismic exposed ear sample as tester and a pooled control ear sample as driver, was designed to be enriched for transcripts that were up-regulated by seismic exposure. The reverse SSH library, using a pooled control ear sample as tester and a pooled seismic exposed ear sample as driver, was designed to be enriched for transcripts that were down-regulated by seismic exposure. Composition of tester and driver for the forward and reverse SSH libraries are shown above.

was added to dissolve the purified ligation mixtures, followed by a 1:4 dilution (1 µl in 4 µl) in TE buffer (10 mM Tris HCl, pH 7.5; 1 mM EDTA). The diluted purified ligation mixtures were stored at -80°C until used in bacterial transformations.

Transformations were performed using ElectroMAX DH5α Electrocompetent *E. coli* Cells (Invitrogen, Burlington, ON). The cells were thawed on ice for ~20 min, and then 50 µl was added to pre-cooled sterile, disposable cuvettes (Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON; Gene Pulser Cuvette 0.1 cm electrode gap, Cat. No. 165-2089) containing 3 µl of the diluted purified ligation mixture from either the forward or reverse SSH cDNA libraries. Cells were electroporated at 1.8 kV, 25 µF, 200 Ω and recovered in 950 µl S.O.C. Medium (supplied with ElectroMAX DH5α Electrocompetent Cells, Invitrogen) for ~1 h at 37°C shaking at 225 rpm in a Max Q Mini 4450 Digital Benchtop Incubated Orbital Shaker. Transformed cells were plated on pre-warmed LB/agar plates (37°C) containing 100 µg/L ampicillin and 40 µl of 2 µg/L x-gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) prepared in N,N-dimethylformamide (ACROS Organics, Thermo Fisher Scientific, New Jersey, USA), and then incubated overnight (~16 h) at 37°C. Remaining transformed cells were transferred to sterile 1.5 ml microcentrifuge tubes, diluted 1:1 in 30% glycerol / S.O.C. Medium (Invitrogen), and aliquots were stored at -80°C.

2.11 DNA sequencing, sequence assembly, and gene identification

Individual bacterial clones from white colonies (containing insert DNA) were inoculated into 1.1 ml LB broth containing 100 µg/L ampicillin in 96-well growth plates (Whatman Cat. No. 7701-5205) followed by incubation at 37°C overnight. In a 96-well microtitre plate, 100 µl of overnight culture was added to 100 µl LB broth / ampicillin / 30% glycerol, sealed and stored at -80°C. cDNA was isolated and purified from the remaining 1.0 ml inoculation broth by: (1) overnight cultures were centrifuged at 1800 x *g* for 10 min, and the supernatant discarded; (2) added 100 µl of 0.05M Tris/HCl, 0.01M EDTA pH7.5, 50 µg/ml RNaseH to each pellet, tubes were sealed and vortexed at moderate speed for 5 min; (3) added 100 µl of 0.2N NaOH, 1% SDS to resuspended pellets, tubes were sealed and gently vortexed for 2 min; (4) added 100 µl of

3.0M KOAc pH5.5 and tubes were gently vortexed for 2 min; (5) lysates were transferred to a 96-well clarification plate (Whatman Cat. No. 7720-2830) secured by adhesive to a 96-well binding/recovery plate (Whatman Cat. No. 7701-1800) with wells pre-filled with 225 µl isopropanol and centrifuged at 2000 rpm for 1 min; (6) clarification plate was removed and discarded, the binding/recovery plate was sealed with aluminum sealing film (Corning, Lowell, MA, USA), inverted several times, then centrifuged at 2250 x *g* for 30 min at room temperature; (7) isopropanol was discarded and pellets were washed with 200 µl of 80% ethanol, centrifuged upside-down at 400 rpm for 15 s at room temperature on a stack of Kimwipes; (8) plates were inverted at a 45° angle on the bench to allow pellets to dry for 1 hr; (9) 50 µl nuclease-free water (Invitrogen) was added to each pellet, the plate was sealed, vortexed for 5 min, centrifuged at 700 rpm for 1 min, then left at 4°C overnight to let the pellets fully dissolve. Initial evaluation of library insert size and complexity was made by comparison of 16 clone restriction fragments from each library using 1.5% agarose gel electrophoresis with a DNA size marker (1 kb ladder; Invitrogen, Burlington, ON). Assessment of cDNA concentration and quality were determined for 18 randomly chosen clones per plate using Nanodrop spectrophotometry, to obtain a general idea of cDNA concentration and purity (assessed by the OD ratio of 260nm/280nm) for the sequencing reactions.

DNA from each clone was amplified using 0.5 µl of sequencing mix (AmpliTaq DNA polymerase, $MgCl_2$, dNTPs and dye terminators) and 2 µl of 5x sequencing buffer (both supplied in Applied Biosystems BigDye® Terminator kit version 3.1), 2 µl of 1.6 pmol/µl T7 primer, 5 µl of DNA template for concentrations less than 100 ng/µl or 3 µl of DNA template for concentrations greater than 100 ng/µl and sufficient nuclease-free water (Invitrogen) for a final volume of 20 µl. Applied Biosystems Gene Amp PCR System 9700 was used to perform cycle sequencing using the following program: 96°C for 6 min then (96°C for 10 s, 50°C for 5 s, and 60°C for 4 min) for 25 cycles. Sequencing reactions were purified to remove excess dye terminator using BigDye® X Terminator Kit (Applied Biosystems) following manufacturer's instructions (CREAIT Network, Memorial University of Newfoundland). Briefly, 20 µl of BigDye X Terminator Solution (Applied

Biosystems) was added to each PCR reaction, followed by 90 µl of SAM Solution (Applied Biosystems), vortexed for 30 min, then centrifuged at 1000 x *g* for 2 min. Sequencing reaction electrophoresis was performed using an Applied Biosystems 3730 DNA Analyzer, detected and converted to electropherograms with DNA Sequencing Analysis software v2.5 (Applied Biosystems Inc., CA, USA).

Sequence information was stored in ABI chromatograph trace files. Sequence data were analyzed using CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA) and base-calling from chromatogram traces was performed by using PHRED (Ewing and Green 1998; Ewing *et al.* 1998). A file containing the adaptor and vector sequences was inserted into CodonCode Aligner and used to trim vector/adaptor sequences. Furthermore, VecScreen was used to detect and then manually remove any residual and partial vector contamination in ESTs. Sequence traces with less than 25 bases, as well as sequence traces with 50 bases having less than Phred 20 quality scores, were automatically discarded. The high-quality, trimmed EST sequences were then used to find overlap assembly of contiguous consensus sequences (contigs) using CodonCode Aligner. First, the "end-to-end alignment" algorithm found potential overlaps between samples by looking for shared 12-nucleotide "words" in the sequence. Then the pair of samples with highest number of shared words was found. Adequate alignments were kept as new contigs, and the consensus sequence calculated; otherwise, the alignment was rejected, and no consensus made. Four criteria were used to determine whether to accept or reject an alignment: (1) minimum percent identity (the minimum percentage of identical bases in the aligned region) ≥70%; (2) minimum overlap length ≥25 bps; (3) minimum alignment score (which takes any mismatches into account) ≥20 bps; and (4) maximum gap size ≤30 bps. Overall, these criteria were relatively relaxed and represent the default setting in Codoncode Aligner. Both contigs and singletons (individual sequence reads) generated in CodonCode Aligner were output in FASTA format and aligned with nonredundant GenBank nucleotide and amino acid sequence databases BLASTN and BLASTX, respectively (Altschul *et al.* 1990). ESTs were compared via BLASTX with annotated protein sequences from the gene ontology (GO) database (August 2009

version; http://www.expasy.ch/sprot/). Sequences with significant matches (*E*-value < 10^{-5}) were classified according to the GO classification(s) of their best (*i.e.* most significant, lowest *E*-value) hits. All EST sequences have been deposited in the GenBank dbEST under accession numbers GT128484-GT129166. Gene name of best BLASTX or BLASTN hit, species and GenBank accession number, *E*-value, percent (%) identity at amino acid or nucleotide level, and contributing number of ESTs are reported for the forward (Table 3.5) and reverse (Table 3.6) SSH cDNA libraries. Identified sequences in Tables 3.5 and 3.6 are listed by functional annotation [based on GO biological process or molecular function terms of best BLAST hit or of related sequences (*e.g.* putative human orthologues), or on reported putative function], then sorted by contributing EST number (*i.e.* contig "depth"). Functional categories in both the forward and reverse SSH cDNA libraries include: DNA binding, transcription, translation; energy metabolism; proteolysis; signaling (including synaptic transmission); ion transport; structural activity; cell death (apoptosis), regeneration (including axonogenesis); post-translational protein modification; stress-response; immune-relevant; otolith development; sensory perception and auditory cell organization; other functions; and unknown and uncharacterized sequences. Complete functional annotations (*i.e.* GO biological process, molecular function and cellular compartment terms and descriptions), sequence lengths and GenBank accession numbers can be found in Supplemental Tables S3 and S4 (Appendices 7.3 and 7.4) for forward and reverse SSH libraries, respectively.

3.0 Results

3.1 Qualitative observations

Qualitative observations made during exposure of animals revealed an initial startle response for approximately the first three airgun blasts, followed by little activity for the remainder of the exposure (~10 minutes; 50 airgun exposures). In addition to the initial startle response, a difference in net-avoidance and swimming speed between control and seismic exposed groups was observed during sampling. Fish from the control group demonstrated little swimming activity

and were therefore very easy to capture, whereas fish from the seismic exposed group demonstrated rapid and erratic swimming activity (compared to controls) during attemped capture.

3.2 Cortisol

Cortisol concentrations measured in individual plasma samples from control salmon and seismic exposed salmon are reported in Table 3.1, with a graphical representation of mean concentrations from control and seismic exposed animals shown in Figure 3.1. The mean plasma cortisol concentration measured in control salmon (n=16) was 68.1 ng/ml (SE 11.5) while the mean plasma cortisol concentration from seismic exposed salmon (n=17) was 77.0 ng/ml (SE 13.8). The mean plasma cortisol concentration was not significantly higher in seismic exposed fish compared to control fish (*P*-value = 0.63).

3.3 Microarray and QPCR

Differential gene expression in seismic exposed and control salmon right ear was determined using a microarray containing 16,006 different cDNAs selected from high complexity, salmonid cDNA libraries (von Schalburg et al., 2005). On the salmonid elements, the average Cy3 signal for all slides was 1171 (SE 65) and the average Cy5 signal for all slides was 1629 (SE 138). The Cy3 mean signal / mean background ratio was 65 (SE 13) and the average Cy5 mean signal / mean background ratio was 4.9 (SE 0.3). A higher signal-to-background ratio noted in Cy3 compared to Cy5 was due to very low background signal in the Cy3 channel. An average of 84% of Cy3-labeled transcripts and 43% of Cy5-labeled transcripts passed the set threshold of 'mean + 3 standard deviations from the mean of the median local background values for all features and were deemed present (see Materials and Methods). A summary of microarray quality statistics for each slide in the study are reported in Table 2.4 (see Materials and Methods).

Table 3.1 Summary of plasma cortisol concentrations

Control Fish		*Exposed Fish*	
Fish ID	*Cortisol (ng/ml)*	*Fish ID*	*Cortisol (ng/ml)*
C1	5.30	E1	35.43
C2	2.70	E2	10.07
C3	28.39	E3	73.29
C4	57.24	E4	6.14
C5	45.82	E5	61.35
C6	77.87	E6	12.97
C7	25.61	E7	4.39
C8	30.49	E8	149.16
C9	81.20	E9	140.03
C10	116.08	E10	117.67
C11	84.76	E11	187.40
C12	142.22	E12	116.08
C13	39.70	E13	25.61
C14	108.67	E14	113.02
C15	146.78	E15	88.59
C16	97.19	E16	117.67
		E17	49.84

Plasma cortisol concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) kit from Neogen Corporation. There was no significant difference between control and exposed groups, measured using students t-test ($P>0.05$).

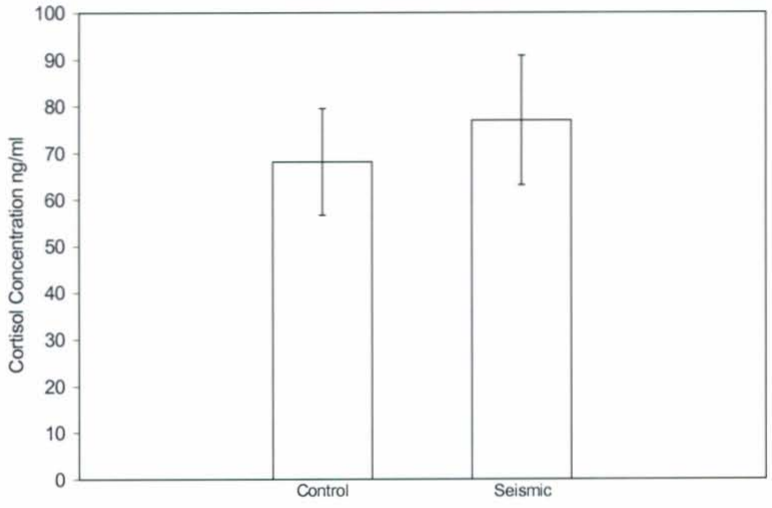


Figure 3.1 Plasma cortisol concentration
Mean cortisol concentration in plasma from control, non-exposed salmon (n=16) and seismic exposed salmon (n=17). Mean fold change in cortisol concentration from seismic exposed fish plasma relative to control, non-exposed fish plasma was 1.13-fold, with a *P*-value = 0.63. Error bars represent standard error from the mean (SE) for each treatment. There was no significant difference between mean concentrations for seismic exposed and control groups.

Atlantic salmon ear transcripts responding reproducibly (as defined in Materials and Methods) to seismic noise exposure are reported in Tables 3.2 and 3.3, and Supplemental Tables S1 and S2 (Appendices 7.1 and 7.2). In the microarray study, 42 different transcripts were reproducibly up-regulated and 37 different transcripts were reproducibly down-regulated >1.75-fold in Atlantic salmon ear following seismic noise exposure. Salmonid cDNAs having significant ($E<10^{-5}$) BLASTX or BLASTN hits against the current GenBank nr or nt databases, as well as unknowns (*i.e.* no significant BLAST hit), are described for reproducibly informative transcripts in response to seismic sound exposure. The degree of similarity (length and percent identity over aligned region) between translation of each salmonid cDNA's expressed sequence tag (EST) and its best (most negative *E*-value) BLASTX hit, or between ESTs and their best BLASTN hits, are also shown and serve to identify potentially informative transcripts. In Tables 3.2 and 3.3, informative transcripts are listed by functional category: DNA/RNA binding, transcription, translation; energy/metabolism; signaling, synaptic transmission; cytoskeleton structure and dynamics; cell cycle, cell death, axonogenesis; protein post-translational modification, degradation and folding; immune response; iron homeostasis; other and unknown. Within each functional category transcripts are listed from highest to lowest mean fold change over all four slides in the study. The mean signal (averaged from all four slides in the study) in the appropriate channel for each reproducibly informative transcript is listed to give an indication of relative abundance; for example, hemoglobin subunit alpha (mean signal 29,332) is a relatively abundant transcript in seismic exposed ear whereas growth hormone 1 (mean signal 712) is a relatively rare transcript in seismic exposed ear (Table 3.2). Multiple entries with the same gene name in an informative transcript list (*i.e.* different microarray features with identical best BLAST hits) represent single genes or closely related paralogs and are indicated by subscripts. The presence of multiple entries of genes in a given informative transcript list provides an internal validation of microarray results. The data from individual microarrays for reproducibly informative transcripts and complete gene ontology are compiled in Supplemental Tables S1-S2 (Appendices 7.1-7.2).

Table 3.2 Transcripts greater than 1.75-fold up-regulated in seismic exposed Atlantic Salmon ear relative to control Atlantic Salmon ear on at least three slides of the study (including at least one dye swap)

EST	*Gene Name of Best BLASTX or BLASTN Hit*	*Acc #*	*Species*	*Align*	*% ID aa or nt*	*E-Value*	*MFC*	*SE*	*Mean Signal*
DNA/RNA binding, transcription, translation									
CA052688	Eukaryotic translation initiation factor 5A-1	BT045750	*Salmo salar*	356/358	99%	0	3.0	1.2	2279
CA041792	Heterogeneous nuclear ribonucleoprotein A0	ACI67551	*Salmo salar*	65/65	100%	1E-31	1.9	0.2	2338
Energy, Metabolism									
CA044613	Succinate-CoA ligase alpha subunit	BT071979	*Salmo salar*	371/424	87%	2E-126	3.0	0.8	2387
CB492469	Nicotinamide riboside kinase 2_2	ACO08546	*Oncorhynchus mykiss*	79/80	98%	4E-41	2.9	0.3	1818
CK991044	Cytochrome c oxidase polypeptide Vic-2	ACI66043	*Salmo salar*	73/76	96%	2E-28	2.9	0.9	6023
CA061344	NADP-dependent malic enzyme	BT058893	*Salmo salar*	148/189	78%	2E-32	2.5	0.8	1202
CB497834	Malate dehydrogenase, cytoplasmic	ACN10417	*Salmo salar*	68/68	100%	2E-32	2.4	0.6	3474
CB505859	Apolipoprotein A-IV precursor	ACI67068	*Salmo salar*	140/144	97%	6E-71	2.1	0.5	3140
CB500653	Adrenodoxin, mitochondrial	BT059432	*Salmo salar*	128/159	80%	6E-33	2.0	0.6	1227
CA047138	NADH dehydrogenase 1 beta subcomplex subunit 1	ACI67195	*Salmo salar*	58/58	100%	1E-26	1.9	0.6	2319
Signalling, Synaptic transmission									
CA045755	Growth hormone I	AAU11453	*Salmo salar*	70/70	100%	7E-35	3.4	0.8	712
CA058330	ADP-ribosylation factor 1	BT045670	*Salmo salar*	597/638	93%	0	2.8	0.9	1769
CA058654	Retinol-binding protein I, cellular	ACI69621	*Salmo salar*	106/108	98%	7E-57	2.3	0.2	2597
CK990828	Transthyretin	CAD66520	*Cyprinus carpio*	89/130	68%	1E-28	2.1	0.6	16616
CB507670	Collagen alpha-2I chain precursor	BT071961	*Salmo salar*	503/575	87%	0	2.1	0.4	1202
CA063898	ADAM metallopeptidase domain 10	BC153863	*Bos taurus*	64/80	80%	2E-08	1.8	0.3	1164
Cytoskeleton structure, Dynamics									
CA050155	Cornin-1A	ACN10578	*Salmo salar*	139/141	98%	5E-55	2.7	0.6	1644
CA055146	Vasodilator-stimulated phosphoprotein	BT047473	*Salmo salar*	461/461	100%	0	1.9	0.1	2741
Cell cycle, cell death, axonogenesis									
CB492165	Histone H1x	BT074265	*Salmo salar*	392/396	98%	0	2.8	0.7	1420
CA060239	Caspase-8 precursor	ACN10768	*Salmo salar*	50/85	58%	1E-18	2.1	0.2	22830
CB517807	Deoxycytidylate deaminase	ACI66682	*Salmo salar*	25/26	96%	1E-06	2.1	0.6	1122
CA062451	catenin beta-1	BT071982	*Salmo salar*	724/724	100%	0	1.9	0.5	2534
Protein Post-Translational Modification, Protein Degradation, Protein Folding									
CA062243	Ubiquitin family domain-containing protein 1	AAH11313	*Mus musculus*	100/110	90%	2E-36	5.0	2.2	1019
CA053740	Ubiquitin carboxyl-terminal hydrolase isozyme L1	ACM09038	*Salmo salar*	88/88	100%	3E-44	2.3	0.4	41323
CB505759	Midasin homolog	CAM26642	*Mus musculus*	41/50	82%	1E-19	1.9	0.3	6470
CA042535	40 kDa peptidyl-prolyl cis-trans isomerase	ACO07506	*Oncorhynchus mykiss*	103/103	100%	1E-46	1.8	0.3	5361
Immune responsive									
CA055856	Ig kappa chain V-IV region B17 precursor	ACN10421	*Salmo salar*	103/103	100%	2E-41	3.2	0.9	1457
CB517114	IGL1E2 gene for immunoglobulin light chain variable region	AJ251651	*Oncorhynchus mykiss*	618/649	95%	0	2.7	0.5	1049
CA056108	C type lectin receptor A	AAT77220	*Salmo salar*	54/54	100%	2E-27	2.4	0.3	1030
Iron Homeostasis									
CK990717	Hemoglobin subunit beta-1	BT060431	*Salmo salar*	83/85	97%	3E-31	2.6	0.7	16933
CA050293	Hemoglobin subunit $beta_3$	ACM08872	*Salmo salar*	148/148	100%	1E-79	2.5	0.4	13827
CA058603	Hemoglobin subunit alpha-4_2	ACO07580	*Oncorhynchus mykiss*	141/145	97%	3E-20	2.3	0.4	24805
CA060701	Hemoglobin subunit $alpha_2$	ACN10007	*Salmo salar*	143/143	100%	3E-76	2.1	0.3	29332
CB505282	Ferritin, middle subunit	ACI69640	*Salmo salar*	95/95	100%	2E-43	2.1	0.5	3070
Other									
CA054421	UPF0527 membrane protein	BT045130	*Salmo salar*	655/657	99%	0	3.9	2.3	1679
CA051567	Coiled-coil domain containing protein 109A	Q08BI9	*Danio rerio*	66/114	57%	9E-38	2.9	1.1	1618
CA045974	UPF0466 protein C22orf32 homolog, mitochondrial	Q5XG64	*Xenopus laevis*	40/70	57%	5E-17	2.8	1.2	3159
CA061818	SH2/SH3 adaptor Crk	BT058800	*Salmo salar*	79/101	78%	5E-10	2.6	0.5	1453
CB517491	UbiA prenyltransferase domain containing 1	Q5ZKS8	*Gallus gallus*	59/76	77%	3E-27	2.4	0.6	1068
Unknown									
CA053832	Unknown						2.1	0.2	1321
CA053773	Unknown						2.1	0.3	21420
CA051670	Unknown						2.3	0.5	4300

Background-corrected, Lowess-normalized (BCLN) signal ratios are sorted by descending mean ratio for seismic exposed salmon ear relative to control salmon ear for all four slides in study for each functional category listed. For the "EST" column, GenBank accession numbers of the expressed sequence tags (ESTs) corresponding to GRASP microarray features is shown. The most significant (lowest E-value) BLASTX hit is shown. If an EST has no significant (E-value <10e-5) BLASTX hit, then the most significant BLASTN hit is shown in blue font. The GenBank nr or nt accession number is shown along with the species. "Length" indicates extent (salmonid EST translation aligned with amino acid (aa) sequence of the best BLASTX hit or EST sequence aligned with nucleotide (nt) sequence of the best BLASTN hit) over the aligned region, along with percent identity (% ID) and the associated *E*-value. "Mean fold change" (MFC) = mean BCLN seismic exposed/control ratio. Number of replicates = 4. Individual slide data along with functional annotation are available in Supplemental Table S1.

Table 3.3 Transcripts greater than 1.75-fold down-regulated in seismic exposed Atlantic salmon ear relative to control Atlantic salmon ear on at least three slides of the study (including at least one dye swap)

EST	Gene Name of Best BLASTX or BLASTN Hit	Accn #	Species	Align	% ID aa or nt	E-Value	MFC	SE	Mean Signal
DNA/RNA binding, transcription, translation									
CB503155	Splicing factor, arginine/serine-rich 2	ACN10350	*Salmo salar*	92/92	100%	7E-39	2.5	0.7	1912
DN047874	TAR DNA-binding protein 43	BT072005	*Salmo salar*	756/756	100%	0	2.5	0.9	2594
CK990619	39S ribosomal protein L36,	ACI69996	*Salmo salar*	111/121	91%	1E-51	2.4	0.5	658
CB505847	Transposase	CAB51372	*Pleuronectes platessa*	108/154	70%	3E-55	2.4	0.9	1938
CB492396	5'-nucleotidase, cytosolic 3	ACH70804	*Salmo salar*	89/90	98%	7E-21	2.3	0.6	717
CB492153	Activated RNA polymerase II transcriptional coactivator p15	ACI67532	*Salmo salar*	65/65	100%	7E-31	2.1	0.2	970
Energy, Metabolism									
CA042992	L-xylulose reductase	BT059117	*Salmo salar*	593/633	93%	0	2.9	1.3	1272
CB497891	Cytochrome c oxidase subunit 5B, mitochondrial precursor	ACN10266	*Salmo salar*	44/60	73%	4E-12	2.5	0.7	2303
CB492183	Succinyl-CoA ligase beta-chain mitochondrial precursor	BT071951	*Salmo salar*	341/472	72%	9E-67	2.3	0.7	858
CB496455	Cytochrome c oxidase polypeptide VIIb, mitochondrial precursor	ACI67848	*Salmo salar*	78/79	98%	1E-31	2.0	0.6	2524
CA051481	Carbonyl reductase 1	BT047504	*Salmo salar*	51/54	94%	5E-13	1.8	0.4	1385
Signalling, Synaptic transmission									
CB510623	Signal recognition particle 19 kDa protein	ACI68109	*Salmo salar*	75/83	90%	1E-34	2.6	1.0	1349
CB502520	calmodulin 2	BT043797	*Salmo salar*	678/856	79%	0	2.1	0.6	1317
CB492269	Purpurin precursor	ACI70127	*Salmo salar*	68/74	91%	2E-11	2.0	0.3	1306
CB511240	Retinoid x receptor beta a	BT043993	*Salmo salar*	593/594	99%	0	1.8	0.1	1771
Cytoskeleton structure, Dynamics									
CA055177	Ezrin-radixin-moesin-binding phosphoprotein 50	BT058849	*Salmo salar*	44/51	86%	6E-06	2.3	0.6	1643
CA059770	Collagen alpha 2 type IV chain	AAX55917	*Danio rerio*	44/52	84%	6E-19	2.0	0.4	1084
CB514261	Motile sperm domain-containing protein 2	BT045370	*Salmo salar*	708/708	100%	0	2.0	0.2	12741
Cell cycle, cell death, axonogenesis									
CB502357	B-cell receptor-associated protein 31	BT045436	*Salmo salar*	797/799	99%	0	2.7	0.8	3098
CB511669	BCCIP homolog	ACI67438	*Salmo salar*	111/113	98%	2E-59	2.6	0.8	3634
CB507647	Protein SET	M93651	*Homo sapiens*	54/59	91%	6E-12	2.5	0.8	1198
CB485925	S100-B	ACM08316	*Salmo salar*	79/80	98%	1E-21	2.3	0.5	1919
CA053033	Nuclear migration protein nudC	ACN10702	*Salmo salar*	135/135	100%	4E-73	2.3	0.3	2352
Protein Post-Translational Modification, Protein Degradation, Protein Folding									
CK990486	Proteasome subunit beta type-1-A	ACI67092	*Salmo salar*	78/96	81%	3E-29	3.0	1.1	1115
CB512698	Proteasome subunit beta type-4 precursor	ACI69686	*Salmo salar*	201/202	99%	7E-112	2.3	0.2	1942
CB516800	Serpin H1 precursor	BT044974	*Salmo salar*	744/745	99%	0	2.0	0.4	3147
CB498113	S-phase kinase-associated protein 1A	ACO07792	*Oncorhynchus mykiss*	149/149	100%	2E-82	2.0	0.6	1338
CA045309	Low molecular weight phosphotyrosine protein phosphatase	ACI67726	*Salmo salar*	36/38	94%	3E-14	2.0	0.5	774
CB517787	Proteasome subunit beta type-5 precursor	ACM09015	*Salmo salar*	154/155	99%	1E-50	1.9	0.5	1874
Immune responsive									
CA050743	4F2 cell-surface antigen heavy chain	ACI33885	*Salmo salar*	69/69	100%	1E-30	2.2	0.6	1006
CB513162	H-2 class II histocompatibility antigen gamma chain	BT071840	*Salmo salar*	399/441	90%	2E-170	1.9	0.6	1346
Other									
CB510977	C17orf37 homolog	BT071863	*Salmo salar*	568/619	91%	0	2.5	0.7	2559
CA057346	C14orf159 protein	AAI55802	*Danio rerio*	82/112	73%	2E-41	2.2	0.3	3898
Unknown									
CA062736	Unknown						2.8	1.1	944
CA052504	Unknown						2.5	0.7	3493
CA051316	Unknown						1.9	0.6	943
CA062981	Unknown						1.9	0.5	1632

Background-corrected, Lowess-normalized (BCLN) signal ratios are sorted by descending mean ratio for control salmon ear relative to seismic exposed salmon ear for all four slides in study for each functional category listed. For the "EST" column, GenBank accession numbers of the expressed sequence tags (ESTs) corresponding to GRASP microarray features is shown. The most significant (lowest E-value) BLASTX hit is shown. If an EST has no significant (E-value <10e-5) BLASTX hit, then the most significant BLASTN hit is shown in blue font. The GenBank nr or nt accession number is shown along with the species. "Length" indicates extent (salmonid EST translation aligned with amino acid (aa) sequence of the best BLASTX hit or EST sequence aligned with nucleotide (nt) sequence of the best BLASTN hit) over the aligned region, along with percent identity (% ID) and the associated *E*-value. "Mean fold change" (MFC) = mean BCLN control/seismic exposed ratio. Number of replicates = 4. Individual slide data along with functional annotation are available in Supplemental Table S2.

Many of the microarray-identified inner ear transcripts responsive to seismic noise exposure are involved in iron homeostasis and energy/metabolism. Reproducibly informative transcripts having functions related to iron homeostasis that were up-regulated in salmon ear in response to seismic noise exposure included: hemoglobin alpha (2.1-fold, SE 0.3; 2.0-fold, SE 0.3), hemoglobin alpha-4 (2.3-fold, SE 0.4; 2.0-fold, SE 0.1), hemoglobin beta (2.5-fold, SE 0.4; 2.3-fold, SE 0.4; 2.0-fold, SE 0.4), hemoglobin beta-1 (2.6-fold, SE 0.7) and ferritin, middle subunit (2.1-fold, SE 0.5) (Table 3.2). In addition to iron homeostasis, hemoglobin is also important in oxygen transport. There are no transcripts with these functions that are reproducibly down-regulated in salmon ear in response to seismic exposure. The hemoglobins are among the most abundant differentially regulated transcripts, indicated by the mean signal from the dominant channel listed in Table 3.2. QPCR confirmation of hemoglobin alpha-4 showed a 3.8-fold (*P*-value <0.05) induction in seismic exposed relative to control salmon ear (Table 3.4; Figure 3.2A).

Several reproducibly informative transcripts important in energy metabolism were differentially regulated in salmon ear in response to seismic sound exposure. Transcripts directly involved in the citric acid cycle that were up-regulated include succinate CoA ligase alpha subunit (3.0-fold, SE 0.8), NADP-dependent malic enzyme (2.5-fold, SE 0.8) and malate dehydrogenase cytoplasmic (2.4-fold, SE 0.6). Succinyl CoA ligase beta chain mitochondrial precursor encoding transcript was down-regulated by seismic sound (2.3-fold, SE 0.7). Transcripts important in the electron transport chain were also differentially regulated in seismic noise exposed salmon ear. Transcripts encoding cytochrome c oxidase VIc 2 (2.9-fold, SE 0.9) and NADH dehydrogenase 1 beta subcomplex subunit 1 (1.9-fold, SE 0.6) were up-regulated in response to seismic noise exposure whereas transcripts encoding cytochrome c oxidase subunit 5B (2.5-fold, SE 0.7) and cytochrome c oxidase polypeptide VIIb, mitochondrial precursor (2.0-fold, SE 0.6) were down-regulated. NADH dehydrogenase 1 beta subcomplex subunit 1 is part of Complex I of the mitochondrial electron transport chain and cytochrome c oxidase subunits form part of Complex IV of the mitochondrial electron transport chain. Other differentially regulated transcripts involved

Table 3.4 Quantitative reverse transcription – polymerase chain reaction (QPCR) validation o selected microarray-identified and SSH-identified transcripts

EST	*Gene Name of Best BLASTX or BLASTN Hit*	*Microarray*		*QPCR*	
		MFC	*SE*	*MFC*	*P-value*
CA045755	Growth hormone I	+3.4	0.8	+7.78	0.24
CB492469	Nicotinamide riboside kinase 22	+2.9	0.3	+1.89	0.02
CA056108	C type lectin receptor A	+2.4	0.3	+2.20	0.14
CA058654	Retinol-binding protein I, cellular	+2.3	0.2	+1.24	0.67
CA060239	Caspase-8 precursor	+2.1	0.2	+1.01	0.89
CB515375	Hemoglobin subunit alpha-4	+2.0	0.1	+3.76	0.01
CB517114	C14orf159 protein	-2.2	0.3	-1.35	0.04
CB512698	Proteasome subunit beta type-4 precursor	-2.3	0.2	-1.07	0.34
GT128561-76[a]	Cytosolic non-specific dipeptidase			+27.3	0.17

QPCR and microarray results for 8 transcripts of interest, normalized to 60S ribosomal L6. Average ratios (mean fold changes (MFC)) and standard errors of the mean (SE) are shown. Numbers of replicates: microarray, n=4 (4 technical replicates including 2 dye swaps using pools of 12 individuals for seismic exposed salmon ear and 12 individuals for control salmon ear with equal amount of mRNA per individual contributing to the pools; QPCR n=2 technical replicates for each of 12 seismic exposed biological replicate and each of 12 control biological replicate salmon ears. '+' indicates up-regulated seismic relative to control (*i.e.* S/C ≥ 1) and '-' indicates down-regulated seismic relative to control (*i.e.* C/S ≥ 1). QPCR of a selected forward SSH library transcript ([a]cytosolic non-specific dipeptidase) normalized to 60S ribosomal L6. Average ratio (MFC) and P-value are shown. Numbers of replicates: n=2 technical replicates for each of 16 seismic exposed biological replicate and each of 16 control biological replicate salmon ears. '+' indicates up-regulated seismic relative to control.

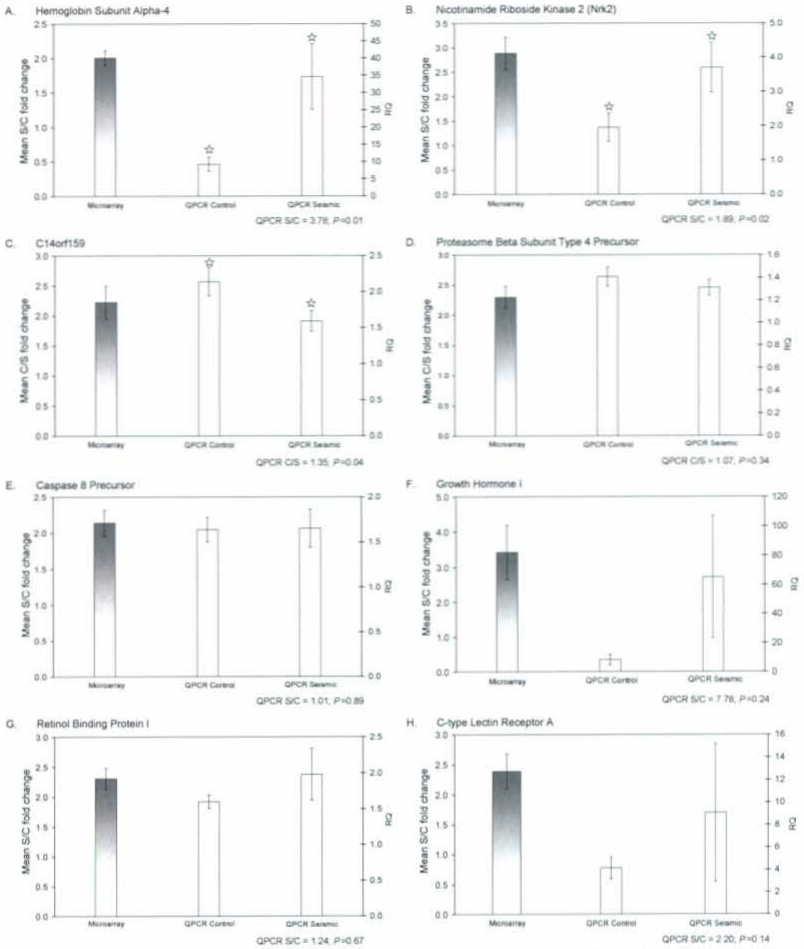


Figure 3.2 Quantitative reverse transcription - polymerase chain reaction (QPCR) validation of microarray results. QPCR validation of cGRASP microarray results for 8 transcripts of interest ((A) hemoglobin subunit alpha-4; (B) nicotinamide riboside kinase 2; (C) C14orf159; (D) proteasome beta subunit type 4 precursor; (E) caspase-8 precursor; (F) growth hormone I; (G) retinol binding protein I; (H) c-type lectin receptor A) normalized to 60S ribosomal L6. Mean fold change (primary Y axis) is shown for microarray data, number of replicates n=4 slides (4 technical replicates including 2 dye swaps) per comparison. Error bars for microarray data are for SE for fold change on all 4 slides in study. Relative Quantity (RQ) (secondary Y axis) is shown for QPCR data from control non-exposed and seismic exposed salmon ear, number of technical replicates: n=2 reactions per individual fish and number of biological replicates: n=12 control and 12 seismic exposed fish. The mean fold change in RQ for QPCR data for seismic / control (S/C) and control / seismic (C/S) for transcripts of interest that are up- and down-regulated, respectively, along with the corresponding P-value is shown below each graph. Error bars for QPCR data are for SE for RQ values. A star indicates statistical significance P<0.05. QPCR amplification products were electrophoresed on an agarose gel to verify product size.

in the citric acid cycle, electron transport chain or energy metabolism include: nicotinamide riboside kinase 2 (2.9-fold up-regulated, SE 0.3; 1.8-fold up-regulated, SE 0.2), which plays a role in a novel salvage pathway to produce NAD^+ (Tempel *et al.*, 2007); adrenodoxin, mitochondrial (2.0-fold up-regulated, SE 0.6), which mediates electron transfer; apolipoprotein A-IV precursor (2.1-fold up-regulated, SE 0.5), which plays a role in lipid transport and regulation of lipid metabolism; and L-xylulose reductase (2.9-fold down-regulated, SE 1.3) and carbonyl reductase (1.8-fold down-regulated, SE 0.4), which are important in carbohydrate and fatty acid metabolism, respectively. Seismic noise-responsive transcripts with no well defined function in metabolism, but known to be important in the mitochondrion, include UPF0466 protein C22orf32 homolog, mitochondrial (2.8-fold up-regulated, SE 1.2) and C14orf159 protein (2.2-fold down-regulated, SE 0.3) (Tables 3.2 and 3.3). QPCR validation of microarray results showed up-regulation of nicotinamide riboside kinase 2 (1.9-fold; *P*-value <0.05) (Table 3.4; Figure 3.2B) and down-regulation of C14orf159 protein (1.4-fold, *P*-value <0.05) (Table 3.4; Figure 3.2C) in response to seismic noise exposure.

The transcript with the highest mean fold-change response to seismic noise exposure was ubiquitin family domain-containing protein 1 (5.0-fold up-regulated, SE 2.2) (Table 3.2). While there are no gene ontology terms assigned to this protein, it is reported to be a polyubiquitin binding protein important in regulation of protein metabolism and function in humans (Fenner *et al.*, 2009). A number of other reproducibly informative transcripts (*i.e.* differentially regulated in response to seismic exposure) had significant BLASTX hits against proteins with important roles in regulation of protein turn-over as well as cell death (apoptosis)/cell regeneration, including axonogenesis. Reproducibly informative transcripts shown to be up-regulated in response to seismic noise that have gene ontologies related to protein modification or protein metabolic process include: ubiquitin carboxyl-terminal hydrolase isozyme L1 (2.3-fold, SE 0.4), midasin homolog (1.9-fold, SE 0.3) and 40 kDa peptidyl-propyl cis-trans isomerase (1.8-fold, SE 0.3) (Table 3.2). Reproducibly informative transcripts down-regulated in the microarray study with important roles in regulated protein turn-over include: proteasome subunit beta type-1A (3.0-fold,

SE 1.1) proteasome subunit beta type-4 precursor (2.3-fold, SE 0.2), s-phase kinase-associated protein 1A (2.0-fold, SE 0.6), low molecular weight phosphotyrosine protein phosphatase (2.0-fold, SE 0.5) and proteasome subunit beta type-5 precursor (1.9-fold, SE 0.5) (Table 3.3). QPCR validation of proteasome beta type-4 precursor (1.1-fold, *P*-value >0.05) revealed no significant down-regulation (Table 3.4; Figure 3.2D), however, consistent down-regulation of three transcripts for proteasome beta subunits (Table 3.3) provides some validation in microarray results, and QPCR for these transcripts should be repeated. Apoptosis is often associated with pathways involved in regulated protein degradation. Differentially regulated transcripts with gene ontologies related to cell death (apoptosis) include: up-regulation of caspase-8 precursor (2.1-fold, SE 0.2) and catenin beta-1 (1.9-fold, SE 0.5) (Table 3.2) as well as down-regulation of B-cell receptor-associated protein 31 (2.7-fold, SE 0.8) (Table 3.3). However, QPCR did not reveal any significant difference in expression of caspase-8 precursor (Table 3.4; Figure 3.2E). Associated with neuronal cell death/regeneration is S100-B and nuclear migration protein, which are reproducibly down-regulated in response to seismic exposure (2.3-fold, SE 0.5 and 2.3-fold, SE 0.3, respectively) (Table 3.3). Several other reproducibly informative transcripts important in cellular turnover as well as regulation of transcription/translation and nucleic acid binding are listed in Tables 3.2 and 3.3.

Several differentially regulated transcripts have important roles related to signaling; microarray-identified transcripts up-regulated by seismic noise include growth hormone 1 (3.4-fold, SE 0.8), retinol binding protein 1 (2.3-fold, SE 0.2), transthyretin (2.1-fold, SE 0.6) (retinol transport), ADP-ribosylation factor 1 (2.8-fold, SE 0.9) (GTPase mediated signal transduction), collagen alpha-2I chain precursor (2.1-fold, SE 0.4) (Rho protein signal transduction) and ADAM metallopeptidase (1.8-fold, SE 0.3) (notch signaling) (Table 3.2). Microarray-identified transcripts down-regulated by seismic noise include: signal recognition particle (2.8-fold, SE 1.0), calcium ion binding, calmodulin 2 (2.1-fold, SE 0.6), purpurin precursor (2.0-fold, SE 0.3), important in retinoid binding and retinoid x receptor (1.8-fold, SE 0.1) (Table 3.3). QPCR validation of growth hormone 1 confirms overall up-regulation in response to seismic noise exposure (7.8-fold) (Table 3.4;

Figure 3.2F); however, there is large individual variability resulting in a high *P*-value (*P*=0.24). QPCR validation of retinol binding protein 1 revealed only a 1.2-fold overall induction in seismic exposed relative to control salmon (*P*-value >0.05) (Table 3.4; Figure 3.2G).

A transcript encoding a hypothetical protein similar to motile sperm domain-containing protein 2, important in cell motility, was down-regulated in response to seismic noise exposure (2.0-fold, SE 0.2) and represented the most abundant down-regulated transcript in the microarray study (Table 3.3). Other microarray-identified transcripts that were differentially regulated with important roles in cytoskeletal structure and dymanics, including actin binding and cross-linking actin filaments as well as extracellular matrix proteins, are listed in Tables 3.2 and 3.3.

Only a few reproducibly informative transcripts identified in the microarray study have functional annotations suggesting important roles in immune responses. The microarray-identified transcript encoding immune-relevant C-type lectin receptor A was up-regulated (2.4-fold, SE 0.3) (Table 3.2) in response to seismic noise exposure. QPCR validation of C-type lectin receptor A revealed an overall induction in response to seismic exposure (2.2-fold) (Table 3.4; Figure 3.2H), but due to the high variability in the seismic exposed group (as observed with other transcripts of interest from the seismic exposed group), the *P*-value ($P = 0.14$) did not indicate that the difference was statistically significant.

Selected reproducibly-informative transcripts identified by the microarray study are shown in a scatterplot of one slide (slide number GG003_014) from the study to give an appreciation of the relative abundance and altered expression of some inner ear transcripts responsive to seismic noise (see Materials and Methods Figure 2.2).

3.4 Reciprocal SSH cDNA Libraries

Reciprocal SSH cDNA libraries were constructed from ear taken from the left side (ear from the right side was used in the microarray study) of seismic exposed and control juvenile Atlantic salmon (*Salmo salar*) smolt for the purpose of discovering genes that are important in the response of salmon to intense noise exposure. The fish used in the control group in this study

were handled in the same manner as the exposed group, except without exposure to seismic blasts, and were siblings of the exposed group from several families.

For SSH cDNA library construction, ears from seismic exposed and control fish were used to create libraries potentially enriched for genes responsive to seismic noise exposure. The "forward" SSH library, using a pooled ear sample from seismic exposed fish as tester and a pooled ear sample from control fish as driver, was designed to be enriched with transcripts up-regulated by seismic noise exposure, and the "reverse" SSH library (the reciprocal of the forward library) was designed to be enriched for transcripts down-regulated by seismic noise exposure. Figure 2.3 provides a summary of SSH library construction. Of the 333 ESTs (GenBank accession numbers GT128484-GT128816) generated from the forward SSH library (library name: ssal_ear_SSH_seismicF), 176 ESTs (average length 664 bp) came from a size-fractionated (500-1200 bp) sub-library and 157 ESTs (average length 435 bp) came from a non-size-fractionated library. Of the 350 ESTs (GenBank accession numbers GT128817-GT129166) generated from the reverse SSH library (library name: ssal_ear_SSH_seismicR), 201 ESTs (average length 706 bp) came from a size-fractionated (500-1200 bp) sub-library and 149 ESTs (average length 525 bp) came from a non-size-fractionated library. After assembly, identification and functional annotation, there were 229 unique sequences [50 contiguous sequences (contigs) and 179 singletons] generated from the forward SSH library, and 243 unique sequences [50 contigs and 193 singletons] generated from the reverse SSH library. The complete list of assembled sequences (contigs and singletons) generated from the forward and reverse ear SSH cDNA libraries are in Tables 3.5 and 3.6, respectively. Tables 3.5 and 3.6 show the most significant (lowest *E*-value) BLASTX or BLASTN hit including species name and corresponding GenBank accession number, number of contributing ESTs, the percent identity over aligned region (amino acid or nucleotide) and assigned functional categories based on GO biological process or molecular function terms of best BLAST hit or of related sequences (*e.g.* putative human

Table 3.5 Genes identified in the forward SSH library (designed to be enriched for ear genes up-regulated by seismic noise)

Gene Name	ESTs	E-Value	% ID	Align (aa or nt)	Accession	Species
DNA/RNA binding, transcription, translation (36 clones)						
28S ribosomal RNA	4	0	96	962/1001	U34341	*Oncorhynchus mykiss*
eukaryotic translation initiation factor 3 subunit A	3	5E-19	80	150/186	ACN10789	*Salmo salar*
poly A binding protein, cytoplasmic 1 b	3	4E-55	91	114/124	AAH59662	*Danio rerio*
transposable element Tc1 transposase	2	1E-46	81	88/108	ACI68988	*Salmo salar*
60S ribosomal protein L8	2	2E-98	99	188/189	ACI68574	*Salmo salar*
40S ribosomal protein S27	1	1E-28	100	59/59	ACI67409	*Salmo salar*
60S ribosomal protein L17	1	8E-65	99	136/137	ACI33750	*Salmo salar*
60S ribosomal protein L7a	1	2E-24	85	51/60	ACH70989	*Salmo salar*
Barrier-to-autointegration factor	1	6E-16	100	40/40	ACI66039	*Salmo salar*
cAMP-responsive element-binding protein-like 2	1	7E-25	96	73/76	BT058965	*Salmo salar*
DNA-directed RNA polymerase II subunit RPB2	1	2E-66	99	122/123	ACN10943	*Salmo salar*
elongation factor 1 gamma	1	2E-47	85	92/107	ACH85371	*Salmo salar*
elongation factor 2	1	3E-22	100	62/62	BT071866	*Salmo salar*
ES1 protein homolog, mitochondrial precursor	1	2E-29	54	62/114	ACM08279	*Salmo salar*
eukaryotic initiation factor 2 alpha subunit	1	0	95	750/786	AF338347	*Oncorhynchus mykiss*
eukaryotic translation initiation factor 3, subunit 5 epsilon 47kDa	1	8E-87	78	153/195	BAF62356	*Pan troglodytes verus*
nuclear factor erythroid derived 2-like protein 1	1	3E-77	99	233/235	ABW77494	*Salmo salar*
oligoribonuclease, mitochondrial precursor	1	5E-14	100	38/38	ACI66405	*Salmo salar*
origin recognition complex subunit 2	1	0	100	487/487	BT059550	*Salmo salar*
orphan nuclear receptor NR1D2 variant	1	4E-18	83	59/71	BAD92328	*Homo sapiens*
ribosomal protein L6	1	1E-28	100	62/62	ABY28366	*Oncorhynchus masou formosanus*
ribosomal protein L7-1	1	2E-100	99	182/183	ACH70988	*Salmo salar*
Splicing factor, arginine /serine-rich 7	1	0	99	784/787	BT044936	*Salmo salar*
TAR DNA-binding protein 43	1	2E-75	98	198/201	ACI33676	*Salmo salar*
Tc1-like transporase	1	2E-25	66	53/80	BAF37936	*Oncorhynchus mykiss*
transposable element Tcb2 transposase	1	3E-18	37	47/127	AAA28149	*Caenorhabditis briggsae*
Zinc finger protein 503	1	4E-85	99	209/210	ACI33520	*Salmo salar*
Energy, Metabolism (33 clones)						
cytochrome c oxidase subunit I	9	8E-81	91	153/167	BAG69248	*Aplocheilus panchax*
cytochrome oxidase subunit II	9	2E-92	95	200/209	AAD04736	*Salmo salar*
cytochrome oxidase subunit I	2	3E-29	91	66/72	AAD04735	*Salmo salar*
glyceraldehyde-3-phosphate dehydrogenase	2	1E-95	95	184/192	ACI33883	*Salmo salar*
alkaline phosphatase	1	6E-75	83	274/328	BT044992	*Salmo salar*
apolipoprotein B	1	2E-34	87	122/139	X81856	*Salmo salar*
cytochrome oxidase subunit I	1	2E-51	91	174/190	ABK06129	*Parupeneus indicus*
fructose-bisphosphate aldolase A	1	3E-81	88	236/267	BT058987	*Salmo salar*
GDP-mannose 4,6 dehydratase	1	0	99	675/676	BT049328	*Salmo salar*
glucose transporter 1A	1	6E-24	63	63/100	AAF75681	*Oncorhynchus mykiss*
iduronate 2-sulfatase	1	1E-27	61	68/110	ABK59973	*Danio rerio*
L-xylulose reductase	1	0	93	564/602	BT059117	*Salmo salar*
NADH dehydrogenase iron-sulfur protein 2, mitochondrial precursor	1	1E-52	100	97/97	ACI33290	*Salmo salar*
NADH dehydrogenase subunit 1	1	3E-44	95	92/96	AAD43230	*Salmo salar*
phosphoglycerate mutase 1	1	0	100	360/360	BT044637	*Salmo salar*
Proteolysis (25 clones)						
cytosolic non-specific dipeptidase	16	0	99	354/357	ACI34318	*Salmo salar*
cytosolic non-specific dipeptidase	3	9E-150	89	394/439	BT046056	*Salmo salar*
cathepsin L1 precursor	2	4E-102	99	171/172	ACN10121	*Salmo salar*
carboxypeptidase X, member 1	1	7E-71	69	135/193	AAI33528	*Bos taurus*
cathepsin A	1	1E-09	61	29/47	AAQ18146	*Branchiostoma belcheri tsingtaunese*
matrix metalloproteinase-2	1	2E-95	96	163/169	BAA78479	*Oncorhynchus mykiss*
salarin gene for cysteine proteinase inhibitor protein, exons 1-13	1	2E-07	100	35/35	AJ550228	*Salmo salar*
Signaling, Synaptic Transmission (23 clones)						
myelin P0 protein precursor	6	0	100	695/695	BT058974	*Salmo salar*
platelet-derived growth factor receptor-like protein precursor	3	0	95	666/700	BT059077	*Salmo salar*
myelin basic protein	2	2E-22	51	70/136	AAH65248	*Homo sapiens*
receptor expression-enhancing protein 5	2	4E-83	99	188/189	ACI67469	*Salmo salar*
calmodulin	1	3E-31	98	81/82	BT060344	*Salmo salar*
growth factor receptor-bound protein 2	1	0	92	605/654	BT058722	*Salmo salar*
growth hormone 1	1	1E-22	92	76/82	EF564440	*Oncorhynchus masou formosanus*
guanine nucleotide binding protein beta 2	1	8E-38	91	64/70	ABC75581	*Ictalurus punctatus*
guanine nucleotide binding protein beta 2	1	1E-75	89	137/153	ABC75581	*Ictalurus punctatus*
main olfactory receptor-like protein	1	8E-23	84	54/64	ABD33498	*Salmo salar*
myostatin 2a gene, promoter region	1	3E-100	89	270/301	DQ138301	*Oncorhynchus mykiss*
protein phosphatase 1, regulatory (inhibitor) subunit 1b-like	1	0	96	671/692	BT043676	*Salmo salar*
serine/threonine kinase receptor	1	5E-37	84	73/86	AAM53074	*Danio rerio*
syntaxin-binding protein 1	1	0	99	353/354	BT072082	*Salmo salar*

Table 3.5 (Continued) Genes identified in the forward SSH library (designed to be enriched for ear genes up-regulated by seismic noise)

Gene Name	*ESTs*	*E-Value*	*% ID*	*Align (aa or nt)*	*Accession*	*Species*
Cytoskeletal / Structural Activity (23 clones)						
Matn4 protein	4	6E-69	83	134/161	AAI63867	*Danio rerio*
collagen alpha-2I chain precursor	2	5E-54	83	226/272	BT071961	*Salmo salar*
dynein cytoplasmic 1 heavy chain 1	2	7E-147	84	267/315	ABC59849	*Danio rerio*
tubulin alpha chain	2	9E-57	96	107/111	ACI33964	*Salmo salar*
tubulin alpha-1	2	9E-65	99	119/120	CAP72044	*Fasciola hepatica*
actin, cytoplasmic 1	1	0	98	363/368	BT045109	*Salmo salar*
alpha tubulin 2	1	5E-27	88	63/71	AAM09674	*Aplysia californica*
beta thymosin	1	2E-120	97	255/262	AJ250180	*Oncorhynchus mykiss*
collagen alpha-2I chain precursor	1	0	85	599/704	BT072251	*Salmo salar*
cytoskeleton-associated protein 5	1	3E-134	99	253/254	ACN58609	*Salmo salar*
fibulin-1	1	2E-27	100	124/124	ACN11181	*Salmo salar*
keratin associated protein 4.12	1	4E-23	36	85/232	CAC27582	*Homo sapiens*
septin-2	1	0	99	786/790	BT071903	*Salmo salar*
simple type II keratin K8a	1	1E-25	97	118/121	CAC45060	*Oncorhynchus mykiss*
tubulin beta chain	1	3E-26	88	97/110	BT044664	*Salmo salar*
twinfilin-1	1	4E-12	90	57/63	BT059739	*Salmo salar*
Cell Cycle, Cell Death, Axonogenesis (17 clones)						
S100-B	3	1E-37	100	77/77	ACM08316	*Salmo salar*
endothelial differentiation-related factor 1 homolog	2	0	100	615/615	BT049693	*Salmo salar*
nucleosome assembly protein 1 like 1	2	0	93	698/744	BT045229	*Salmo salar*
optineurin	2	2E-122	93	244/261	ACI33853	*Salmo salar*
catenin beta-1	1	8E-66	100	144/144	ACN58608	*Salmo salar*
cyclin-dependent kinase 2-associated protein 2	1	3E-14	83	85/102	BT044623	*Salmo salar*
ependymin precursor	1	1E-34	98	69/70	ACI33774	*Salmo salar*
insulin-like growth factor binding protein 2	1	0	91	469/513	DQ146968	*Oncorhynchus mykiss*
Neuroblast differentiation-associated protein	1	0	97	881/901	BT072112	*Salmo salar*
NF-kappa-B inhibitor alpha	1	1E-53	100	119/119	ACI67986	*Salmo salar*
parvalbumin, thymic CPV3 (synonym: oncomodulin)	1	3E-47	100	94/94	ACM09534	*Salmo salar*
translocon-associated protein subunit alpha precursor	1	1E-65	100	148/148	ACM08230	*Salmo salar*
Ion Channel Activity, Ion Transport (14 clones)						
integral membrane transporter protein	7	4E-12	100	34/34	CAB81951	*Homo sapiens*
voltage-gated sodium channel alpha type IV	3	5E-80	74	192/259	ABP98938	*Oncorhynchus mykiss*
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	1	4E-78	100	150/150	ACH70848	*Salmo salar*
chloride intracellular channel 4	1	2E-14	73	34/46	AAH51622	*Danio rerio*
Na/K ATPase alpha subunit isoform 2	1	3E-82	94	194/205	AY319387	*Oncorhynchus mykiss*
vacuolar ATP synthase subunit e 2	1	5E-55	91	153/168	BT057637	*Salmo salar*
Stress Responsive (10 clones)						
heat shock protein 8	3	3E-104	100	236/236	ACH70651	*Salmo salar*
glutathione S-transferase A	2	3E-68	100	126/126	ACM08643	*Salmo salar*
Selenoprotein Pa precursor	2	0	99	777/779	BT072678	*Salmo salar*
cold-inducible RNA-binding protein	1	1E-140	98	281/284	BT057118	*Salmo salar*
hypoxia-inducible gene1 domain family member 1A	1	3E-28	83	62/74	ACQ57876	*Anoplopoma fimbria*
Selenoprotein Pa precursor	1	0	97	837/855	BT072678	*Salmo salar*
Protein Post-Translational Modification, Protein Degradation, Protein Folding (11 clones)						
26S protease regulatory subunit 4	1	4E-85	99	157/158	ACI68908	*Salmo salar*
26S protease regulatory subunit 7	1	2E-99	98	197/199	ACH70710	*Salmo salar*
chaperonin containing TCP1, subunit 7	1	2E-88	89	168/188	AAI63866	*Danio rerio*
CMP-N-acetylneuraminate-beta-galactosamide-alpha-2, 3-	1	1E-141	99	279/280	BT072382	*Salmo salar*
FK506-binding protein 2 precursor	1	4E-66	99	137/138	ACI68911	*Salmo salar*
glutamine synthetase	1	8E-140	98	230/234	AAM73660	*Oncorhynchus mykiss*
proteasome subunit beta type-4 precursor	1	3E-65	100	122/122	ACI67540	*Salmo salar*
ubiquitin-conjugating enzyme 16	1	2E-46	97	103/106	AAY24555	*Homo sapiens*
ubiquitin-conjugating enzyme E2 L3	1	0	96	572/590	BT056382	*Salmo salar*
ubiquitin-conjugating enzyme E2 L3	1	1E-15	88	68/77	BT072278	*Salmo salar*
ubiquitin-conjugating enzyme E2 R1	1	8E-83	92	205/222	BT058795	*Salmo salar*
Immune Responsive (10 clones)						
beta-2-microglobulin precursor	3	0	99	714/720	BT046530	*Salmo salar*
Ig-superfamily protein	3	1E-07	37	46/124	BAA03716	*Gallus gallus*
CD9 antigen	1	4E-19	72	42/58	ACI66221	*Salmo salar*
grancalcin	1	5E-87	99	159/160	ACI66013	*Salmo salar*
integrin beta-1 precursor	1	3E-83	97	142/146	ACN11424	*Salmo salar*
serum amyloid A-5 protein	1	7E-16	97	37/38	ACM09349	*Salmo salar*
Otolith Development (7 clones)						
SPARC precursor	3	0	99	650/652	BT045906	*Salmo salar*
SPARC precursor	2	2E-86	97	169/174	ACI34168	*Salmo salar*
SPARC precursor	1	6E-19	100	47/47	ACI34168	*Salmo salar*
chondromodulin-I	1	3E-20	78	39/50	AAA30445	*Bos taurus*
Sensory Perception of Sound (3 clones)						
integral membrane protein 2B	1	6E-49	98	98/100	ACI33731	*Salmo salar*
peripheral myelin protein 22	1	2E-34	90	74/82	ACI33730	*Salmo salar*
peripheral myelin protein 22	1	0	97	842/863	BT045468	*Salmo salar*

Table 3.5 (Continued) Genes identified in the forward SSH library (designed to be enriched for ear genes up-regulated by seismic noise)

Gene Name	*ESTs*	*E-Value*	*% ID*	*Align (aa or nt)*	*Accession*	*Species*
Other (30 clones)						
senescence-associated protein	4	3E-29	77	67/87	ACA04850	*Picea abies*
sulfate transporter	2	9E-47	87	154/177	BT059146	*Salmo salar*
claudin domain-containing protein 1	2	0	99	652/656	BT058944	*Salmo salar*
microfibular-associated protein 2 precursor	2	0	99	602/605	BT049030	*Salmo salar*
serine incorporator 1	2	2E-20	100	47/47	ACI32887	*Salmo salar*
1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-3	1	0	95	542/567	BT048157	*Salmo salar*
1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-3	1	6E-65	89	187/209	BT048157	*Salmo salar*
abhydrolase domain-containing protein 10, mitochondrial precursor	1	0	99	657/662	BT045396	*Salmo salar*
active breakpoint cluster region-related protein	1	5E-138	85	429/500	BT071954	*Salmo salar*
annexin A11	1	6E-150	99	293/294	BT072167	*Salmo salar*
brain protein 44-like protein	1	1E-42	96	84/87	ACI66404	*Salmo salar*
C7orf57 homolog	1	0	100	393/393	BT045484	*Salmo salar*
CA protein	1	7E-16	84	84/100	AF515834	*Schistosoma japonicum*
calumenin precursor	1	3E-145	96	312/325	BT044805	*Salmo salar*
gametogenetin-binding protein 2	1	8E-64	92	166/180	BT056828	*Salmo salar*
Heme-binding protein 2	1	0	92	502/540	BT059063	*Salmo salar*
high affinity copper uptake protein 1	1	1E-64	88	192/216	BT059358	*Salmo salar*
iron-sulfur cluster assembly enzyme ISCU, mitochondrial precursor	1	0	98	380/384	BT049908	*Salmo salar*
PDZ and LIM domain protein 2	1	5E-71	92	180/195	BT044685	*Salmo salar*
pleiotrophic factor-alpha-2 precursor	1	0	99	760/764	BT057895	*Salmo salar*
serine/threonine-protein phosphatase PP1-beta catalytic subunit	1	6E-125	100	247/247	BT072371	*Salmo salar*
serine/threonine-protein phosphatase PP1-beta catalytic subunit	1	2E-58	98	195/198	ACI69393	*Salmo salar*
tripartite motif-containing protein 16	1	0	98	617/628	BT045784	*Salmo salar*
Unknown (14 clones)						
BAC CHORI214-8I14	3	0	92	939/1020	EF427379	*Salmo salar*
mitochondrion complete genome	2	0	99	658/664	AF133701	*Salmo salar*
novel cds	2	1E-147	90	387/430	BT072520	*Salmo salar*
BAC CH214-397C7	1	0	99	766/771	AC203456	*Salmo salar*
large open reading frame novel cds	1	2E-84	98	179/181	BT071909	*Salmo salar*
large open reading frame novel cds	1	2E-21	85	99/116	BT072783	*Salmo salar*
novel cds	1	0	99	364/365	BT072673	*Salmo salar*
novel cds	1	1E-142	93	333/357	BT071844	*Salmo salar*
OclMSU21 sequence microsatellite	1	4E-22	82	112/135	GQ249048	*Oncorhynchus clarkia*
VMRC26-21K14, complete sequence	1	0	91	183/200	AC187703	*Gasterosteus aculeatus*
Uncharacterized (77 clones)	69					

ESTs from the forward SSH cDNA library from salmon ear designed to be enriched for transcripts up-regulated in response to seismic noise. If an EST has no significant (E-value <10e-5) BLASTX hit, then the most significant BLASTN hit is shown in blue font. The GenBank nr or nt accession number is shown along with the species. Out of 50 contigs: 29 have a significant BLASTX hit (E<1e-05); 13 have a significant BLASTN hit (E<1e-05); and 8 have no significant BLASTX or BLASTN hit (E<1e-05). Out of 179 singletons: 60 have a significant BLASTX hit (E<1e-05); 58 have a significant BLASTN hit (E<1e-05); 61 have no significant BLASTX or BLASTN hit (E<1e-05). "Align (aa or nt)" indicates extent (salmonid EST translation aligned with amino acid (aa) sequence of the best BLASTX hit or EST sequence aligned with nucleotide (nt) sequence of the best BLASTN hit) over the aligned region, along with percent identity (% ID) and the associated E-value. Functional categories are based on GO biological process or molecular function terms of best BLAST hit or of related sequences (*e.g.* putative human orthologues), or based on reported putative function. Forward Library name is ssal_ear_SSH_seismicF.

Table 3.6 Genes identified in the reverse SSH library (designed to be enriched for ear genes down-regulated by seismic noise)

Gene Name	*ESTs*	*E-Value*	*% ID*	*Align (aa or nt)*	*Accession*	*Species*
DNA/RNA binding, transcription, translation (56 clones)						
28S ribosomal RNA	10	0	99	852/860	U34341	*Oncorhynchus mykiss*
transposable element Tc1 transposase	6	2E-57	86	69/80	ACI68988	*Salmo salar*
ribosomal protein L6	3	1E-118	98	252/256	ACH70868	*Salmo salar*
DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	2	2E-71	97	181/185	ACH85363	*Salmo salar*
DEAD-box RNA-dependent helicase p68	2	3E-124	88	237/269	AAX22124	*Carassius auratus*
nanos homolog 1	2	2E-122	99	225/226	ACI70036	*Salmo salar*
poly A binding protein, cytoplasmic 1 b	2	6E-148	87	275/316	AAH59662	*Danio rerio*
ReO_6	2	5E-25	53	31/58	BAB83841	*Oryzias latipes*
40S ribosomal protein S14	1	1E-66	95	128/134	ACN09966	*Salmo salar*
60S ribosomal protein L13	1	1E-18	100	64/64	ACI68750	*Salmo salar*
60S ribosomal protein L3	1	2E-82	99	149/150	ACI68315	*Salmo salar*
60S ribosomal protein L35	1	7E-48	100	122/122	ACI66532	*Salmo salar*
60S ribosomal protein L35a	1	4E-27	100	110/110	ACN10038	*Salmo salar*
60S ribosomal protein L8	1	9E-98	100	188/188	ACN10341	*Salmo salar*
deoxyribonuclease gamma precursor	1	6E-07	70	28/40	ACM08762	*Salmo salar*
DNA-directed RNA polymerase III subunit RPC4	1	7E-74	99	168/169	ACI33723	*Salmo salar*
eukaryotic translation initiation factor 3 subunit I	1	3E-69	98	213/217	ACI32945	*Salmo salar*
eukaryotic translation initiation factor 3, subunit 5 epsilon	1	3E-68	82	122/148	BAF62356	*Pan troglodytes verus*
FACT complex large subunit	1	1E-76	88	176/199	AAI63334	*Danio rerio*
FACT complex subunit SSRP1	1	0	99	543/547	BT059584	*Salmo salar*
Gmpr2 protein	1	2E-48	83	93/112	AAH90559	*Xenopus tropicalis*
piggyBac transposase Uribo1	1	6E-20	42	58/138	BAF82019	*Silurana tropicalis*
probable ATP-dependent RNA helicase DDX5	1	0	99	492/494	BT059556	*Salmo salar*
probable peptidyl-tRNA hydrolase 2	1	0	97	561/576	BT072330	*Salmo salar*
ribosomal protein S12	1	7E-13	98	77/78	ACI66971	*Salmo salar*
RNA-binding protein 5	1	0	99	562/565	BT072055	*Salmo salar*
transcription factor BTF3 homolog 4	1	6E-53	99	138/139	ACI69060	*Salmo salar*
transposable element Tcb1 transposase	1	6E-19	72	45/62	ACN11391	*Salmo salar*
transposable element Tcb1 transposase	1	1E-20	54	53/98	ACN11475	*Salmo salar*
transposase	1	1E-59	69	83/120	CAB51372	*Pleuronectes platessa*
transposase	1	2E-23	76	38/50	ABB83608	*Siniperca chuatsi*
transposase	1	1E-35	82	75/91	ABV31711	*Salmo salar*
transposase	1	2E-35	65	54/83	AAP49009	*Rana pipiens*
U4/U6 small nuclear ribonucleoprotein Prp31	1	5E-18	100	47/47	ACN11428	*Salmo salar*
UMP-CMP kinase	1	5E-17	80	46/57	ACO14015	*Esox lucius*
Energy, Metabolism (37 clones)						
cytochrome oxidase subunit II	13	3E-95	94	201/212	AAD04736	*Salmo salar*
cytochrome oxidase subunit I	3	1E-123	90	221/244	AAD04735	*Salmo salar*
epididymal secretory protein E1 precursor	3	4E-49	100	123/123	ACM09246	*Salmo salar*
glycogen phosphorylase, brain form	3	0	99	590/591	BT072327	*Salmo salar*
cytochrome b	2	6E-87	92	174/188	AAF06986	*Salmo salar*
NADH dehydrogenase 1 alpha subcomplex subunit 8	2	4E-99	98	170/172	ACI67834	*Salmo salar*
neighbor of COX4	2	3E-109	100	191/191	ACI69932	*Salmo salar*
creatine kinase B-type	1	2E-34	90	77/85	ACI32901	*Salmo salar*
cytochrome c oxidase subunit I	1	7E-26	90	93/103	CAP40321	*Salmo trutta trutta*
cytochrome oxidase subunit III	1	8E-23	95	60/63	ABZ01931	*Salmo salar*
ferritin, heavy subunit	1	4E-51	81	107/132	ACI67950	*Salmo salar*
isocitrate dehydrogenase 2-2 (NADP+), mitochondrial	1	7E-123	100	213/213	ACH70947	*Salmo salar*
NADH dehydrogenase subunit 1	1	7E-63	96	149/155	AAD43230	*Salmo salar*
NADH dehydrogenase subunit 1	1	8E-33	98	98/100	AAD43227	*Salmo salar*
pyruvate kinase	1	4E-42	100	82/82	ACI34219	*Salmo salar*
retinol dehydrogenase 3	1	1E-177	99	297/298	ACI67927	*Salmo salar*
Proteolysis (5 clones)						
72 kDa type IV collagenase precursor	1	2E-34	98	68/69	ACI33172	*Salmo salar*
glia-derived nexin precursor	1	8E-81	100	166/166	ACI33531	*Salmo salar*
leucine aminopeptidase	1	3E-31	37	93/247	CAZ30678	*Schistosoma mansoni*
lysostaphin	1	1E-18	23	39/169	AAB53783	*Staphylococcus simulans*
methionine aminopeptidase 2	1	2E-64	70	109/155	ACI33485	*Salmo salar*
Signaling, Synaptic Transmission (21 clones)						
growth hormone 1	4	0	99	1028/1032	EU621898	*Salmo salar*
follistatin-related protein 1 precursor	3	2E-131	94	222/236	ACI33002	*Salmo salar*
follistatin-related protein 1 precursor	2	2E-42	92	116/125	BT046049	*Salmo salar*
poliovirus receptor-related protein 1 precursor (Salmo salar)	2	1E-29	87	64/73	ACM09386	*Salmo salar*
follistatin-related protein 1 precursor	1	9E-42	82	188/228	BT072046	*Salmo salar*
Gnl3 protein	1	2E-28	75	63/83	AAH93001	*Danio rerio*
GTP-binding protein SAR1a	1	3E-101	100	183/183	ACI33991	*Salmo salar*
myelin P0 protein	1	0	100	559/559	BT045194	*Salmo salar*
myelin P0 protein precursor	1	0	100	583/583	BT058974	*Salmo salar*
phosphodiesterase 5A, cGMP-specific	1	1E-115	88	201/227	CAQ14723	*Danio rerio*
PKC-delta1 protein	1	3E-55	77	102/132	AAH78019	*Xenopus laevis*
stathmin	1	2E-51	100	144/144	ACI69595	*Salmo salar*
thyroid hormone receptor interactor 12, isoform CRA_l	1	2E-50	95	99/104	EAW70907	*Homo sapiens*
tyrosine-protein kinase RYK	1	2E-16	67	61/91	CAA49591	*Homo sapiens*

Table 3.6 (Continued) Genes identified in the reverse SSH library (designed to be enriched for ear genes down-regulated by seismic noise)

Gene Name	ESTs	E-Value	% ID	Align (aa or nt)	Accession	Species
Cytoskeleton Structurre and Dynamics (16 clones)						
matn4 protein	6	3E-80	81	154/188	AAI63867	*Danio rerio*
beta-tubulin	3	3E-48	98	241/245	ABO28786	*Mesenchytraeus solifugus*
tubulin alpha-1A chain	2	1E-94	99	196/197	ACI69569	*Salmo salar*
actin, cytoplasmic 1	1	5E-69	100	130/130	ACI67042	*Salmo salar*
cofilin-2	1	4E-54	99	104/105	ACI68104	*Salmo salar*
envelope polyprotein	1	2E-26	51	71/138	ABB52640	*Cercopithecus aethiops*
simple type II keratin K8a	1	2E-158	97	286/294	CAC45060	*Oncorhynchus mykiss*
thymosin beta-12	1	2E-07	100	42/42	ACI66596	*Salmo salar*
Cell Cycle, Cell Death, Axonogenesis (22 clones)						
reticulon 4	2	1E-66	100	148/148	DAA05186	*Salmo salar*
insulin-like growth factor binding protein 2	2	0	93	717/770	DQ146968	*Oncorhynchus mykiss*
anterior gradient protein 2 homolog precursor	1	1E-30	76	70/92	ACI68219	*Salmo salar*
APEX nuclease 1	1	2E-121	94	211/223	ACH70702	*Salmo salar*
BNIP2 motif-containing molecule at the C-terminal region 1	1	5E-53	92	137/148	ACI33014	*Salmo salar*
C8orf4 homolog	1	0	100	825/825	BT047158	*Salmo salar*
development and differentiation-enhancing factor 2	1	3E-13	100	46/46	BT071907	*Salmo salar*
ependymin	1	2E-91	100	165/165	AAA49551	*Salmo salar*
ependymin-2 precursor	1	5E-21	100	51/51	ACI67122	*Salmo salar*
extracellular matrix protein 1 precursor	1	8E-56	100	144/144	ACN10906	*Salmo salar*
insulin-like growth factor binding protein 5a	1	4E-19	95	64/67	BC066725	*Danio rerio*
insulin-like growth factor-binding protein 7 precursor	1	1E-135	93	230/245	ACN11329	*Salmo salar*
insulin-like growth factor-binding protein 7 precursor	1	0	99	731/738	BT059616	*Salmo salar*
myotrophin	1	4E-61	100	117/117	ACN10623	*Salmo salar*
NDRG3	1	0	99	835/839	BT072295;	*Salmo salar*
parvalbumin, thymic CPV3 (synonym: oncomodulin)	1	7E-53	97	106/109	ACM09534	*Salmo salar*
testis enhanced gene transcript (BAX inhibitor 1)	1	9E-24	96	55/57	ACH70693	*Salmo salar*
testis enhanced gene transcript (BAX inhibitor 1)	1	0	99	374/376	BT043578	*Salmo salar*
tetraspanin-3	1	4E-72	100	127/127	ACI33238	*Salmo salar*
Zinc finger protein ZIC 2	1	5E-42	96	96/99	ACN11131	*Salmo salar*
Ion Channel Activity, Ion Transport (18 clones)						
sodium/potassium-transporting ATPase subunit beta-233	3	1E-36	98	73/74	ACN11046	*Salmo salar*
ATPase subunit 6	2	1E-79	94	204/216	AAF61383	*Salmo salar*
kainate receptor beta subunit	2	3E-08	51	47/92	AAA21577	*Carassius auratus*
voltage-dependent anion-selective channel protein 2	2	0	100	374/374	BT045741	*Salmo salar*
ATP synthase subunit alpha	1	6E-101	100	188/188	ACH70752	*Salmo salar*
ATPase H+ transporting V1 subunit D	1	1E-21	100	66/66	ACH70735	*Salmo salar*
FXYD domain containing ion transport regulator 5a	1	0	99	380/383	BK006253	*Salmo salar*
glutamate receptor U1 precursor	1	0	98	458/464	BT059539	*Salmo salar*
K-Cl cotransporter	1	2E-52	76	113/148	BAD86529	*Canis familiaris*
Na/K ATPase alpha subunit isoform 1b	1	6E-90	100	181/181	AAQ82789	*Oncorhynchus mykiss*
Na/K ATPase alpha subunit isoform 3	1	0	90	549/604	AY319388	*Oncorhynchus mykiss*
potassium channel subunit	1	1E-58	86	115/133	AAM18770	*Gallus gallus*
sodium/potassium-transporting ATPase subunit beta-233	1	2E-29	100	62/62	ACN11038	*Salmo salar*
Stress Responsive (10 clones)						
heat shock protein 10, mitochondrial	3	8E-48	100	99/99	ACM09432	*Salmo salar*
heat shock protein hsp90 beta	2	7E-97	98	201/205	AAD30275	*Salmo salar*
cold-inducible RNA-binding protein	1	2E-39	97	81/83	ACI68319	*Salmo salar*
cold-inducible RNA-binding protein	1	0	98	768/776	BT058753	*Salmo salar*
heat shock 70 kDa protein	1	1E-78	100	146/146	ACI34374	*Salmo salar*
HSC71	1	3E-117	91	263/288	AAB21658	*Oncorhynchus mykiss*
microsomal epoxide hydrolase	1	3E-76	61	141/229	AAC41694	*Homo sapiens*
Protein Post-Translational Modification, Protein Degradation, Protein Folding (9 clones)						
poly synthetase 3	3	2E-75	99	143/144	ACI33214	*Salmo salar*
proteasome subunit alpha type-3	2	1E-123	99	195/196	ACI66058	*Salmo salar*
FK506-binding protein 1A	1	2E-57	100	107/107	ACO07946	*Oncorhynchus mykiss*
signalosome complex subunit COP94	1	4E-97	91	182/198	ACN11367	*Salmo salar*
S-phase kinase-associated protein 1	1	2E-41	99	99/100	ACM09794	*Salmo salar*
ubiquitin and ribosomal protein S27a precursor	1	4E-40	97	84/86	ABC75552	*Ictalurus punctatus*
Immune Responsive (7 clones)						
4F2 cell-surface antigen heavy chain	1	5E-26	98	62/63	ACI33885	*Salmo salar*
CD209 antigen-like protein	1	0	99	611/614	BT049669	*Salmo salar*
complement receptor-like protein 1	1	2E-12	79	109/137	AJ620466	*Oncorhynchus mykiss*
cytokine-like protein 1 precursor	1	0	100	513/513	BT057798	*Salmo salar*
lysosome-associated membrane glycoprotein 1 precursor	1	3E-23	97	68/70	BT058886	*Salmo salar*
MHC class I	1	9E-47	95	91/95	AAO14685	*Salmo salar*
NOD-like receptor C	1	2E-18	49	58/117	ABU89560	*Danio rerio*
Otolith Development (2 clones)						
otolith matrix macromolecule-64	1	3E-22	94	56/59	BAG14384	*Oncorhynchus mykiss*
otolin 1	1	2E-29	80	67/83	CAN88052	*Danio rerio*

Table 3.6 (Continued) Genes identified in the reverse SSH library (designed to be enriched for ear genes down-regulated by seismic noise)

Gene Name	ESTs	E-Value	% ID	Align (aa or nt)	Accession	Species
Sensory Perception of Sound, Auditory Receptor Cell Organization (9 clones)						
integral membrane protein 2B	3	2E-145	100	256/256	ACI33031	*Salmo salar*
Alstrom syndrome protein 1	1	8E-26	40	78/193	Q8TCU4	*Homo sapiens*
coagulation factor C homolog, cochlin	1	4E-131	65	233/356	AAI62194	*Danio rerio*
integral membrane protein 2B	1	0	98	921/938	BT045039	*Salmo salar*
N-cadherin precursor	1	3E-72	82	153/185	CAA47890	*Danio rerio*
peripheral myelin protein 22	1	0	95	743/774	BT045468	*Salmo salar*
stereocilin	1	1E-16	47	46/96	AAL35321	*Mus musculus*
Other (44 clones)						
senescence-associated protein	16	3E-31	76	69/90	ACA04850	*Picea abies*
heme-binding protein 2	3	2E-105	100	183/183	ACI66639	*Salmo salar*
hemoglobin subunit alpha-4	3	0	99	666/668	BT059981	*Salmo salar*
glutamine synthetase	2	2E-125	99	232/234	AAM73660	*Oncorhynchus mykiss*
iron-sulfur cluster assembly enzyme ISCU, mitochondrial precursor	2	3E-13	75	40/53	ACI69709	*Salmo salar*
active breakpoint cluster region-related protein	1	3E-136	85	428/500	BT071954	*Salmo salar*
brain protein 44	1	3E-12	100	33/33	ACH70732	*Salmo salar*
collagen triple helix repeat-containing protein 1 precursor	1	4E-07	100	77/77	ACI66329	*Salmo salar*
dolichyl-P-Man :Man7GlcNAc2-PP-dolichyl-alpha-1, 6-mannosyltransferase	1	5E-114	100	221/221	ACI34126	*Salmo salar*
fatty acid-binding protein, heart	1	5E-72	100	135/135	ACI66142	*Salmo salar*
gap junction alpha-1 protein	1	0	98	777/786	BT059188	*Salmo salar*
heme-binding protein 2	1	9E-43	97	82/84	ACN10776	*Salmo salar*
hemoglobin subunit beta	1	1E-64	99	124/125	ACI68762	*Salmo salar*
hemoglobin subunit beta-1	1	1E-46	96	84/87	ACI66559	*Salmo salar*
histidine-rich glycoprotein precursor, putative	1	4E-11	46	53/115	EDP36098	*Brugia malayi*
integral membrane protein GPR177 precursor	1	0	98	531/541	BT059614	*Salmo salar*
leucine rich repeat containing 40	1	3E-80	99	154/155	ACH70736	*Salmo salar*
matrix-remodeling-associated protein 8 precursor	1	4E-106	90	202/224	ACN10981	*Salmo salar*
membrane protein	1	2E-19	100	57/57	BT049454	*Salmo salar*
prothymosin	1	5E-145	92	337/363	BT057594	*Salmo salar*
Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 precursor	1	4E-65	100	139/139	BT072024	*Salmo salar*
tensin-like C1 domain-containing phosphatase	1	0	99	652/654	BT072145	*Salmo salar*
transmembrane protein 43	1	1E-94	97	190/194	ACN11108	*Salmo salar*
Unknown (8 clones)						
BAC S0188I22 partial sequence	3	0	90	451/499	DQ156150	*Salmo salar*
DNA, dispersed repeat HpaI	2	7E-68	92	173/187	D32149	*Thymallus thymallus*
unknown large open reading frame	2	0	99	891/896	BT072322	*Salmo salar*
unknown large open reading frame	1	0	99	696/700	BT071900	*Salmo salar*
Uncharacterized (86 clones)	76	76				

ESTs from the reverse SSH cDNA library from salmon ear designed to be enriched for transcripts down-regulated in response to seismic noise. If an EST has no significant (E-value <10e-5) BLASTX hit, then the most significant BLASTN hit is shown in blue font. The GenBank nr or nt accession number is shown along with the species. Out of 50 contigs: 30 have a significant BLASTX hit (E<1e-05); 11 have a significant BLASTN hit. (E<1e-05); and 9 have no significant BLASTX or BLASTN hit (E<1e-05). Out of 193 singletons: 100 have a significant BLASTX hit (E<1e-05); 28 have a significant BLASTN hit (E<1e-05); 65 have no significant BLASTX or BLASTN hit (E<1e-05). "Align (aa or nt)" indicates extent (salmonid EST translation aligned with amino acid (aa) sequence of the best BLASTX hit or EST sequence aligned with nucleotide (nt) sequence of the best BLASTN hit) over the aligned region, along with percent identity (% ID) and the associated E-value. Functional categories are based on GO biological process or molecular function terms of best BLAST hit or of related sequences (e.g. putative human orthologues), or based on reported putative function. Reverse Library name is ssal_ear_SSH_seismicR.

orthologues), or based on reported putative function. In addition to this, Supplemental Tables S3 and S4 show the sequence length, GenBank accession numbers for individual (unassembled) sequences, and functional annotation including GO terms and description.

Functional categories from both the forward and reverse SSH cDNA libraries include: DNA binding, transcription and translation; energy metabolism; proteolysis; signaling (including synaptic transmission); ion transport; structural activity; cell death (apoptosis), regeneration (including axonogenesis); post-translational protein modification; stress-response; immune-relevant; otolith development; sensory perception and auditory cell organization; other functions; and unknown and uncharacterized sequences. A summary of the number and percentage of sequences assigned to each functional category is provided in Table 3.7 and Figure 3.3.

Sequences identified as having importance in DNA/RNA binding, transcription and translation represent the largest percentage of identified ESTs in both the forward and reverse SSH libraries, contributing 10.8% and 16.0% of all ESTs, respectively (Table 3.7; Figure 3.3). Of particular interest is the large number of sequences identified as transposases (12 ESTs overall) in the reverse library compared to the forward library (4 ESTs overall). In addition to transposases identified as salmonid genes, several are identified as putative orthologues of transposase genes from Leopard frog (*Rana pipiens*), Chinese perch (*Siniperca chuatsi*), European plaice (*Pleuronectes platessa*) (Table 3.6), and nematode (*Caenorhabditis briggsae*) (Table 3.5). Identification of these novel, potentially seismic-responsive, genes in Atlantic salmon ear signifies an interesting discovery in salmonid and seismic research.

The largest contig (*i.e.* having the highest number of contributing ESTs) in the forward SSH library had sixteen contributing sequences and was identified as Atlantic salmon cytosolic non-specific dipeptidase (CNDP) (GenBank accession number ACI34318; 99% identity over 357 aligned amino acids) (Table 3.5). A non-overlapping portion of Atlantic salmon CNDP sequence, identified by BLASTN, was also present in the forward SSH library with three contributing ESTs (GenBank accession number BT046056; 89% identity over 439 aligned nucleotides) (Table 3.5). Gene ontology for CNDP indicates metallopeptidase activity and importance in proteolysis. There

Table 3.7 Summary of sequenced ESTs and functional classification of salmon inner ear ESTs from forward and reverse SSH cDNA libraries

Functional Classification	*Forward Library*		*Reverse Library*		*All*
	# of ESTs	*ESTs (%)*	*# of ESTs*	*ESTs (%)*	*ESTs (%)*
DNA/RNA binding, transcription, translation	36	10.8	56	16.0	13.5
Energy, metabolism	33	9.9	37	10.6	10.3
Proteolysis	25	7.5	5	1.4	4.5
Signalling, synaptic transmission	23	6.9	21	6.0	6.2
Cytoskeleton structure and dynamics	23	6.9	16	4.6	5.7
Cell cycle, cell death, axonogenesis	17	5.1	22	6.3	5.7
Ion channel activity, ion transport	14	4.2	18	5.1	4.7
Protein post-translational modification, protein degradation, protein folding	11	3.3	9	2.6	2.3
Stress responsive	10	3.0	10	2.9	2.9
Immune responsive	10	3.0	7	2.0	2.5
Otolith development	7	2.1	2	0.6	1.3
Sensory perception of sound, auditory receptor cell organization	3	0.9	9	2.6	1.8
Other	30	9.0	44	12.6	10.8
Unknown	14	4.2	8	2.3	3.2
Uncharacterized	77	23.1	86	24.6	23.9
Total characterized ESTs	256	76.9	264	75.4	76.1
ESTs encoding a putative protein previously characterized in salmonids	203	79.3	200	75.8	77.5
ESTs encoding a putative protein not previously characterized in any salmonid	53	20.7	64	24.2	22.5
Total ESTs	333		350		

Functional categories are based on GO biological process or molecular function terms of best BLAST hit or of related sequences (e.g. putative human orthologues), or based on reported putative function.

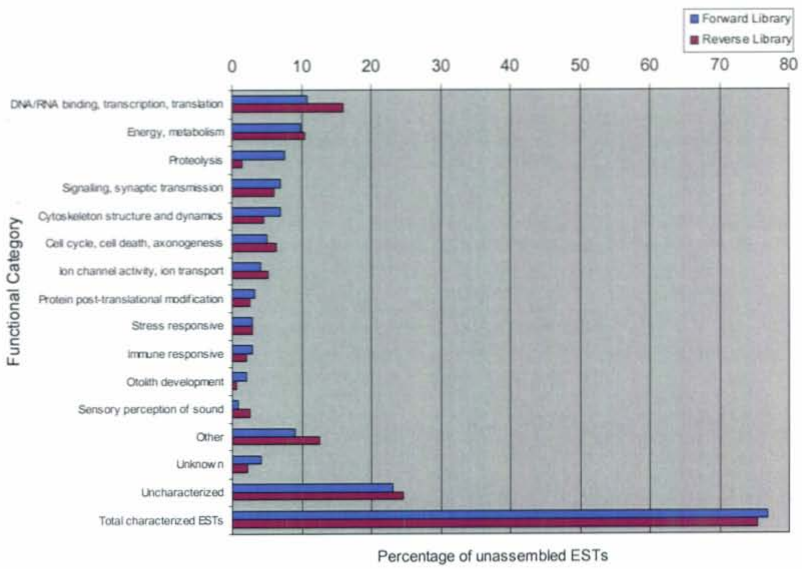


Figure 3.3 Gene classification of Atlantic salmon inner ear SSH cDNA libraries
Gene classification of inner ear suppression subtractive hybridization libraries from Atlantic salmon based on Gene Ontology biological process terms, molecular function or reported putative function. GO annotations were obtained from Swiss-Prot http://us.expasy.org/sprot.

are no sequences identified as CNDP in the reverse SSH library. QPCR analysis of this sequence revealed a 27.3 fold overall induction in seismic exposed compared to control ear (Table 3.4; Figure 3.4); however, due to high biological variability of CNDP transcript expression in seismic exposed individuals (SE 504.9) compared to control individuals (SE 17.7), the difference in relative quantity between groups is not statistically significant (P=0.19). A number of other sequences have been identified in the forward and reverse libraries as having important roles in proteolysis (Tables 3.5 and 3.6). Overall, ESTs identified as having importance in proteolysis account for 7.5% and 1.4% of total ESTs in the forward and reverse SSH cDNA library, respectively (Table 3.7; Figure 3.3).

One of the two second largest contigs in the forward library each having nine contributing ESTs was identified as a putative orthologue of Blue panchax (*Aplocheilus panchax*) cytochrome c oxidase subunit I (Genbank accession number BAG69248; 91% identity over 167 aligned amino acids). While Atlantic salmon (*Salmo salar*) cytochrome c oxidase subunit I was identified in the forward (2 ESTs) and the reverse (3 ESTs) SSH libraries, the Blue panchax (*Aplocheilus panchax*) putative orthologue of this gene was not identified in the reverse library, suggesting discovery of a novel seismic responsive salmon cytochrome c oxidase gene. The other second largest contig in the forward SSH library was identified as Atlantic salmon (*Salmo salar*) cytochrome c oxidase subunit II (GenBank accession number AAD04736; 95% identity over 209 aligned amino acids) (Table 3.5). This gene was also identified as the second largest contig in the reverse SSH library, however, with thirteen contributing ESTs (GenBank accession number AAD04736; 94% identity over 212 aligned amino acids) (Table 3.6). The presence of this transcript in both forward and reverse SSH libraries suggests that it was an abundant transcript in the tester and driver RNA samples used to generate the reciprocal SSH libraries. When sorted by gene ontology, identified ESTs with functions related to energy metabolism represent 9.9% and

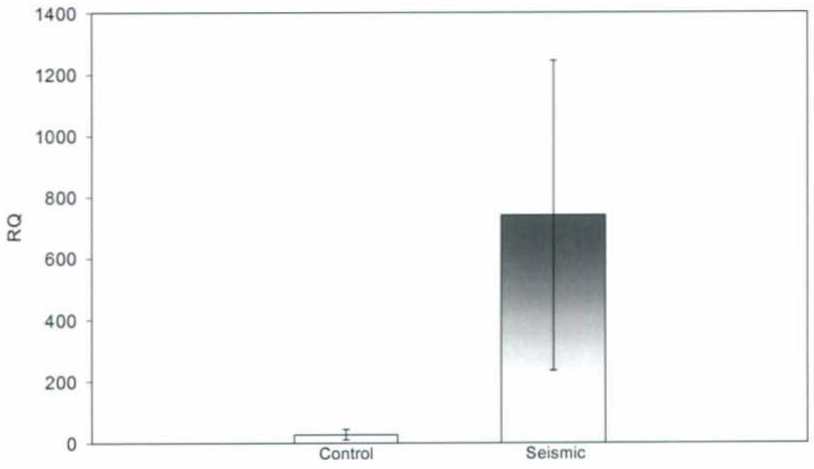


Figure 3.4 QPCR results for cytosolic non-specific dipeptidase (CNDP)
QPCR results for CNDP from salmon ear forward SSH cDNA library. There were n=2 reactions per individual fish, 16 control and 16 seismic exposed salmon, normalized to 60S ribosomal L6. Relative Quantity (RQ) is shown on the Y axis. Mean fold change in RQ and corresponding *P*-value is shown below graph. A '+' prefix for the RQ ratio indicates up-regulated in seismic compared to control (*i.e.* seismic mean / control mean > 1). Error bars for are for SE. QPCR amplification products were run on an agarose gel to verify product size (see Materials and Methods).

10.6% of unassembled sequences from the forward and reverse SSN cDNA libraries, respectively (Table 3.7; Figure 3.3).

There are approximately the same number of sequences identified with gene names or functional annotation suggesting importance in signaling, including synaptic transmission, in both the forward and reverse SSH cDNA libraries; however, there are different genes (or different relative abundance of specific genes) identified in each library. The largest contig in the forward SSH cDNA library with a signaling-relevant functional annotation was identified as myelin P0 protein precursor with six contributing sequences (GenBank accession number BT058974; 100% identity over 695 aligned nucleotides). Myelin P0 protein precursor (GenBank accession number BT058974; 100% identity over 583 aligned nucleotides) was also identified in the reverse library but with only one contributing EST. Other notable differences in signaling-relevant genes are the identification of platelet-derived growth factor receptor-like protein precursor (3 ESTs) (gene ontology classification: olfactory receptor activity) in the forward SSH library, but not in the reverse SSH library, and identification of follistatin-related protein 1 precursor, represented by six ESTs (not all from the same contig) in the reverse SSH cDNA library, but not in the forward SSH cDNA library. Also, growth hormone-1 had four contributing ESTs in the reverse library and only one EST in the forward SSH library. Novel potentially seismic responsive signaling-relevant genes identified in either the forward or reverse SSH cDNA library include (but are not limited to) human (*Homo sapiens*) putative orthologues for myelin basic protein (identified only in the forward SSH cDNA library), thyroid hormone receptor interactor 12, isoform CRA_l (reverse SSH cDNA library only) and tyrosine-protein kinase RYK (identified only in the reverse SSH cDNA library).

Several potentially different transcripts were identified in both the forward and reverse SSH cDNA libraries with gene ontologies or reported functions related to cell death/regeneration (often associated with stress response) and with regulated protein turnover. S100-B (GenBank accession number ACM08316; 100% identity over 124 aligned amino acids) (Table 3.5) was identified in the forward SSH library with three contributing sequences, but was not identified in

the reverse SSH library. S100-B has the gene ontology term: axonogenesis and cell proliferation. Also, the reverse SSH library had a larger proportion of sequences identified as insulin-like growth factor-binding proteins (1.5%) than the forward SSH library (0.3%). Several other sequences identified as playing a role in apoptosis and cell death/proliferation were identified in both libraries (Tables 3.5 and 3.6). Stess-responsive genes, often associated with processes related to cell death, were identified in the forward and reverse SSH libraries. Of particular interest is the relatively large number of different heat shock protein-encoding transcripts identified in the reverse SSH library (7 ESTs of HSP10, HSP90beta, HSP70, HSC71) compared to the forward SSH library (3 ESTs of HSP8). Also, genes important in oxidative stress response predominate in the forward compared to the reverse SSH library (Tables 3.5 and 3.6).

In addition to genes identified as having important roles in cell death and stress response, there were a number of genes identified in both the forward and reverse SSH libraries that are important in post-translational protein modification (regulation of protein turnover). Different genes important in the ubiquitin-proteasome pathway are represented in the forward and reverse SSH libraries. In the forward library, four different transcripts were identified as ubiquitin-conjugating enzymes, including a putative orthologue of human ubiquitin-conjugating enzyme 16, with 97% identity at the amino acid level; in the reverse library, a putative orthologue of catfish (*Ictalurus punctatus*) ubiquitin and ribosomal protein S27a precursor (1 EST), and s-phase kinase-associated protein 1 (1 EST) which were identified as reproducibly down-regulated greater than 1.75-fold in the microarray study (Table 3.3). A few genes identified as different proteasome subunits are present in both libraries (Tables 3.5 and 3.6).

While there were relatively few immune responsive genes identified in the SSH libraries (2.5%) (Table 3.7; Figure 3.3), it is interesting to note that two of the largest contigs in this category were only in the forward SSH library. These two contigs were identified as beta-2-microglobulin precursor (3 ESTs) and a putative orthologue of chicken (*Gallus gallus*) Ig-superfamily protein (3 ESTs), which is a salmon transcript encoding a putative protein that has not been characterized in Atlantic salmon (*Salmo salar*) (as evidenced by the absence of that

salmon protein sequence in the GenBank non-redundant amino acid sequence database). Also interesting to note is that the largest contig important in ion transport was in the forward SSH cDNA library only and is a putative orthologue of human integral membrane transporter protein (7 ESTs) (GenBank accession number CAB81951; 100% identity over 34 amino acids). While this gene was not identified in the reverse SSH library, several genes with roles in ion channel activity and ion transport were present in both forward and reverse SSH libraries (Tables 3.5 and 3.6).

Forward and reverse libraries contain sequences with gene ontology terms related specifically to ear, including sensory perception of sound, auditory cell development and otolith development. Together, genes assigned to these functional categories represent the least relative abundant (*i.e.* rare) transcripts: 1.8% for sensory perception of sound, auditory receptor cell organization and 1.3% for genes important in otolith development (Table 3.7; Figure 3.3). In the forward SSH cDNA library there are three sequences identified as important in sensory perception of sound: two separate ESTs identified as Atlantic salmon (*Salmo salar*) peripheral myelin protein 22 (GenBank accession number ACI33730; 90% identity over 82 aligned amino acids; GenBank accession number BT045468, 97% identity over 863 aligned nucleotides) and Atlantic salmon (*Salmo salar*) integral membrane protein 2B (GenBank accession number ACI33031; 98% identity over 100 aligned amino acids) (Table 3.5). In contrast, there are seven genes identified in the reverse SSH library (9 ESTs) with important functions in sensory perception or auditory cell organization, including two sequences identified as Atlantic salmon (*Salmo salar*) integral membrane protein 2B (4 ESTs) (GenBank accession number ACI33031; 100% identity over 256 aligned amino acids) and peripheral myelin protein 22 (1 EST, GenBank accession number BT045468; 95% identity at the nucleotide level), both also identified in the forward library. Other sensory-relevant genes identified in the reverse SSH library were salmon putative orthologues of zebrafish (*Danio rerio*) coagulation factor C homolog, cochlin (GenBank accession number AAI62194; 65% identity over 356 aligned amino acids), zebrafish (*Danio rerio*) N-cadherin precursor (GO terms auditory receptor cell stereocilium organization and semicircular canal morphogenesis; GenBank accession number CAA47890; 82% identity over 185 aligned

amino acids), human Alstrom syndrome protein 1 (GenBank accession number Q8TCU4; 40% identity over 193 aligned amino acids) and mouse (*Mus musculus*) stereocilin (GenBank accession number AAL35321; 47% identity over 96 aligned amino acids) (Table 3.6). Identification of these novel Atlantic salmon ear sequences potentially responsive to seismic noise exposure will contribute significantly to salmonid literature.

A few genes were identified in both the forward and reverse SSH cDNA libraries that have roles in the biological process of otolith development, or are reported to be important in otolith development. In the forward SSH cDNA library these include three sequences identified as SPARC precursor (6 ESTs) and a putative orthologue of cow (*Bos taurus*) chondromodulin-I (1 EST) (Nemoto *et. al.*, 2007; GenBank accession number AAA30445; 78% identity at the amino acid level) (Table 3.5). In the reverse SSH cDNA library genes identified as having importance in otolith development include putative orthologues of rainbow trout (*Oncorhynchus mykiss*) otolith matrix macromolecule-64 (1 EST) (GenBank accession number BAG14384; 94% identity at the amino acid level) and zebrafish (*Danio rerio*) otolin 1 (1 EST) (GenBank accession number CAN88052; 80% identity at the amino acid level) (Table 3.6).

Several sequences were identified as genes with important functions related to cytoskeletal structure and dynamics, important in hair cell structure and function, with a larger percentage of ESTs identified in the forward SSH cDNA library (6.9%) compared to the reverse SSH cDNA library (4.6%) (Table 3.7; Figure 3.3) The largest contig listed under this functional category present in the forward and reverse SSH libraries is a putative orthologue of zebrafish (*Danio rerio*) matrilin-4 gene (matn4), with four contributing ESTs in the forward SSH cDNA library (GenBank accession number AAI63867; 83% identity at the amino acid level) and six contributing ESTs in the reverse SSH cDNA library (GenBank accession number AAI63867; 81% identity at the amino acid level). Matn4 has been reported to be an important structural protein in mouse inner ear (Klockars *et. al.*, 2002).

Identified sequences with little or no known function, or not fitting into the functional categories listed are reported under "other" (Tables 3.5 and 3.6). These sequences represent

about 11% of all 683 ESTs from both libraries (Table 3.7; Figure 3.3). Included in this functional category is the largest contig in the reverse library (*i.e.* having the highest number of contributing ESTs) which was identified by BLASTX as a putative orthologue of Norway spruce (*Picea abies*) senescence-associated protein (16 ESTs) (GenBank accession number ACA04850, 76% identity over 90 amino acids). This contig was also identified in the forward library with only four contributing ESTs (GenBank accession number ACA04850; 77% identity over 87 amino acids) (Table 3.6). Sequences with a significant BLASTN hit but with no assigned gene name are listed under "unknown" and represent about 3% of all 683 ESTs while those sequences with no significant BLASTX or BLASTN hit are listed as "uncharacterized", and represent about 24% of all 683 ESTs. Atlantic salmon (*Salmo salar*) sequences encoding a putative protein that have not been characterized in any salmonid account for 23% of the 520 characterized ESTs (Tables 10-12).

4.0 Discussion

The purpose of this study was to investigate the physiological stress response as well as changes in gene expression in Atlantic salmon (*Salmo salar*) inner ear induced by intense noise exposure generated from a seismic airgun. Qualitative observations were also made on behavioral changes in response to noise at the following pre-sampling time points: during seismic noise exposure; immediately following exposure; and sixteen hours post exposure. Physiological stress response was determined by measurement of plasma cortisol concentration, which demonstrated no detectable difference between control and exposed groups. Transcript expression response to seismic stress was studied using 16K salmonid cDNA microarrays as well as through the creation and characterization of reciprocal SSH cDNA libraries designed to be enriched for genes up-regulated or down-regulated by seismic noise exposure in inner ear of Atlantic salmon. The microarray study revealed 42 different transcripts reproducibly up-regulated and 37 different transcripts reproducibly down-regulated in response to seismic noise exposure in salmon inner ear (Tables 3.2 and 3.3). Gene lists provided insights into effects of noise induced

stress on salmon inner ear, and revealed alteration in the expression of transcripts with functional annotations important in metabolism, signaling, iron homeostasis, structural scaffolding, regulation of transcription/translation, and stress response [including cell cycle/cell death (apoptosis), post-translational protein modification and immune response]. Candidate molecular biomarkers of seismic noise induced stress were selected from the lists of reproducibly informative genes for validation by QPCR (Table 3.4; Figure 3.2). Development and characterization of reciprocal SSH cDNA libraries from seismic exposed salmon ear provided: 1) general information on the molecular activities that may be altered in ear in response to noise exposure; 2) at least one potential molecular biomarker (cytosolic non-specific dipeptidase) of noise induced stress (confirmed by QPCR) (Table 3.4; Figure 3.4); and 3) discovery and characterization of novel genes expressed in salmon inner ear.

4.1 Physiological Stress Response

Physiological stress response was determined by measurement of plasma cortisol concentration. Blood plasma cortisol concentration is often used as a primary indicator of stress (Mazeaud *et al.*, 1977) and has been used to demonstrate a biochemical response to acoustical stress in various fish species (*e.g.* Santulli *et al.*, 1999; Smith *et al.*, 2004; Wysocki *et al.*, 2006; Buscaino *et al.*, 2010). Primary stress response is the perception of an altered state by the central nervous system (CNS) and the release of cortisol along with catecholamines into the bloodstream by the endocrine system (Randall and Perry, 1992). In the present study the mean plasma cortisol concentrations were 68.1 ng/ml (SE 11.5) and 77.0 ng/ml (SE 13.8) for control (n=16) and seismic exposed fish (n=17), respectively (Table 3.1; Figure 3.1). While the mean cortisol concentration was slightly greater in seismic exposed fish compared to control fish, the difference was not statistically significant (P=0.63). The range of plasma cortisol concentration was 2.7 - 146.8 ng/ml for control and 4.4 - 187.4 ng/ml for seismic exposed fish. While cortisol levels of 150 ng/ml or more have been recorded in salmonids (Barton *et al.*, 1986; Pickering and Pottinger, 1989), normal cortisol concentration for non-stressed salmonids is considered to be <10 ng/ml

(Pickering and Pottinger, 1989). In the present study, higher than normal cortisol concentration in individuals from the control group, as well as the exposed group, are reported.

Primary stress responses, such as cortisol release, may be the result of confinement, dip net capture, and handling (Wedemeyer *et al.*, 1976; Strange *et al.*, 1977; Waring *et al.*, 1992; Demers and Bayne, 1997; Barton *et al.*, 2005; Veiseth *et al.*, 2006; Fast *et al.*, 2008; Hori *et al.*, 2010). Rotllant and Tort (1997) suggested that cortisol is released in similar quantities after acute stress in red porgy (*Pagrus pagrus*) regardless of previous exposure to chronic stress. This may also be the case in our study on Atlantic salmon where elevated cortisol levels from both control and exposed groups may be due to stress from acute handling during capture. However, Hori *et al.* (2010) demonstrated a significant elevation of plasma cortisol in cod (*Gadus morhua*) at 3, 12 and 24 hours following heat stress/handling compared to control fish subjected only to handling. The plasma cortisol response (and degree of response) may be different for different types of stress and in different species.

In addition to possible effects of handling on plasma cortisol in the current study, it is possible that we have missed peak cortisol levels since it is reported in studies on acute stress that the cortisol response in most fish is rapid, reaching maximum concentration within 1 hour after being stressed (Iwama *et al.*, 2006). A significant increase in plasma cortisol might have been detected in the current study had we sampled at earlier time points post-stress, however, the goal of the study was to identify molecular biomarkers of seismic stress (*i.e.* that could be useful for assessing the potential impact of seismic noise on wild fish), in which case a gene that responds within a few hours and rapidly goes back to pre-stress levels would not be very useful. By choosing the sixteen hour post-stress time point, we increased the likelihood of identifying molecular biomarkers of seismic stress exposure that might be useful with field-collected fish ear tissue samples. So while cortisol response is a useful measure of primary stress in acute stress experiments (Martinez-Porchas *et al.*, 2009), the timing of plasma cortisol response (and degree of response) may be different for different types of stress and in different species. To fully study

cortisol response in Atlantic salmon to seismic noise, a dedicated experiment that measures plasma cortisol before, during, and at several time points post-stress would be necessary.

Qualitative observations made during exposure of animals revealed an initial startle response for approximately the first three airgun blasts, followed by little activity for the remainder of the exposure (~10 minutes; 50 airgun blasts). Exposure to intense noise has been shown to induce a startle response in fishes (*e.g.*, Blaxter *et al.*, 1981; Pearson *et al.*, 1992; Wardle *et al.*, 2001; Hassel *et al.*, 2004; Smith *et al.*, 2004). In addition to the initial startle response, a difference in net-avoidance and swimming speed between control and seismic exposed groups was observed during sampling. Fish from the control group demonstrated little swimming activity and were therefore very easy to capture, whereas fish from the seismic exposed group demonstrated rapid and erratic swimming activity (compared to controls) during attemped capture. An increase in swimming speed in response to noise induced stress has also been noted in six-lined trumpter (*Pelates sexlineatus*; McCauley *et al.*, 2000), goldfish (*Carassius auratus*; Smith *et al.*, 2004), European sea bass (*Dicentrarchus labrax* L.; Buscaino *et al.*, 2010) and gilthead bream (*Sparus aurata* L.; Buscaino *et al.*, 2010). An increase in swimming activity indicates an increase in overall metabolic rate which implies a higher demand for oxygen. Increased swimming activity and the associated metabolic costs could compromise other biological activities, such as food acquisition, homeostasis due to environmental stressors, migration and reproduction (Buscaino *et al.*, 2010).

4.2 Alteration in Gene Expression

The hearing capabilities of fishes are quite refined; the basic functions of the fish auditory system are very similar to those of terrestrial vertebrates including mammals (Fay and Popper 2000). The principal difference in the acoustic system in fishes compared to that of more recently evolved vertebrates is that fish only have an inner ear (a membranous sac). In most fishes this consists of three semicircular canals and three otolithic end organs (utricle, saccule, and lagena) (Popper and Coombs, 1980; Platt and Popper, 1981) while higher vertebrates have an outer and

middle ear, in addition to highly elaborate inner ear structures for sound detection and analysis (basilar papilla in birds, cochlea in mammals) (Fay and Popper, 2000). The sensory structures of the semicircular canals and otolithic end organs, cristae and macula, respectively, contain mechanoreceptive hair cells which are associated with branches of the eighth auditory nerve (Saidel and Popper, 1983). These sensory hair cells which detect sound waves and acceleration are remarkably conserved among vertebrates despite differences in the ear structure between fishes and other vertebrates (Platt 1993; Popper and Fay 1993).

The development of high-throughput methods in functional genomics has permitted new advances in uncovering some of the molecular events in the mammalian inner ear following noise exposure, ranging from transient metabolic stress in the cells to mechanical disruption of the sensory epithelium (Lomax *et al.* 2001; Taggart *et al.*, 2001; Cho *et al.*, 2004; Kirkegaard *et al.*, 2006; Sun *et al.*, 2008). While studies in fish have demonstrated the behavioral, physiological and anatomical changes associated with intense noise exposure (reviewed in Popper and Hastings, 2009), the changes in gene expression that underlie these biological changes have been investigated in only one study to date, on zebrafish (*Danio rerio*) (Schuck *et al.*, 2009; meeting abstract). To fill this knowledge gap, a functional genomics approach was taken to investigate the potential effects of seismic noise exposure on gene expression in Atlantic salmon inner ear, and to develop a set of potential biomarkers for noise induced stress. Genomics research tools and techniques used in this study are (1) cDNA microarrays and (2) SSH cDNA library construction and characterization.

This is the first study to examine changes in gene expression in response to seismic noise exposure in any fish species and only the second study to investigate the changes in gene expression in fish in response to high intensity sound using a functional genomics approach. This is also the first time cDNA libraries from salmon inner ear have been constructed, and novel genes discovered represent a significant contribution to salmonid genomics research.

4.2.1 Microarray-identified reproducibly informative gene set

In this study, successful application of 16K salmonid cDNA microarrays to study the impact of seismic sound exposure on global gene expression in inner ear from pre-reproductive (smolt) Atlantic salmon (*Salmo salar*) was demonstrated. DNA microarrays have been used to identify genes responsible for deafness in human cochlear and vestibular tissues (Abe *et al.*, 2003) as well as genes responsive to intense noise exposure in rat cochlea (Lomax *et al.*, 2001; Cho *et al.*, 2004; Kirkegaard *et al.*, 2006), chinchilla cochlea (Taggart *et al.*, 2001) and more recently zebrafish whole inner ear (Schuck *et al.*, 2009; meeting abstract).

4.2.1.1 Energy and metabolism. Differential expression of several transcripts encoding genes important in cellular respiration, including those with roles in oxygen transport, the glycolytic pathway, the citric acid cycle, and the electron transport chain are reported in Tables 3.2 and 3.3. Four different transcripts encoding hemoglobin genes were identified in the microarray study as being up-regulated in salmon ear in response to seismic noise exposure: hemoglobin beta-1 (2.6-fold), hemoglobin beta (2.5-fold), hemoglobin alpha-4 (2.3-fold) and hemoglobin alpha (2.1-fold). These were represented by a total of eight features (*i.e.* spots) on the microarray. QPCR validated the increase in the transcript encoding hemoglobin alpha-4 showing an overall increase of 3.8-fold (P=0.01) in seismic exposed ear relative to control ear (Table 3.4; Figure 3.2A). Higher levels of hemoglobin in ear following exposure to intense noise may be due to tissue rupture or hemorrhage. High level acoustic exposures have been shown to cause hemorrhage and physical damage to fishes (*reviewed in* Hastings and Popper, 2005), including damage to hair cells (Enger, 1981; Hastings *et al.*, 1996; McCauley *et al.*, 2003). Increased hemoglobin may also imply a higher demand for oxygen in the seismic exposed fish compared to control fish, due to an increase in metabolic activity.

The tricarboxylic acid (TCA) cycle is a central pathway of metabolism and also functions in a biosynthetic capacity, including synthesis of heme through succinyl-coenzyme A (CoA). Depletion of glucose appears to be the major mechanism for the increased expression of TCA

cycle enzymes necessary for oxidative metabolism, leading to a 3- to 10-fold increase in TCA cycle mRNAs (DeRisi *et al.*, 1997). Several transcripts representing genes important in energy metabolism with important roles in the citric acid cycle or electron transport chain were differentially regulated in response to seismic noise exposure in Atlantic salmon ear (Tables 3.2 and 3.3). Changes in metabolism have been reported in relation to noise-induced stress and in a previous microarray study; a time-dependent (measured up to six hours) noise induced change in genes important in the citric acid cycle and the electron transport chain were reported following intense noise exposure in chinchilla cochlea (Taggart *et al.*, 2001). Cytochrome c oxidase, which is highly expressed in teleost hair cells (Saidel and Crowder, 1997), has been shown by immunohistochemistry to decrease in the cochlea of albino guinea pigs in response to acoustic trauma (Hsu *et al.*, 1998). Hsu *et al.*, (1998) suggested that a decrease in cytochrome c oxidase activity implies that metabolic damage may play a role in noise-induced hearing loss. The microarray results presented here show altered regulation of different subunits of cytochrome c oxidase encoding transcripts in salmon ear, further confirming the potential for metabolic disturbance in response to intense noise exposure. Of particular interest is the transcript encoding succinyl-CoA ligase beta subunit, which I have shown was down-regulated in response to noise exposure (Table 3.3). A mutation in the gene encoding the beta subunit of succinyl-CoA ligase has been reported to be responsible for a specific mitochondrial brain disease, characterized by severe hearing impairment (Ostergaard *et al.*, 2007).

Neurons require large amounts of energy to support their survival and function, and are therefore susceptible to excitotoxicity, a form of cell death involving bioenergetic stress (Liu *et al*, 2009). Nicotinamide adenine dinucleotide (NAD+) is an essential co-factor for metabolic and gene regulatory pathways that control life and death. Nicotinamide riboside, a recently discovered third vitamin precursor of NAD+ in eukaryotes, is converted to NAD+ in a novel salvage pathway, the nicotinamide riboside kinase (Nrk) pathway (Tempel *et al.*, 2007). It has been reported that Nrk2 was induced >20-fold at 14 days after neuronal injury, and other NAD-synthesizing enzymes increased 2- to 8-fold, in mouse neuronal cell culture, suggesting that pathways that synthesize

NAD+ are activated after neuronal injury. These pathways enable increased NAD+ production for downstream functions associated with the neuronal response to injury (Sasaki *et al.*, 2006). Two transcripts for Nrk2 are reproducibly up-regulated (2.9-fold, SE 0.3; 1.8-fold, SE 0.2) in salmon ear in response to seismic noise exposure in the microarray study (Table 3.2). QPCR confirmed up-regulation of this gene (1.9-fold; P=0.02) (Table 3.4; Figure 3.2B). Nrk2 up-regulation in response to noise exposure in salmon ear points to evidence for neuronal protection in ear through increased production of NAD+: Nrk2 may potentially be an important biomarker for seismic noise exposure in salmonids and other fish species.

4.2.1.2 Protein post-translational modification or degradation. The ubiquitin–proteasome pathway (UPP), which functions in the process of protein turnover in eukaryotic cells, consists of the combined action of enzymes that link chains of ubiquitin onto proteins to mark them for degradation by the 26S proteasome, a very large multicatalytic protease complex that degrades ubiquitinated proteins to small peptides (Lecker *et al.*, 2006). Three enzymatic components are required to link chains of ubiquitin onto proteins that are destined for degradation: ubiquitin-activating enzyme (E1), ubiquitin conjugating-enzymes (E2), and ubiquitin ligase (E3), which is the key enzyme in the process because it recognizes a specific protein substrate and catalyzes the transfer of activated ubiquitin to it (Alberts *et al.*, 2008). Induction of the gene encoding E3 ubiquitin ligase has been previously reported in injured tissues of chick cochlea immediately following noise exposure (Lomax *et al.*, 2000). Lomax *et al.* (2001) infer that E3 ubiquitin ligase may play a protective role in either the classic stress response or in the stress response invoked by oxidative damage, as it is homologous to an oxidative stress responsive gene. However, microarray results presented here reveal a down-regulation of s-phase kinase associated protein 1A (a putative orthologue of human E3 ubiquitin ligase (100% identity) (2.0-fold, SE 0.6) (Table 3.3) in salmon ear in response to seismic noise exposure. In addition to down-regulation of E3 ligase-encoding transcript, microarray results point to an overall suppression of genes important in the UPP in response to noise exposure, whereas there is an induction of genes important in

other pathways of post-translational protein modification. Additional genes important in the UPP down-regulated in response to intense noise exposure in salmon ear include proteasome subunit beta type-1A (3.0-fold, SE 1.1), proteasome subunit beta type-4 precursor (2.3-fold, SE 0.2) and proteasome subunit beta type-5 precursor (1.9-fold, SE 0.5) (Table 3.3). Ubiquitin carboxyl terminal hydrolase isozyme L1 was up-regulated in response to noise exposure in salmon (2.3-fold, SE 0.4) (Table 3.2). Ubiquitin C-terminal hydrolases (UCHs) are thought to be essential for deubiquitination activity by releasing ubiquitin from its substrates (Kwon *et al.*, 2004). UCHs have been shown to be highly expressed in the peripheral nervous system of mammalian species, including in the sensory and motor nerves, and are important in preventing neurodegeneration (Chen *et al.*, 2010). Down-regulation of genes important in the UPP, along with increased expression of a transcript important in deubiquination (UCH), points to an overall down-regulation of regulated protein degradation machinery via the UPP in response to noise induced stress in salmon ear. It is interesting to note that Payne *et al.* (2007) have reported a decrease in serum protein in lobster (*Homarus americanus*) exposed to seismic noise suggesting a disturbance in protein synthesis. In the microarray study, ubiquitin family domain-containing protein 1 was the most up-regulated transcript (5.0-fold, SE 2.2) (Table 3.2) in response to noise induced stress in salmon ear. While there are no gene ontology terms assigned to this protein, it is reported to be important in protein modification (Fenner *et al.*, 2009). Peptidyl-propyl cis-trans isomerase, important in post-translational protein modification, was also up-regulated in salmon ear in response to seismic noise exposure (1.8-fold, SE 0.3) (Table 3.2) and has previously been reported to be up-regulated in a time-dependent manner in response to intense noise exposure in chinchilla cochlea (Taggart *et al.*, 2001). Cells contain multiple proteolytic systems to carry out the degradation process and complex regulatory mechanisms to ensure that the continual proteolytic processes are highly selective; therefore, excessive breakdown of cell constituents is prevented (Lecker *et al.*, 2006). Together, differential regulation of genes important in regulated protein modification point to evidence for depression of UPP genes and an increase in alternative processes involved in protein modification that may affect important physiological processes in

the cell in response to noise exposure, which may include an additional source of amino acids for new protein synthesis or energy production.

4.2.1.3 Cell cycle / cell death, axonogenesis. Throughout the life of eukaryotes many cells die, either during tissue remodeling, or due to injury or mechanical stress. Regulated cell death (*i.e.* apoptosis) is important during development and maintenance of tissues (Cambi and Figdor, 2009). Caspases are a family of cysteine proteases that are critical in destruction of cell constituents during apoptosis (Masumichi *et al.*, 2006). Smith *et al.* (2006) observed increased apoptotic activity in goldfish (*Carassius auratus*) ear tissues 0-2 days following noise exposure, coinciding with the period of greatest hair cell loss, suggesting that hair cells were dying due to cell death. Also, altered expression of genes involved in regulation of apoptosis has been reported in mammalian ear tissues following noise exposure (discussed in Kirkegaard *et al.*, 2006). Microarray-identified transcripts encoding genes involved in apoptosis that are differentially-regulated in salmon ear following seismic noise exposure include caspase-8 precursor (2.1-fold up-regulated, SE 0.2) (Table 3.2) and B-cell receptor-associated protein 31 (2.7-fold down-regulated, SE 0.8) (Table 3.3). However, QPCR results did not confirm up-regulation of caspase 8 precursor-encoding transcript (Table 3.4; Figure 3.2E). Several other transcripts encoding genes important in cell cycle and neuroregeneration were identified in the microarray experiment as being differentially expressed in seismic exposed and control salmon ears (Tables 3.2 and 3.3). Of particular interest is down-regulation of the transcript encoding calcium binding protein S100-B (2.3-fold, SE 0.5) (Table 3.3) in the microarray study (right ear) and the identification of the transcript encoding calcium binding protein S100-B (3 ESTs) in the forward SSH cDNA library only (left ear), which was designed to be enriched for transcripts up-regulated in response to seismic noise exposure (Table 3.5). Calcium binding protein S100 has been identified in the neurons and secretory cells of the developing avian ear (Fermin *et al.*, 1995). Early induction of S100 protein has been demonstrated in response to: 1) intense noise exposure in chinchilla cochlea (Taggart *et al.*, 2001); and 2) destruction of the vestibular

epithelium in guinea pig. In the latter case early enhanced expression of S100 in the side contralateral to the lesion was followed by a decrease one day post-tauma indicating a shift in S100 in response to inner ear trauma (Rickmann *et al.*, 1995). While QPCR would be required to determine if this gene is up- or down-regulated by seismic noise exposure, differences in gene expression would be expected between right and left ear if the fish received more exposure on one side relative to the other due to the animal's orientation with respect to the source. Assymetric exposure could cause increased trauma or injury in one side relative to the other due to increased exposure.

4.2.1.4 Immunity. Unlike apoptosis, non-programmed cell death: 1) is induced by injury, leading to necrosis; 2) is characterized by disturbance of energy metabolism, disruption of cellular membranes and the release of cytoplasmic and nuclear components into the extracellular environment; and 3) can induce inflammatory immune responses leading to defense and repair mechanisms (Cambi and Figdor, 2009). Recent studies have shown that C-type lectins, a family of surface receptors known to recognize microbial carbohydrate moieties, also sense products from dying cells and transduce inflammatory signals that modulate the immune system (Cambi and Figdor, 2009). Our microarray study showed a 2.4-fold induction of the transcript encoding C-type lectin receptor A in seismic exposed ear compared with control ear, and QPCR confirmed the up-regulation of this transcript (Table 3.4; Figure 3.2H). As seen with other noise induced genes reported in this study there is a high biological variability among individuals from the seismic exposed group, suggesting that there are differences in the sensitivity of different individual ears to stress-induced by noise exposure. Other transcripts with immune-relevant functional annotations that reproducibly responded to seismic noise exposure in salmon inner ear are listed in Tables 3.2 and 3.3.

4.2.1.5 Signaling, synaptic transmission. The neuroendocrine system provides an interface between external stimuli and organ systems so that physiological challenges (*i.e.*, stresses) are met with adaptive reactions, initiated by neuroendocrine signals (Dorshkind and Horseman, 2001). Multiple studies have suggested that growth hormone is produced during stress and that growth hormone, together with insulin growth factors, act as antagonists to glucocorticoids, playing a role in the stress response and immune function (Kelley and Dantzer, 1991). Our microarray results revealed a 3.4-fold (SE 0.8) increase in growth hormone 1-encoding transcript in salmon ear in response to noise exposure, with an increase of 7.8-fold demonstrated by QPCR (Table 3.4; Figure 3.2F). Again, there was high biological variability in the QPCR data for the seismic exposed group (the *p*-value was greater than the set threshold of 0.05). This high biological variability in biomarker transcript expression may be a reflection of individual variability in response to stress, and/or differences in timing of transcript induction between individuals (*i.e.* some animals may have passed peak expression of growth hormone-encoding transcript or may not have reached peak expression at time of sampling). Shuck *et al.* (2009) demonstrated a 30-fold increase in growth hormone encoding transcript in zebrafish inner ear two days post noise exposure with levels decreasing four days post exposure, whereas a decrease in growth hormone gene expression has been detected in mammalian ear in early response to noise exposure associated with a temporary threshold shift (TTS) (Cho *et al.*, 2004). Growth hormone induction in salmon ear in response to seismic noise exposure may act to restore homeostasis in inner ear in response to stress, possibly through stimulation of the immune system.

In addition to growth hormone, thyroid hormones can act as immunostimulatory mediators, which are particularly important during the response to environmental and physiological stress. Transthyretin is a carrier for both thyroxine (a thyroid hormone), as well as for retinol through binding to the retinol-binding protein, and it has been demonstrated to play an important role in nerve regeneration (Fleming *et al.*, 2007). Microarray-identified transcripts encoding transthyretin and retinol binding protein 1 were up-regulated (2.1-fold, SE 0.6 and 2.3-fold, SE 0.2, respectively) (Table 3.2) in seismic exposed salmon ear, while retinoid x receptor

beta a-encoding transcript was down-regulated (1.8-fold, SE 0.1) (Table 3.3). Cellular retinol-binding protein type I and retinoid x receptors are important in retinoic acid biosynthesis, which is reported in the developing sensory epithelium of rat inner ear (Ylikoski *et al.*, 1994). Retinoic acid may play a role in damage-induced hair cell regeneration in developing and mature inner ear organs as well as in the developing auditory organ (Ylikoski *et al.*, 1994) and is implicated in playing a role in anti-apoptosis in mammalian systems in response to intense noise exposure in outer hair cells (Ahn *et al.*, 2005). Alteration of expression of transcripts important in retinoic acid biosynthesis and thyroid hormone transport in inner ear of salmon may point to protective mechanisms via immunostimulation or anti-apoptosis, as well as a role in neuroregeneration.

Calmodulin, important in the G-protein coupled receptor protein signaling pathway (gene ontology biological process term of a human putative orthologue), is a major intracellular calcium receptor which maintains intracellular calcium homeostasis in many tissues including hair cells, and as a result protects the cytoskeleton from degradation (Zuo *et al.*, 2008). Calmodulin participates in the automatic movement of outer hair cells and regulates hearing acuity based on the calcium sensitive contraction process. Microarray-identified calmodulin 2-encoding transcript was down-regulated (2.3-fold, SE) (Table 3.3) in salmon ear in response to seismic noise exposure. However, acoustic overstimulation has been shown to cause an increase in calmodulin 3 gene expression in chinchilla inner ear (Taggart *et al.*, 2001) and an increase in calmodulin protein expression in guinea pig cochlear hair cells (Zuo *et al.*, 2008). Direct comparison of gene expression between this study on salmon inner ear and studies on mammalian ear is difficult since studies on mammalian systems target specific organs or tissues within the ear, and the salmon study presented here includes the whole inner ear. Gene profiles of two subregions of rat cochlea show differential expression of several genes, including calmodulin expression (Cho *et al.*, 2001).

4.2.1.6 Structural genes. Cytoskeletal proteins contribute to the highly organized structure of the inner ear (Flock *et al.*, 1981), however, noise can result in many types of cytoskeletal protein

changes, including disruption of the three-dimensional organization of skeletal proteins (Avinash *et al.*, 1993). The structural integrity of the inner ear clearly depends on the synthesis of cytoskeletal proteins to replenish those degraded over time and by environmental stressors such as noise exposure (Taggart *et al.*, 2001). Altered expression of transcripts encoding genes with cytoskeletal roles have been previously observed in zebrafish inner ear (Schuck *et al.*, 2008; meeting abstract), rat cochlea (Chen, 2006), chinchilla cochlea (Taggart *et al.*, 2001), chick ear tissues (Cotanche *et al.*, 1994; Adler *et al.*, 1995; Gong *et al.*, 1996; Lomax *et al.*, 2000) and guinea pig cochlear hair cells (Zuo *et al.*, 2008) following noise exposure. While the studies conducted on mammalian and avian ear tissues demonstrate primarily elevated levels of cytoskeletal transcripts following exposure, the cytoskeletal relevant transcripts of interest in the zebrafish study were significantly down-regulated following noise exposure. In the current study, microarray-identified reproducibly informative transcripts encoding genes important in structural scaffolding were differentially regulated in response to seismic noise exposure in salmon ear. Transcripts up-regulated included coronin-1A (2.7-fold, SE 0.6) and vasodilator-stimulated phosphoprotein (1.9-fold, SE 0.1) (Table 3.2), while transcripts reproducibly down-regulated included ezrin-radixin-moesin-binding phosphoprotein 50 (2.3-fold, SE 0.6) which plays a role in crosslinking actin filaments with plasma membranes; collagen alpha 2 type IV chain (2.0-fold, SE 0.4) which forms part of the extracellular matrix; and motile sperm domain-containing protein 2 (2.0-fold, SE 0.2) (Table 3.3), the most abundant down-regulated transcript identified in the microarray study, which is a motor protein important in cell motility. Motor proteins are known to be important in normal auditory function in mammals (Lomax *et al.*, 2001). Coronin-1A, which belongs to the WD40 repeat family, is associated with F-actin and has been implicated in a variety of cytoskeleton-dependent processes (Mugnier *et al.*, 2008). WDR1 gene, which encodes a WD40 repeat protein believed to be involved in actin dynamics, is one of the genes suspected to play an important role in hair cell regeneration in birds (Adler *et al.*, 1999).

4.2.1.7 Other and unknown. Transcripts that are not currently classified or have no known function are also identified in Tables 3.2 and 3.3, and also represent important transcripts potentially responsive to noise induced stress in salmon inner ear. The microarray-identified transcript encoding C14orf159 protein is down-regulated (2.2-fold, SE 0.3) (Table 3.3) in response to seismic noise exposure in salmon ear and validated by QPCR (-1.4-fold, *P*-value = 0.04) (Table 3.4; Figure 3.2C). C14orf159 protein has no well-defined function, but is known to be important in the mitochondrion. Mitochondria are dynamic organelles essential for cellular life, death, and differentiation (Pagliarini *et al.*, 2008). C14orf159 has been shown to be up-regulated in salmon gill infected with amoebic gill disease immediately following exposure (Morrison *et al.*, 2006) and has been associated with estrogen receptor alpha in human breast cancer cells (Creekmore *et al.*, 2007). These results suggest a role for C14orf159 protein in early stress response possibly associated with regulation of cell death, so C14orf159 may be an important biomarker of noise induced stress in salmon ear.

4.2.2 Genes identified in reciprocal SSH cDNA libraries

Effects of noise can range from transient metabolic stress in the cells to mechanical disruption to sensory epithelium, stereocilia, hair cell loss and disruption of synapses (Kirkegaard *et al.*, 2006). Microarray results on seismic noise induced stress in salmon inner ear point to overall changes in cellular energy, structural integrity, signaling and synaptic transmission, as well as cell death and regeneration. While the microarray study allowed the identification of potential candidate molecular markers through analysis of thousands of genes, these results are based on the particular 16,006 genes that were present on the array (von Schalburg *et al.*, 2005). The cGRASP 16K microarray does not contain transcripts that were developed from salmonid ear-specific cDNA libraries (Rise *et al.*, 2004; von Schalburg *et al.*, 2005), hence transcripts encoding genes with important ear-relevant functions responsive to seismic noise exposure were likely not included in its construction. Therefore, to complement the microarray study, a small scale targeted gene discovery study was conducted on seismic exposed and control salmon inner ear

through construction and characterization of reciprocal SSH cDNA libraries. SSH library construction has the advantages of novel gene discovery and discovery of potential important biomarkers that would not otherwise be detected. This is the first study where cDNA libraries from inner ear of salmonids have been described, and only the second study describing cDNA library construction from inner ear of fish. The first was development of a subtracted cDNA library from zebrafish inner ear (Coimbra *et al.*, 2002).

In this study, 333 and 350 ESTs were generated from the forward and reverse SSH cDNA libraries, respectively. The highest proportion of genes represented in the forward (11%) and reverse (16%) SSH cDNA libraries have functional annotations involving nucleotide binding, transcription and translation (Table 3.7; Figure 3.3). The reverse SSH cDNA library has a higher proportion of genes in this functional category than the forward SSH cDNA library, suggesting that seismic noise may down-regulate transcripts encoding transcription/translational machinery proteins, 16 hours post-exposure. An increase in transcriptional/translational machinery and ribosomal transcripts associated with protein synthesis has been reported in mammalian cochlea in an early response (3 to 6 h) to moderate noise exposure (Taggart *et al.*, 2001) and an early response (3 h) to intense noise exposure (Kirkegaard *et al.*, 2006); however, a delayed response to intense noise exposure (24 h) in mammalian cochlea did not show an induction of transcripts associated with translational machinery (Kirkegaard *et al.*, 2006). The suppression of transcriptional and translational machinery-relevant transcripts observed in salmon ear exposed to seismic noise may point to cellular damage to inner ear tissues.

Also of particular interest is the large number of sequences identified as transposases (13 ESTs overall) in the reverse SSH cDNA library (Table 3.6) compared to the forward SSH cDNA library (4 ESTs overall) (Table 3.5). Recent high-throughput sequencing of salmonid cDNA libraries has revealed a surprisingly large number of transposon transcripts; microarray studies have shown that transcription of rainbow trout transposons is activated by external stimuli such as toxicity, stress, and bacterial antigens, suggesting that these genes can be used as sensitive molecular biomarkers of acute conditions in salmonid fish (Krasnov *et al.*, 2005). In addition to

sequences that have been previously characterized as salmonid transposases identified in both the forward and the reverse SSH cDNA libraries, putative orthologues of transposase genes from Tropical clawed frog (*Silurana tropicalis*), Leopard frog (*Rana pipiens*), Chinese perch (*Siniperca chuatsi*) and European plaice (*Pleuronectes platessa*) was identified in the reverse library (Table 3.6), and from nematode (*Caenorhabditis briggsae*), in the forward library (Table 3.5). Identification of novel, potentially seismic responsive genes in Atlantic salmon ear is a significant discovery in salmonid and seismic research.

The second largest functional classification of SSH library sequences was energy metabolism, with 9.9% of the forward library ESTs and 10.6% of the reverse library ESTs associated with this functional category (Table 3.7; Figure 3.3). As previously discussed, several microarray-identified genes important in energy metabolism (including electron transport, TCA cycle and glycolysis) were differentially regulated in response to seismic exposure in salmon ear (Tables 3.2 and 3.3). A putative orthologue of Blue panchax (*Aplocheilus panchax*) cytochrome c oxidase subunit I was identified in the forward SSH cDNA library (9 ESTs) (Table 3.5) but not in the reverse SSH cDNA library (Table 3.6), suggesting discovery of a novel seismic noise-responsive salmon cytochrome c oxidase gene. Several other genes important in energy metabolism were present in both forward and reverse libraries, lending support for alteration in metabolism in salmon ear in response to seismic noise exposure. Metabolic changes have been previously reported in ear in response to noise induced stress in albino guinea pigs (Hsu *et al.*, 1998), chinchilla (Taggart *et al.*, 2001) and are reviewed in Konings *et al.* (2009).

ESTs identified as important in proteolysis accounted for 7.5% and 1.4% (Table 3.7; Figure 3.3) of total ESTs in the forward and reverse SSH cDNA libraries, respectively, suggesting a potential up-regulation of such transcripts in response to seismic noise exposure. The largest contig in the forward salmon ear SSH library (designed to be up-regulated by seismic noise exposure), with sixteen contributing ESTs, and an additional contig composed of three ESTs, were of cytosolic non-specific dipeptidase (CNDP) (Table 3.5). There were no ESTs identified as CNDP in the reverse SSH cDNA library. QPCR revealed an overall 27-fold induction of CNDP in

seismic exposed salmon ear relative to control salmon ear, with a high variability among noise-exposed individuals (Figure 3.4). Gene ontology terms associated with a putative orthologue of CNDP from Nile tilapia (*Oreochromis niloticus*) indicate metallopeptidase activity and importance in proteolysis. A study examining the differential expression of genes within mouse cochlea identified CNDP as being preferentially expressed in the organ of Corti, indicating it may play a crucial role in hearing (Morris *et al.*, 2005). Carnosine is susceptible to hydrolysis by CNDP, releasing essential amino acids important in the TCA cycle (β–alanine, L-histidine) and in histamine synthesis (L-histidine) (Otani *et al.*, 2008). Histamine is known to be regulated by various physiological factors and stress (Mochizuki *et al.*, 1992). A peptide related to carnosine, homocarnosine, is also susceptible to hydrolysis by CNDP, releasing γ-aminobutyric acid (GABA) (Otani *et al.*, 2008), an essential inhibitory neurotransmitter (Balion et al., 2007). Carnosine is reported to have anti-inflammatory effects (Fleisher-Berkovich *et al.*, 2009), play a role in proton buffering, heavy metal chelating, and have anti-crosslinking and neurotransmitter properties, acting as both a neuromodulator and a neuroprotective agent (Boldyrev 2000). Of particular interest is a study demonstrating the ability of carnosine to protect the auditory apparatus in rats exposed to acute noise trauma (Zhuravskii *et al.*, 2004). Up-regulation of CNDP in seismic noise exposed salmon ear may be the result of an increased cellular demand for energy, by providing essential amino acids in the TCA cycle. An increase in transcripts important in electron transfer and the TCA cycle were identified also in the current salmon ear microarray study, including up-regulation of transcript encoding nrk2 which produces NAD+ by a novel scavenge pathway (Tempel *et al.*, 2007). CNDP up-regulation may also indicate an inflammatory response or protection against excitotoxicity through release of the important inhibitory neurotransmitter, GABA. Finally, high variability of CNDP transcript expression in the seismic noise exposed group may indicate different individual thresholds for response to noise induced stress, or that peak expression of this gene was missed. Further work is required to determine the time-response pattern of CNDP mRNA in ear following noise exposure to determine the suitability of this gene as a molecular biomarker in early or delayed noise-induced stress.

So far I have described a potential down-regulation of protein synthesis machinery and a potential increase in proteolysis in seismic noise-exposed salmon ear. There also seems to be an indication of transcript expression response of genes important in post-translational protein modification, as indicated in the microarray study. The microarray results presented here reveal down-regulation of s-phase kinase-associated protein 1 (a ubiquitin-ligase) (Table 3.3), which is also present in the reverse SSH cDNA ear library and not in the forward SSH CDNA ear library, lending further support to potential down-regulation of this gene. Several ESTs identified as ubiquitin-conjugating enzymes appear in the forward SSH cDNA library, suggesting differential regulation of components of the ubiquitin-proteasome pathway (UPP) in seismic noise exposed salmon ear. E3 ubiquitin ligase has been previously shown to be up-regulated in ear immediately following noise exposure in chicks (Lomax *et al.*, 2000) and reported to exhibit a higher expression in the organ of Corti relative to other cochlear tissues in mouse (Morris *et al.*, 2005), suggesting it may play a crucial role in hearing. Differential regulation of various components of the ubiquitin-proteasome pathway in relation to cell differentiation in rat lens epithelia has also been previously reported; a constant level of ubiquitin-activating enzyme, increased expression of several ubiquitin-congugating enzymes and down-regulation of E3 ligase (Guo *et al.*, 2004). In the current study, the potential increase in gene expression of several ubiquitin-conjugating enzymes, and concurrent decrease in gene expression of ubiquitin-ligase, indicate that there is a fundamental reorganization of the ubiquitin conjugation system in salmon ear following noise-induced stress. Novel transcripts encoding genes important in regulated protein turnover identified in the SSH cDNA libraries include a highly conserved putative orthologue of human ubiquitin-conjugating enzyme 16 (97% identity at the amino acid level) (Table 3.5) and catfish (*Ictalurus punctatus*) ubiquitin and ribosomal protein S27a precursor (Table 3.6).

Genes with important functions in cell cycle, cell death (including apoptosis) and axon regeneration are present in both libraries, with a greater percentage appearing in the reverse library (6.3%) compared to the forward library (5.1%) (Table 3.7; Figure 3.3). A number of genes present in the reverse library (*i.e.* enriched for ear transcripts down-regulated by seismic noise)

are important in negative regulation of apoptosis or cell death, such as insulin-like growth factor binding proteins, reticulon-4, testis enhanced gene transcript (BAX inhibitor 1) and NDRG3. Genes appearing in the forward library (*i.e.* enriched for ear transcripts up-regulated by seismic noise) including S100B, optineurin, catenin-beta and NF-kappa-B inhibitor alpha, have functional annotations suggesting important roles in axon/nerve regeneration and positive regulation of apoptosis. The down-regulation of transcripts with roles in negative regulation in apoptosis, and up-regulation of transcripts with roles in positive regulation of apoptosis, may indicate an overall increase in apoptosis or regulated cell death in seismic noise-exposed salmon ear. Smith *et al.* (2006) observed increased apoptotic activity in goldfish ear tissues 0-2 days following noise exposure, which coincided with greatest hair cell loss suggesting that hair cells were dying due to programmed cell death.

Other candidate seismic noise-responsive transcripts identified in the current study include those encoding chaperones, *e.g.* members of the heat shock protein gene family (HSPs). In addition to their role as molecular chaperones (which assist in protein folding and assembly), there are many families of HSPs produced in response to stress in a wide variety of cells, in order to protect cells from even more severe stresses (*e.g.* Welch, 1992; Tytell *et al.*, 1993). There are three ESTs for HSP8 in the forward ear SSH cDNA library (Table 3.5), and a total of seven HSP-encoding ESTs (3 HSP10, 2 HSP90 beta, 1 HSP70, and 1 HSC71) in the reverse SSH cDNA library (Table 3.6). Up-regulation of HSPs have been reported in rat cochlea following ishcemia (HSP72; Myers *et al.*, 1992), in rat cochlea following exposure to a noise level that produces a TTS (HSP72; Lim *et al.*, 1993), and in guinea pig cochlea in response to noise exposure as well as pre-conditioning to noise (HSP70; Zuo *et al.*, 2008). Up-regulation of genes encoding HSPs have been reported in rat auditory cortex (HSP27 and HSP70; Sun *et al.*, 2008), and in chinchilla cochlea (HSP74; Taggart *et al.*, 2001) following exposure to noise. A potential increase in HSP8 and a decrease in HSP70 and other HSPs in salmon inner ear following noise exposure may indicate replacement of damaged cells through denaturation of damaged proteins and new protein biosynthesis.

Other genes with stress-relevant functional annotations identified in the salmon ear SSH cDNA libraries included genes potentially involved in response to oxidative stress (glutathione S-transferase A and selenoprotein Pa precursor), which were present in the forward SSH library (Table 3.5) and absent in the reverse SSH cDNA library. One of the early events in noise trauma may be the formation of reactive oxygen species (ROS, *e.g.* 'free radicals') to a level that varies with the intensity of exposure (Le Prell *et al.*, 2003). Evidence for oxidant stress as a consequence of noise trauma in ear tissues is compelling (*Reviewed in* Le Prell *et al.*, 2003). While there were only a few immune-responsive genes identified in the salmon ear SSH libraries, it is interesting to note that the two contigs in this functional classification (beta-2 microglobulin precursor and a transcript related to chicken Ig-superfamily protein, 3 ESTs each) (Table 3.5) were in the forward SSH cDNA library (*i.e.* potentially up-regulated by seismic stress). Since the best BLASTX hit for the Ig-superfamily-like salmon ear transcript was closest to *Gallus gallus* (chicken), this represents a novel, potentially seismic stress-responsive salmon transcript identified in the current study.

The structural integrity of the inner ear depends on the synthesis of cytoskeletal proteins to replace those damaged or degraded over time and by environmental stressors, such as noise (Taggart *et al.*, 2001). Microarray-identified genes in salmon ear differentially regulated in response to seismic noise exposure with functional annotations suggesting important roles in cytoskeletal structure and dynamics are described above (Tables 3.2 and 3.3). Sequences with importance in cytoskeletal structure and dynamics were also identified in the forward and reverse SSH cDNA libraries from salmon ear, with a larger percentage in the forward ear SSH cDNA library (6.9%) compared to the reverse ear SSH cDNA library (4.6%) (Table 3.7; Figure 3.3). Altered expression of genes with cytoskeletal roles have been previously observed in zebrafish inner ear (Schuck *et al.*, 2008, meeting abstract), rat cochlea (Chen, 2006), chinchilla cochlea (Taggart *et al.*, 2001), chick ear tissues (Cotanche *et al.*, 1994; Adler *et al.*, 1995; Gong *et al.*, 1996; Lomax *et al.*, 2000) and guinea pig cochlear hair cells (Zuo *et al.*, 2008), following noise exposure. The largest contig classified in this functional category for both salmon ear SSH

libraries was a putative orthologue of zebrafish matrilin-4 (Matn4 protein) (Table 3.5), a novel salmon gene identified in the current study. Matrilin-4-encoding transcript was previously reported in mouse inner ear (Klockars *et al.*, 2002) and may therefore be an important structural protein in inner ear.

While there were similar proportions of genes in the 'signaling, synaptic transmission' functional annotation category in both salmon ear SSH libraries (Tables 3.5 and 3.6), there were differences in the genes identified in each library or a different relative abundance of genes (determined by number of contributing ESTs) identified in each library. For example, myelin P0 protein precursor,-encoding transcript had six ESTS in the forward ear SSH cDNA library (Table 3.5) and one EST in the reverse ear SSH cDNA library (Table 3.6). The auditory nerve is normally surrounded by peripheral myelin, and a decrease in the density of myelin protein P0 labeling in rat cochlea has been associated with sensorineural hearing loss (Hurley *et al.*, 2007). The potential up-regulation of this Atlantic salmon transcript following seismic noise exposure may indicate a need for myelin replacement due to damage or stress in the auditory nerve. Novel, potentially seismic stress-responsive genes [*e.g.* putative orthologues of human myelin basic protein (forward SSH library), thyroid hormone receptor interactor 12 and tyrosine-protein kinase RYK (reverse SSH library)], with functional annotations suggesting important roles in signaling, were also identified in the current study (Tables 3.5 and 3.6).

Also important in ear are specific genes for sensory perception of sound, auditory receptor cell organization and otolith development. Together, genes assigned to these functional categories represented 1.8% for sensory perception of sound and auditory receptor cell organization and 1.3% for genes important in otolith development (Table 3.7; Figure 3.3). Integral membrane protein 2B (1 EST in the forward library; 3 ESTs in the reverse library) and peripheral myelin protein 22 (2 ESTs in the forward library; 1 EST in the reverse library), transcripts with functional annotations in the 'sensory perception of sound' category, were identified in both the forward library (Table 3.5) and the reverse library (Table 3.6). The human putative orthologue of integral membrane protein 2B (transmembrane protein BRI) has gene ontology (GO) biological

process terms 'nervous system development' and 'sensory perception of sound', and the human putative orthologue of peripheral myelin protein 22 has GO biological process terms 'mechanosensory behavior'; 'sensory perception of sound' and 'synaptic transmission'. There was a larger proportion of genes with important auditory roles identified in the reverse library (2.6%) compared to the forward library (0.9%). In addition to integral membrane protein and peripheral myelin protein 22 (also identified in the forward library), these genes include: a salmon transcript related to human Alstrom syndrome protein 1 (40% identity at the amino acid level); putative orthologues of zebrafish cochlin (65% identity at the amino acid level) and N-cadherin (82% identity at the amino acid level), and a putative orthologue of mouse stereocilin (47% identity at the amino acid level). In humans, mutations in the gene encoding cochlin leads to sensorineural deafness and balance problems (Robertson *et al.*, 1998), while N-cadherin has GO biological process terms including 'auditory receptor cell organization' and 'semicircular canal morphogenesis' (Table S4). Stereocilin is expressed only in the sensory hair cells in mouse inner ear and is associated with the stereocilia, the stiff microvilli forming the structure for mechanoreception of sound stimulation (Verpy *et. al.*, 2001). A potential down-regulation of salmon ear transcripts important in sensory perception of sound in response to intense noise exposure may be the result of cell death or damage, and/or may be a part of pathways involved in protection, repair or recovery. With the exception of integral membrane protein 2B and peripheral myelin protein 22, these novel salmon genes with hearing-relevant functional annotations were discovered in the current study. Salmon ear-specific transcripts identified in this study may be valuable in future research on the effects of noise exposure in fish, and in future research on hearing in fish.

Otoliths, homologous to otoconia in other vertebrates, are essential to the sense of balance and hearing by conveying information to the sensory hair cells of the inner ear (Hudspeth, 1989). Actinopterygii (ray-finned fishes) contain three large otoliths that continue to grow throughtout life (Campana and Thorrold, 2001). Genes with specific functions relating to otolith development identified in the forward library included three sequences identified as

SPARC precursor (6 ESTs in total), which are required for normal otolith growth in zebrafish (Kang *et al.*, 2008) and may also function in the salmon lens (Tröβe *et al.*, 2010), as well as a putative orthologue of cow chondromodulin-1, which has been shown to be an ear-specific marker in medaka (Nemoto *et al.*, 2008) (Table 3.5). Putative orthologues of rainbow trout otolith matrix macromolecule-64 and zebrafish otolin-1 (a collagenous otolith specific protein; Murayama *et al.*, 2002) were present as singletons in the reverse library (Table 3.6). The presence of SPARC precursor-encoding transcript in the forward SSH library may point to alteration in otolith regeneration in response to seismic noise-induced stress in salmon ear.

Together, genes assigned in the functional classifications of sensory perception of sound, auditory receptor cell organization and otolith development, represented the least abundant ESTs in both the forward and reverse SSH libraries combined (Table 3.7; Figure 3.3). The large number of different cell types in the inner ear, along with the low percentage of target cell types (*e.g.*, sensory hair cells), within the inner ear, help to explain the relatively low proportions of transcripts with ear-specific functional annotations in the salmon ear SSH libraries. However, the discovery of genes in these salmon SSH libraries with important roles in sensory perception or inner ear development will potentially aid future research on the impact of seismic noise exposure on fish and represent an important contribution to salmon gene discovery.

5.0 Conclusions

In conclusion, this study demonstrates the successful application of a functional genomics approach to determine the potential impact of seismic noise exposure on fish inner ear. One of the major advantages of using microarray technology is that changes in expression of thousands of genes can be analyzed simultaneously to identify candidate molecular biomarkers as well as gain insights into pathways and processes that may be altered as a result of exposure to environmental stressors. Microarray-identified genes responsive to seismic noise-induced stress in salmon ear that were validated by QPCR included: nicotinamide riboside kinase 2, important in a novel pathway to generate NAD^+ in energy production as well as implicated in

nerve tissue repair mechanisms (Tempel *et al.*, 2007); hemoglobin subunit alpha-4, indicating possible tissue damage or an increase in oxygen demand as a result of elevated metabolic activity; and C14orf159, a mitochondrial gene associated with human cancer cells (Creekmore *et al.*, 2007) as well as immune response in salmon (Morrison *et al.*, 2006).

Identification of potential seismic stress-responsive genes in salmon ear was also conducted through the construction and characterization of reciprocal SSH cDNA libraries. CNDP-encoding transcript, the largest contig in the forward SSH cDNA library (enriched for ear transcripts up-regulated by seismic stress), was shown by QPCR to be over 27-fold induced in seismic noise-exposed ear compared to control ear. It should be noted that the up-regulation of CNDP transcript was not statistically significant, due to high individual variability in exposed animals, which itself is an interesting result. Induction of CNDP in some animals may be the result of an increased cellular demand for energy or may indicate an inflammatory response for protection against excitotoxicity. CNDP has also been implicated to play a crucial role in hearing (Morris *et* al., 2005).

QPCR results for many of the genes tested demonstrate a high variability of gene expression in salmon ear for individuals exposed to seismic generated noise. A large individual variability in susceptibility to noise (ISO1999) is noted as one of the most remarkable features of noise induced hearing loss in humans; after an identical exposure to noise, some individuals develop extensive hearing loss while others have no effect on hearing (Konings *et al.*, 2009). In addition to differences in susceptibility to noise, other possibilities for high variability in gene expression in ear among exposed animals include distance from the source as well as orientation with respect to the source.

Multiple intracellular pathways can come into play in the response to noise over stimulation and the specific stress pathways initiated would depend on the severity of the noise exposure (Le Prell *et al.*, 2003). Microarray results presented here point primarily to alteration of genes classified as being important in: metabolic processes (including increased demand for oxygen), pathways for regulated protein turnover (including apoptosis), signaling, and

cytoskeleton structure and dynamics, in response to seismic noise exposure in salmon ear. Analysis of genes present in forward and reverse SSH cDNA libraries, designed to be potentially up- and down-regulated in response to seismic noise exposure, respectively, validate and complement results from the microarray study. In addition to alteration of processes/pathways identified in the microarray study in response to seismic noise exposure in salmon ear, genes important in transcription/translational machinery; stress-response (including several genes for HSPs); as well as sensory perception and auditory receptor cell organization, are identified in the reciprocal SSH cDNA libraries. Targeted gene discovery in salmon ear following exposure to seismic airgun noise allowed for identification of novel sensory-relevant and ear-specific genes, and represents a significant contribution to salmonid hearing research.

There are guidelines in place for seismic surveys but it is recognized that they were formulated on the basis of limited empirical data. More comprehensive studies on the impacts of seismic surveys on fish are required to link molecular changes with behavioral, physiological and anatomical changes, as well as establish distance/exposure relationships to determine the size of injury zones, to help bridge the knowledge gap. Taken together, the results here indicate changes in global gene expression that point to alteration of cellular metabolism, regulated protein degradation, programmed cell death, oxidative stress, signaling, and sensory perception in Atlantic salmon ear following acoustic stimulation. These novel results on Atlantic salmon ear indicate that microarray and SSH library technologies hold considerable potential for more fundamental studies on seismic sounds.

6.0 References

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7.0 Appendices

7.1 Supplemental Table S1 *Transcripts greater than 1.75-*fold *up-regulated in seismic exposed Atlantic salmon ear relative to control Atlantic salmon ear on at least three slides of study*

EST	Gene Name of Top BLASTX or BLASTN Hit (Acc#; Species)	Length (% ID aa or nt)	E-Value	GG003_011		GG003_012		GG003_013		GG003_014		MFC	SE	Mean Signal	Putative Function of Top BLAST Hit (Gene Ontology)
				S/C	Signal	S/C	Signal	S/C	Signal	S/C	Signal				
CA053740	Ubiquitin carboxyl-terminal hydrolase isozyme L1 (ACM09038; Salmo salar)	88/88 100%	3.E-44	1.82	26960	1.38	32721	3.28	57448	2.55	48165	2.26	0.42	41323	ubiquitin-dependent protein catabolic process(BP) (GO0006511); ubiquitin thiolesterase activity (MF) (GO:0004221); hydrolase activity (MF) (GO:00016787); intracellular (CC) (GO:0005622) [B9ENE1]
CA050426	Hemoglobin subunit alpha (ACN10007; *Salmo salar*)	143/143 100%	4.E-76	2.41	31427	2.02	34548	2.31	37881	1.30	38152	2.01	0.25	35502	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); iron ion binding (MF) (GO:0005506); heme binding (MF) (GO:0020037); oxygen binding (MF) (GO:0019825); oxygen transporter activity (MF) (GO:0005344) [Q98974] *O. mykiss* E value = 1e-72
CB515375	Hemoglobin subunit alpha-4 (ACI66666; *Salmo salar*)	143/143 100%	8.E-76	1.91	29707	2.28	35874	2.03	40366	1.80	28115	2.01	0.10	33515	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); heme binding (MF) (GO:0020037); oxygen binding (MF) (GO:0019825); oxygen transporter activity (MF) (GO:0005344) [C1BEX7]
CA060701	Hemoglobin subunit alpha (ACN10007; *Salmo salar*)	143/143 100%	4.E-76	1.43	18533	2.81	42054	1.80	26480	2.25	30263	2.07	0.30	29332	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); iron ion binding (MF) (GO:0005506); heme binding (MF) (GO:0020037); oxygen binding (MF) (GO:0019825); oxygen transporter activity (MF) (GO:0005344) [Q98974] *O. mykiss* E value = 1e-72

CA058603	Hemoglobin subunit alpha-4 (ACO07580; *Oncorhynchus mykiss*)	141/145 97%	3.E-20	2.61	20931	1.25	22946	1.95	22117	3.22	33226	2.26	0.43	24805	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); heme binding (MF) (GO:0020037); oxygen binding (MF) (GO:0019825); oxygen transporter activity (MF) (GO:0005344) [C1BEX7]
CA060239	Caspase-8 precursor (ACN10768; *Salmo salar*)	50/85 58%	1.E-18	1.94	31331	2.25	22683	2.59	20653	1.78	16652	2.14	0.18	22830	proteolysis (BP) (GO:0006508); regulation of apoptosis (BP) (GO:0042981); cysteine-type endopeptidase activity (MF) (GO:0004197); protein binding (MF) (GO:0005515) [B6CGU5a] *H. sapiens* E-value = 6e-18
CA053773	Unknown			2.44	19642	1.42	18623	2.61	23633	1.75	23782	2.06	0.28	21420	
CK990717	Hemoglobin subunit beta-1 (BT060431; *Salmo salar*)	83/85 97%	3.E-31	4.01	13715	2.83	29578	0.75	8664	2.74	15775	2.58	0.68	16933	oxygen transport (BP) GO:0015671);hemoglobin complex(CC) (GO:0005833);heme binding (MF) (GO:0020037);oxygen binding (MF) (GO:0019825);oxygen transporter activity (MF) (GO:0005344) [Q91470]
CK990828	Transthyretin (CAD66520; *Cyprinus carpio*)	89/130 68%	1.E-28	2.37	24054	2.53	16661	0.31	13845	3.32	11904	2.13	0.64	16616	transport (BP) (GO:0006810); cytoplasm (CC) (GO:0005737); extracellular region (CC) (GO:0005576); hormone activity (MF) (GO:0005179) [P02766] *H. sapiens* E value = 2e-14
CA050293	Hemoglobin subunit beta (ACM08872; *Salmo salar*)	148/148 100%	1.E-79	3.56	239	2.28	17625	2.01	19681	1.99	17763	2.46	0.37	13827	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); iron ion binding (MF) (GO:0005506) [B9EMX5]
CA036686	Hemoglobin subunit beta (ACM08872; *Salmo salar*)	148/148 100%	1.E-79	2.20	3345	1.40	7996	2.34	4649	3.13	9968	2.27	0.35	6489	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); iron ion binding (MF) (GO:0005506) [B9EMX5]
CB505759	Midasin homolog (CAM26642; *Mus musculus*)	41/50 82%	1.E-19	1.34	1805	2.55	6934	1.83	11524	1.87	5618	1.90	0.25	6470	regulation of protein complex assembly (BP) (GO:0043254); nucleus (CC) (GO:0005634) ; ATP binding (MF) (GO:0005524); ATPase activity (MF) (GO:0016687) [A2ANY6]

CK991044	Cytochrome c oxidase polypeptide VIc-2 (ACI66043; *Salmo salar*)	73/76 96%	2.E-28	3.43	9453	1.03	7579	5.10	3105	1.91	3956	2.87	0.89	6023	cytochrome-c oxidase activity (MF) (GO:0004129) [B5X5C3]
CA042535	40 kDa peptidyl-prolyl cis-trans isomerase (ACO07506; *Oncorhynchus mykiss*)	103/103 100%	1.E-46	1.22	3671	1.75	5029	2.44	5915	1.82	6830	1.81	0.25	5361	protein folding (BP) (GO:0006457); binding (MF) (GO:0005488); peptidyl-prolyl cis-trans isomerase activity (MF) (GO:0003755) [C1BEQ3]
CA049313	Hemoglobin subunit beta (ACI68762; *Salmo salar*)	122/123 99%	3.E-71	2.25	1916	2.58	4841	0.96	5032	2.38	7823	2.04	0.37	4903	oxygen transport (BP) GO:0015671); hemoglobin complex(CC) (GO:0005833); iron ion binding (MF) (GO:0005506) [B9EMX5]
CA051670	Unknown			3.57	1902	1.82	10383	2.16	1515	1.56	3399	2.28	0.45	4300	
CB497834	Malate dehydrogenase, cytoplasmic (ACN10417; *Salmo salar*)	68/68 100%	2.E-32	1.94	1165	3.98	5410	2.12	5302	1.48	2019	2.35	0.55	3474	glycolysis (BP) (GO:0006096); malate metabolic process (BP) (GO:0006108); oxidation reduction (BP) (GO:0055114); tricarboxylic acid cycle (BP) (GO:0006099); cytosol (CC) (GO:0005829); L-malate dehydrogenase activity (MF) (GO:0030060); malic enzyme activity (MF) (GO:0004470) [P40925] *H. sapiens* E-value = 1-19
CA045974	UPF0466 protein C22orf32 homolog, mitochondrial (Q5XG64; *Xenopus laevis*)	40/70 57%	5.E-17	0.38	1339	1.84	5744	3.21	4314	5.92	1241	2.84	1.18	3159	integral to membrane (CC) (GO:0016021);mitochondrion (CC) (GO:0005739)
CB505859	Apolipoprotein A-IV precursor (ACI67068; *Salmo salar*)	140/144 97%	6.E-71	0.97	526	1.95	4351	3.39	6454	1.93	1231	2.06	0.50	3140	lipid transport (BP) (GO:0006869); lipoprotein metabolic process (BP) (GO:0042157); extracellular region (CC) (GO:0005576); lipid binding (MF) (GO:0008289) [B5X898]
CB505282	Ferritin, middle subunit (ACI69640; *Salmo salar*)	95/95 100%	2.E-43	3.02	1857	0.68	2930	1.79	3595	2.73	3899	2.06	0.53	3070	cellular iron ion homeostasis (BP) (GO:0006879); ferric iron binding (MF) (GO:0008199) [B5XFM0]

CA055146	Vasodilator-stimulated phosphoprotein (BT047473; *Salmo salar*)	461/461 100%	0.E+00	1.86	2082	2.12	2944	1.61	4792	2.10	1147	1.92	0.12	2741	actin cytoskeleton (CC) (GO:0015629); cytosol (CC) (GO:0005829); filopodium membrane (CC) (GO:0031527); focal adhesion (CC) (GO:0005925); lamellipodium membrane (CC) (GO:0031258); SH3 domain binding (MF) (GO:0017124); actin binding (MF) (GO:0003779) [P50552] *H. sapiens*
CA058654	Retinol-binding protein I, cellular (ACI69621; *Salmo salar*)	106/108 98%	7.E-57	2.36	1592	2.16	2715	2.76	4260	1.93	1821	2.30	0.17	2597	transport (BP) (GO:0006810); lipid binding (MF) (GO:0008289); transporter activity (MF) (GO:0005215) [B5XFK1]
CA062451	catenin beta-1 (BT071982; *Salmo salar*)	724/724 100%	0.E+00	2.85	2289	0.50	1955	1.96	4901	2.16	993	1.87	0.49	2534	binding (MF) (GO:0005488) [C0PU30]
CA044613	Succinate-CoA ligase alpha subunit (BT071979; *Salmo salar*)	371/424 87%	2.E-126	1.39	1147	3.26	2630	5.18	3358	2.03	2412	2.97	0.83	2387	tricarboxylic acid cycle (BP) (GO:0006099); ATP citrate synthase activity (MF) (GO:0003878); binding (MF) (GO:0005488); succinate-CoA ligase (ADP-forming) activity (MF) (GO:0004775) [C0PU29]
CA041792	Heterogeneous nuclear ribonucleoprotein A0 (ACI67551; *Salmo salar*)	65/65 100%	1.E-31	2.01	588	1.12	1086	2.54	6099	1.90	1578	1.89	0.18	2338	ribonucleoprotein complex (CC) (GO:0030529); nucleic acid binding (MF) (GO:0003676) [B5X9N1]
CA047138	NADH dehydrogenase 1 beta subcomplex subunit 1 (ACI67195; *Salmo salar*)	58/58 100%	1.E-26	0.24	273	1.88	3508	2.73	3797	2.87	1701	1.93	0.61	2319	mitochondrion (CC) (GO:0005739); NADH dehydrogenase activity (MF) (GO:0003954) [B5X8M5]

CA052688	Eukaryotic translation initiation factor 5A-1 (BT045750; *Salmo salar*)	356/358 99%	0.E+00	2.35	3450	1.24	892	1.83	2649	6.63	2125	3.01	1.23	2279	peptidyl-lysine modification to hypusine (BP) (GO:0008612);positive regulation of translational elongation (BP) (GO:0045901);positive regulation of translational termination (BP) (GO:0045905);translational frameshifting (BP) (GO:0006452);RNA binding (MF) (GO:0003723);ribosome binding (MF) (GO:0043022);translation elongation factor activity (MF) (GO:0003746);translation initiation factor activity (MF) (GO:0003743) [B5DGQ3]
CA063916	Nicotinamide riboside kinase 2 (ACI68758; *Salmo salar*)	86/88 97%	3.E-45	2.07	1077	2.05	1573	1.82	3687	1.30	1961	1.81	0.18	2075	kinase activity (MF) (GO:0016301); [B5X9J5] Salvage pathway to produce NAD+ (Tempel *et al.*, 2007)
CB492469	Nicotinamide riboside kinase 2 (ACO08546; *Oncorhynchus mykiss*)	79/80 98%	2.E-41	3.56	1445	2.20	2040	2.46	2929	3.32	857	2.89	0.33	1818	kinase activity (MF) (GO:0016301); [B5X9J5] Salvage pathway to produce NAD+ (Tempel *et al.*, 2007)
CA058330	ADP-ribosylation factor 1 (BT045670; *Salmo salar*)	597/638 93%	0.E+00	0.60	288	4.85	3952	3.12	2087	2.54	751	2.78	0.87	1769	small GTPase mediated signal transduction (BP) (GO:0007264); intracellular (CC) (GO:0005622); GTP binding (MF) (GO:0005525) [B5X3Q1]
CA054421	UPF0527 membrane protein (BT045130; *Salmo salar*)	655/657 99%	0.E+00	0.85	277	1.96	938	10.9	4688	1.79	814	3.86	2.34	1679	integral to membrane (CC) (GO:0016201) [B5X349] Peripheral myelin protein (*S. salar*) E value = 5e-09
CA050155	Coronin-1A (ACN10578; *Salmo salar*)	139/141 98%	5.E-55	3.84	1242	2.09	601	1.20	2248	3.54	2485	2.67	0.62	1644	actin binding (MF) (GO:0003779)
CA051567	Coiled-coil domain containing protein 109A (Q08BI9; *Danio rerio*)	66/114 57%	9.E-38	5.84	1408	2.65	2172	2.54	1897	0.63	997	2.91	1.08	1618	integral to membrane (CC) (GO:0016201)

CA055856	Ig kappa chain V-IV region B17 precursor (ACN10421;*Salmo salar*)	103/103 100%	2.E-41	1.14	457	4.36	2883	4.92	1222	2.21	1264	3.16	0.89	1457	
CA061818	SH2/SH3 adaptor Crk (BT058800; *Salmo salar*)	79/101 78%	5.E-10	2.99	549	3.65	3096	1.31	1421	2.38	746	2.58	0.50	1453	protein binding (MF) (GO:0005515)
CB492165	Histone H1x (BT074265; *Salmo salar*)	392/396 98%	0.E+00	3.06	982	2.78	2642	4.30	1273	0.90	784	2.76	0.70	1420	nucleosome assembly (BP) (GO:0006334);nucleosome (CC) (GO:0000786);nucleus (CC) (GO:0005634);DNA binding (MF) (GO:0003677) [C1BF13]
CA053832	Unknown			1.57	1407	2.62	1341	1.98	1300	2.23	1234	2.10	0.22	1321	
CB500653	Adrenodoxin, mitochondrial (BT059432; *Salmo salar*)	128/159 80%	6.E-33	0.26	160	2.24	1461	3.06	1808	2.30	1479	1.97	0.60	1227	2 iron, 2 sulfur cluster binding (MF) (GO:0051537); electron carrier activity (MF) (GO:0009055); iron ion binding (MF) (GO:0005506) [C0HAS6]
CA061344	NADP-dependent malic enzyme (BT058893; *Salmo salar*)	148/189 78%	2.E-32	2.09	250	0.50	1282	4.33	2515	3.24	762	2.54	0.82	1202	malate metabolic process (BP) (GO:0006108); oxidation reduction (BP) (GO:0055114); NAD or NADH binding (BP) (GO:0051287); malic enzyme activity (MF) (GO:0004470); metal ion binding (MF) (GO:0046872); oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor (MF) (GO:0016616) [C0H987]

CB507670	Collagen alpha-2I chain precursor (BT071961; *Salmo salar*)	503/575 87%	0.E+00	1.44	366	1.84	1819	1.86	1617	3.04	1005	2.05	0.35	1202	Rho protein signal transduction (BP) (GO:0007266); blood vessel development (BP) (GO:0001568); collagen fibril organization (BP) (GO:0030199); regulation of blood pressure (BP) (GO:0008217); transforming growth factor beta receptor signaling pathway (BP) (GO:0007179); extracellular space (CC) (GO:0005615); plasma membrane (CC) (GO:0005886); extracellular matrix structural constituent (MF) (GO:0005201); identical protein binding (MF) (GO:0042802); platelet-derived growth factor binding (MF) (GO:0048407); protein binding, bridging (MF) (GO:0030674) [P08123] *H. sapiens* E value = 1e-15
CA063898	ADAM metallopeptidase domain 10 (BC153863; *Bos taurus*)	64/80 80%	2.E-08	2.34	958	1.90	1198	0.93	1711	2.17	788	1.84	0.32	1164	notch signaling pathway (MP) (GO:0007219);negative regulation of cell adhesion (BP) (GO:0007162);protein amino acid phosphorylation (BP) (GO:0006468);golgi membrane (CC) (GO:0000139);golgi-associated vesicle (CC) (GO:0005798);cell surface (CC) (GO:0009986);integral to membrane (CC) (GO:0016021);nucleus (CC) (GO:0005634);plasma membrane (CC) (GO:0005886);SH3 domain binding (MF) (GO:0017124);metalloendopeptidase activity (MF) (GO:0004222);protein homodimerization activity (MF) (GO:0042803);protein kinase binding (MF) (GO:0019901);zinc ion binding (MF) (GO:0008270) [Q10741]

CB517807	Deoxycytidylate deaminase (AC166682; *Salmo salar*)	25/26 96%	1.E-06	0.59	338	2.24	1986	3.59	1429	1.99	737	2.11	0.61	1122	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds (MF) (GO:0016810); zinc ion binding (MF) (GO:0008270) [B5X762]
CB517491	UbiA prenyltransferase domain containing 1 (Q5ZKS8; Gallus gallus)	59/76 77%	3.E-27	3.49	670	2.98	1206	2.18	1418	0.77	980	2.36	0.59	1068	integral to membrane (CC) (GO:0016021); prenyltransferase activity (MF) (GO:0004659) [Q5ZKS8]
CB517114	IGL1E2 gene for immunoglobulin light chain variable region (AJ251651; *Oncorhynchus mykiss*)	618/649 95%	0.E+00	3.14	687	3.83	1156	2.05	1259	1.81	1093	2.71	0.47	1049	
CA056108	C type lectin receptor A (AAT77220; *Salmo salar*)	54/54 100%	2.E-27	2.58	488	2.13	791	1.75	1434	3.09	1406	2.39	0.29	1030	integral to membrane (CC) (GO:0016021); receptor activity (MF) (GO:0004872); sugar binding (MF) (GO:0005529) [Q68S98]
CA062243	Ubiquitin family domain-containing protein 1 (AAH11313; *Mus musculus*)	100/110 90%	2.E-36	0.33	144	4.06	341	4.91	2719	10.8	873	5.03	2.17	1019	Novel polyubiquitin binding protein important in protein modification (Fenner *et al.*, 2009)
CA045755	Growth hormone I (AAU11453; *Salmo salar*)	70/70 100%	7.E-35	1.39	270	5.07	522	3.35	1348	3.88	708	3.42	0.77	712	extracellular region (CC) (GO:0005576); hormone activity (MF) (GO:0005179) [Q5SDS1]

Background-corrected, Lowess-normalized (BCLN) signal ratios are sorted by descending mean signal from the dominant channel for all four slides in study. For the "EST" column, GenBank accession numbers of the expressed sequence tags (ESTs) corresponding to GRASP microarray features is shown. The most significant (lowest E value) BLASTX hit is shown. If an EST has no significant (E value <10-5) BLASTX hit, then the most significant BLASTN hit is shown. The GenBank nr or nt accession number is shown in parentheses, along with the species. "Length" indicates extent (salmonid EST translation aligned with amino acid sequence of top BLASTX hit, or EST sequence aligned with top BLASTN hit) over the aligned region, along with percent identity and the associated E value. "Mean fold change" (MFC) = mean BCLN seismic exposed/control ratio. Number of replicates = 4. Individual slide data are reported for Slides GG003_011, 012, 013, 014. The "Function" is that associated with the EST top BLAST hit or an annotated putative *Homo sapiens* orthologue, from Swiss-Prot http://us.expasy.org/sprot/), with Gene Ontology (GO) identifiers in parentheses and Swiss-Prot accession numbers in brackets. All GOannotation was performed by the Gene Ontology Consortium (http://www.geneontology.org/): MF is GO molecular function; CC is GO cellular component; BP is GO biological process. BLAST hits were up-dated May 2010.

7.2 Supplemental Table S2 *Transcripts greater than 1.75-*fold *down-regulated in seismic exposed Atlantic salmon ear relative to control Atlantic salmon ear on at least three slides of study*

EST	Gene Name of Top BLASTX or BLASTN Hit (Acc#, *Species*)	Align (%ID aa or nt)	E-Value	GG003_011		GG003_012		GG003_0 13		GG003_014		MFC	SE	Mean Signal	Putative Function of Top BLAST Hit (Gene Ontology)
				C/S	Signal	C/S	Signal	C/S	Signal	C/S	Signal				
CB514261	Motile sperm domain-containing protein 2 (BT045370; *Salmo salar*)	708/708 100%	0.E+00	2.14	5625	2.28	20490	1.27	14071	2.18	10780	2.0	0.2	12741	
CA057346	C14orf159 protein (AAI55802; *Danio rerio*)	82/112 73%	2.E-41	1.79	2491	2.98	6131	2.30	4368	1.82	2582	2.2	0.3	3898	mitochondrion[c] (GO: GO:0005739) [Q7Z3D6[a]] *H. Sapiens* E value = 9e-33
CB511669	BCCIP homolog (ACI67438; *Salmo salar*)	111/113 98%	2.E-59	1.96	3857	0.74	1440	3.58	6826	4.28	2413	2.6	0.8	3634	DNA repair (BP) (GO:0006281); cell cycle (BP) (GO:0007049); regulation of cyclin-dependent protein kinase activity (BP) (GO:0000079); nuclear cyclin-dependent protein kinase holoenzyme complex (CC) (GO:0019908); kinase regulator activity (MF) (GO:0019207); protein binding (MF) (GO:0005515) [Q9P287] *H. sapiens* E value 5e-30
CA052504	Unknown			3.68	1620	3.01	7567	0.66	3060	2.54	1727	2.5	0.7	3493	
CB516800	Serpin H1 precursor (BT044974; *Salmo salar*)	744/745 99%	0.E+00	2.66	2639	1.01	3336	2.35	3798	2.05	2815	2.0	0.4	3147	serine-type endopeptidase inhibitor activity (MF) (GO:0004867); [B5X1Q5] response to unfolded protein (BP) (GO:0006986) P50454 H. sapiens.
CB502357	B-cell receptor-associated protein 31 (BT045436; *Salmo salar*)	797/799 99%	0.E+00	1.84	960	1.02	2498	4.24	4187	3.76	4739	2.7	0.8	3098	apoptosis (BP) (GO:0006915); immune response (BP) (GO:0006955); intracellular protein transport (BP) (GO:0006886); endoplasmic reticulum (CC) (GO:0005783); integral to membrane (CC) (GO:0016021); receptor activity (MF) (GO:0004872) [P51572] *H. sapiens.*
DN047874	TAR DNA-binding protein 43 (BT072005; *Salmo salar*)	756/756 100%	0.E+00	2.67	3028	4.68	1377	0.61	401	1.88	5571	2.5	0.9	2594	DNA binding (MF) (GO:0003677); nucleotide binding (MF) (GO:0000166) [C0PU38]
CB510977	C17orf37 homolog (BT071863; *Salmo salar*)	568/619 91%	0.E+00	2.57	2190	2.69	4089	0.56	524	4.17	3435	2.5	0.7	2559	

CB496455	Cytochrome c oxidase polypeptide VIIb, mitochondrial precursor (ACI67848; *Salmo salar*)	78/79 98%	1.E-31	3.14	3573	2.17	4169	2.41	2096	0.19	258	2.0	0.6	2524	mitochondrial respiratory chain (CC) (GO:0005746); cytochrome-c oxidase activity (MF) (GO:0004129) [B5XAH8]
CA053033	Nuclear migration protein nudC (ACN10702; *Salmo salar*)	135/135 100%	4.E-73	2.87	1453	1.65	1667	1.82	4557	2.70	1731	2.3	0.3	2352	cell division (BP) (GO:0051301); cell proliferation (BP) (GO:0008283); mitosis (BP) (GO:0008283); multicellular organismal development (BP) (GO:0007275); microtubule (CC) (GO:0005874); nucleus (CC) (GO:0005634); protein binding (MF) (GO:0005515) [Q9Y266] *H. sapiens* E value = 4e-60
CB497891	Cytochrome c oxidase subunit 5B, mitochondrial precursor (ACN10266; *Salmo salar*)	44/60 73%	4.E-12	2.31	1034	4.36	3177	2.46	3962	1.00	1040	2.5	0.7	2303	mitochondrial envelope (CC) (GO:0005740); cytochrome-c oxidase activity (MF) (GO:0004129) [C0H897]
CB512698	Proteasome subunit beta type-4 precursor (ACI69686; *Salmo salar*)	201/202 99%	7.E-112	2.75	570	1.96	2190	2.04	4284	2.42	725	2.3	0.2	1942	ubiquitin-dependent protein catabolic process (BP) (GO:0006511); cytosol (CC) (GO:0005829); proteasome core complex (CC) (GO:0005839); threonine-type endopeptidase activity (MF) (GO:0004298) [B5XFR6]
CB505847	Transposase (CAB51372; *Pleuronectes platessa*)	108/154 70%	3.E-55	4.83	1981	2.62	2805	0.33	1621	1.86	1343	2.4	0.9	1938	regulation of cellular transcription, DNA-dependentd (GO:0006355); transcription initiationd (GO:0006352); sigma factor activityb (GO:0016987); transcription factor activityb (GO:0003700) [Q9PW00]
CB485925	S100-B (ACM08316; *Salmo salar*)	79/80 98%	1.E-21	3.01	971	2.39	4120	0.82	689	2.93	1897	2.3	0.5	1919	calcium ion binding (MF) (GO:0005509) [B9ELB9]
CB503155	Splicing factor, arginine/serine-rich 2 (ACN10350; *Salmo salar*)	92/92 100%	7.E-39	2.19	733	2.16	1844	1.15	1790	4.41	3282	2.5	0.7	1912	nucleic acid binding (MF) (GO:0003676); nucleotide binding (MF) (GO:0000166) [C0H8I1]

CB517787	Proteasome subunit beta type-5 precursor (ACM09015; *Salmo salar*)	154/155 99%	1.E-50	2.60	1776	2.07	2103	0.48	647	2.23	2970	1.9	0.5	1874	proteolysis involved in cellular protein catabolic process (BP) (GO:0051603); cytoplasm (CC) (GO:0005737); nucleus (CC) (GO:0005634); proteasome core complex (CC) (GO:0005839); threonine-type endopeptidase activity (MF) (GO:0004298) [B5X8C2]
CB511240	Retinoid x receptor beta a (BT043993; *Salmo salar*)	593/594 99%	0.E+00	1.79	554	1.92	2236	1.88	2994	1.44	1299	1.8	0.1	1771	regulation of transcription, DNA-dependent (BP) (GO:0006355); nucleus (CC) (GO:0005634); sequence-specific DNA binding (MF) (GO:0043565); steroid binding (MF) (GO:0005496); steroid hormone receptor activity (MF) (GO:0003707); transcription factor activity (MF) (GO:0003700); zinc ion binding (MF) (GO:0008270) [B5RI69]
CA055177	Ezrin-radixin-moesin-binding phosphoprotein 50 (BT058849; *Salmo salar*)	44/51 86%	6.E-06	3.52	869	2.87	2747	2.02	2157	0.88	802	2.3	0.6	1643	
CA062981	Unknown			1.90	635	0.71	1528	1.91	2234	3.00	2131	1.9	0.5	1632	
CA051481	Carbonyl reductase 1 (BT047504; *Salmo salar*)	51/54 94%	5.E-13	1.77	1195	0.84	1082	2.34	1713	2.31	1551	1.8	0.4	1385	oxidation reduction (BP) (GO:0055114); oxidoreductase activity (MF) (GO:0016491) [B5X5V8]
CB510623	Signal recognition particle 19 kDa protein (ACI68109; *Salmo salar*)	75/83 90%	1.E-34	2.39	826	0.57	1491	2.16	2261	5.17	817	2.6	1.0	1349	SRP-dependent cotranslational protein targeting to membrane (BP) (GO:0006614); signal recognition particle (CC) (GO:0048500); 7S RNA binding (MF) (GO:0008312) [B5XB89]
CB513162	H-2 class II histocompatibility antigen gamma chain (BT071840; *Salmo salar*)	399/441 90%	2.E-170	3.16	1178	1.86	870	0.35	680	2.20	2657	1.9	0.6	1346	antigen processing and presentation[d] (GO:0019882); immune responsed (GO:0006955); intracellular protein transportd (GO:0006886); membranec (GO:0016020); MHC class II protein bindingb (GO:0042289) [Q8JFN5] *O. mykiss* invariant chain S25-7 E Value = 3e-143

CB498113	S-phase kinase-associated protein 1A (ACO07792; *Oncorhynchus mykiss*)	149/149 100%	2.E-62	2.15	480	2.77	3747	0.41	627	2.67	500	2.0	0.6	1338	ubiquitin-dependent protein catabolic process (BP) (GO:0006511); kinase activity (MF) (GO:0016301); protein binding (MF) (GO:0005515) [C1BFI9]
CB502520	calmodulin 2 (BT043797; *Salmo salar*)	678/856 79%	0.E+00	2.96	569	2.32	1804	2.62	2364	0.44	530	2.1	0.6	1317	calcium ion binding (MF) (GO:0005509); kinase activity (MF) (GO:0016301); [B5DGN6] G-protein coupled receptor protein signaling pathway (BP) (GO:0007816) (*H. sapiens*).
CB492269	Purpurin precursor (ACI70127; *Salmo salar*)	68/74 91%	2.E-11	1.16	801	2.31	1267	2.26	1972	2.42	1185	2.0	0.3	1306	transport (BP) (GO:0006810); retinoid binding (MF) (GO:0005501); transporter activity (MF) (GO:0005215) [B5XH07]
CA042992	L-xylulose reductase (BT059117; *Salmo salar*)	593/633 93%	0.E+00	6.56	566	1.84	1215	2.04	2008	0.94	1298	2.9	1.3	1272	oxidation reduction (BP) (GO:0055114); oxidoreductase activity (MF) (GO:0016491) [B5X5V8]
CB507647	Protein SET (M93651; *Homo sapiens*)	54/59 91%	6.E-12	2.08	1221	4.01	1546	3.22	1551	0.47	475	2.5	0.8	1198	cellular DNA replication (BP) (GO:0006260); negative regulation of histone acetylation (BP) (GO:0035067); nucleocytoplasmic transport (BP) (GO:0006913); nucleosome assembly (BP) (GO:0006334); nucleosome disassembly (BP) (GO:0006337); cytosol (CC) (GO:0005829); endoplasmic reticulum (CC) (GO:0005783); nucleoplasm (CC) (GO:0005654); perinuclear region of cytoplasm (CC) (GO:0048471); histone binding (MF) (GO:0042393); phosphoprotein phosphatase inhibitor activity (MF) (GO:0004864); protein phosphatase type 2A regulator activity (MF) (GO:0008601)[Q01105]
CK990486	Proteasome subunit beta type-1-A (ACI67092; *Salmo salar*)	78/96 81%	3.E-29	6.00	1373	0.54	1117	2.79	1012	2.81	959	3.0	1.1	1115	proteolysis involved in cellular protein catabolic process (BP) (GO:0051603); cytoplasm (CC) (GO:0005737); nucleus (CC) (GO:0005634); proteasome core complex (CC) (GO:0005839); threonine-type endopeptidase activity (MF) (GO:0004298) [B5X8C2]

CA059770	Collagen alpha 2 type IV chain (AAX55917; *Danio rerio*)	44/52 84%	6.E-19	2.05	740	1.79	1643	1.19	860	3.05	1091	2.0	0.4	1084	collagen (CC) (GO:0005581); binding (MF) (GO:0005488); extracellular matrix structural constituent (MF) (GO:0005201) [Q58FS6]
CA050743	4F2 cell-surface antigen heavy chain (ACI33885; *Salmo salar*)	69/69 100%	1.E-30	2.15	566	0.67	1411	2.79	1524	3.20	524	2.2	0.6	1006	carbohydrate metabolic process (BP) (GO:0005975); catalytic activity (MF) (GO:0003824); cation binding (MF) (GO:0043169) [B5X3K4]; Multiple functions including immune response (Devés and Boyd, 2000)
CB492153	Activated RNA polymerase II transcriptional coactivator p15 (ACI67532; *Salmo salar*)	65/65 100%	7.E-31	1.68	866	1.93	1270	2.10	1024	2.67	719	2.1	0.2	970	regulation of transcription, DNA-dependent (BP) (GO:0006355); DNA binding (MF) (GO:0003677); transcription coactivator activity (MF) (GO:0003713) [B5X9L2]
CA062736	Unknown			0.41	558	2.85	1585	5.76	853	2.35	782	2.8	1.1	944	
CA051316	Unknown			2.09	582	0.22	942	2.04	793	3.32	1455	1.9	0.6	943	
CB492183	Succinyl-CoA ligase beta-chain mitochondrial precursor (BT071951; *Salmo salar*)	341/472 72%	9.E-67	3.37	584	2.00	1453	0.50	653	3.21	743	2.3	0.7	858	
CA045309	Low molecular weight phosphotyrosine protein phosphatase (ACI67726; *Salmo salar*)	36/38 94%	3.E-14	2.13	1041	3.03	941	0.71	602	1.92	512	2.0	0.5	774	protein amino acid dephosphorylation (BP) (GO:0006470); cytoplasm (CC) (GO:0005737); acid phosphatase activity (MF) (GO:0003993); non-membrane spanning protein tyrosine phosphatase activity (MF) (GO:0004726) [B5XA56]
CB492396	5'-nucleotidase, cytosolic 3 (ACH70804; *Salmo salar*)	89/90 98%	7.E-21	2.20	592	2.45	1471	0.71	422	3.71	382	2.3	0.6	717	cytoplasm (CC) (GO:0005737); 5'-nucleotidase activity (MF) (GO:0008253); magnesium ion binding (MF) (GO:0000287) [B5DGD0]

CK990619	39S ribosomal protein L36 (ACI69996; *Salmo salar*)	111/121 91%	1.E-51	1.34	603	3.66	1065	2.83	425	1.82	540	2.4	0.5	658	translation (BP) (GO:0006412); ribosome (CC) (GO:0005840); structural constituent of ribosome (MF) (GO:0003735) [B5XGM6]

Background-corrected, Lowess-normalized (BCLN) signal ratios are sorted by descending mean signal from the dominant channel for all four slides in study. For the "EST" column, GenBank accession numbers of the expressed sequence tags (ESTs) corresponding to GRASP microarray features is shown. The most significant (lowest E value) BLASTX hit is shown. If an EST has no significant (E value <10-5) BLASTX hit, then the most significant BLASTN hit is shown. The GenBank nr or nt accession number is shown in parentheses, along with the species. "Length" indicates extent (salmonid EST translation aligned with amino acid sequence of top BLASTX hit, or EST sequence aligned with top BLASTN hit) over the aligned region, along with percent identity and the associated E value. "Mean fold change" (MFC) = mean BCLN control/seismic exposed ratio. Number of replicates = 4. Individual slide data are reported for Slides GG003_011, 012, 013, 014. The "Function" is that associated with the EST top BLAST hit or an annotated putative [a]*Homo sapiens* orthologue, from Swiss-Prot http://us.expasy.org/sprot/), with Gene Ontology (GO) identifiers in parentheses and Swiss-Prot accession numbers in brackets. All GOannotation was performed by the Gene Ontology Consortium (http://www.geneontology.org/): MF is GO molecular function; CC is GO cellular component; BP is GO biological process. BLAST hits were up-dated May 2010.

7.3 Supplemental Table S3 ESTs from forward SSH cDNA Atlantic salmon ear library (ssal_ear_SSH_seismicF)

contig #	# ESTs	BLAST identification of salmon cDNA gene name (acc # of hit; species name)	GO term	GO description	Seq length (bp)	% ID align	E-value	Accession number
29.1 29.2 29.3 29.4 29.5 29.6 29.7 29.8 29.9 29.10 29.11 29.12 29.13 29.14 29.15 29.16	16	cytosolic non-specific dipeptidase (ACI34318; *Salmo salar*)	GO:0008237 GO:0046983 GO:0006508	metallopeptidase activity (MF) protein dimerization activity (MF) proteolysis (BP) anserinase (BAE45262; *Oreochromis niloticus*) 2e-96	1097	354/357 (99%)	0.0	GT128561 GT128562 GT128563 GT128564 GT128565 GT128566 GT128567 GT128568 GT128569 GT128570 GT128571 GT128572 GT128573 GT128574 GT128575 GT128576
44.1 44.2 44.3 44.4 44.5 44.6 44.7 44.8 44.9	9	cytochrome c oxidase subunit I (BAG69248; *Aplocheilus panchax*)	GO:0005739	mitochondrion (CC)	546	153/167 (91%)	8e-81	GT128611 GT128612 GT128613 GT128614 GT128615 GT128616 GT128617 GT128618 GT128619
30.1 30.2 30.3 30.4 30.5 30.6 30.7 30.8 30.9	9	cytochrome oxidase subunit II (AAD04736;*Salmo salar*)	GO 0016021 GO:0005746 GO:0005507 GO:0004129 GO:0009055 GO:0020037 GO:0022904 GO:0006810	integral to membrane (CC). mitochondrial respiratory chain (CC) copper ion binding (MF) cytochrome-c oxidase activity (MF) electron carrier activity (MF) heme binding (MF) respiratory electron transport chain (BP) transport (BP)	630	200/209 (95%)	2e-92	GT128577 GT128578 GT128579 GT128580 GT128581 GT128582 GT128583 GT128584 GT128585
8.1 8.2 8.3 8.4 8.5 8.6	7	integral membrane transporter protein (CAB81951; *Homo sapiens*)	GO:0016021 GO:0012505 GO:0006810	integral to membrane (CC) endomembrane system (CC) transport (BP)	1243	34/34 (100%)	4e-12	GT128501 GT128502 GT128503 GT128504 GT128505 GT128506

8.7								GT128507
18.1 18.2 18.3 18.4 18.5 18.6	6	myelin P0 protein precursor (BT058974; *Salmo salar*)	GO:0016020	membrane (CC)	712	695/695 (100%)	0.0	GT128530 GT128531 GT128532 GT128533 GT128534 GT128535
14.1 14.2 14.3 14.4	4	Matn4 protein (AAI63867; *Danio rerio*)	GO:0005576	extracellular region (CC) FUNCTION: Major component of the extracellular matrix of cartilage Cytoskeletal structural protein in inner ear (Klockars *et. al.*, 2002)	777	134/161 (83%)	6e-69	GT128519 GT128520 GT128521 GT128522.
48.1 48.2 48.3 48.4	4	senescence-associated protein (ACA04850; *Picea abies*)			399	67/87 (77%)	3e-29	GT128627 GT128628 GT128629 GT128630
4.1 4.2 4.3 4.4	4	28S ribosomal RNA gene (U34341; *Oncorhynchus mykiss*)			995	962/1001 (96%)	0.0	GT128491 GT128492 GT128493 GT128494
12.1 12.2 12.3	3	S100-B (ACM08316; *Salmo salar*)	GO:0007409 GO:0008283 GO:0007417 GO:0007611 GO:0005737 GO:0005634 GO:0001726 GO:0048154 GO:0005509 GO:0048306 GO:0042803 GO:0048156 GO:0008270	axonogenesis (BP) cell proliferation (BP) central nervous system development (BP) learning or memory (BP) cytoplasm (CC) nucleus (CC) ruffle (CC) S100 beta binding (MF) calcium ion binding (MF) calcium-dependent protein binding (MF) protein homodimerization activity (MF) tau protein binding (MF) zinc ion binding (MF) S100 calcium binding protein B (AAH01766; *Homo sapiens*) 7e-19	792	77/77 (100%)	1e-37	GT128514 GT128515 GT128516
19.1 19.2 19.3	3	heat shock protein 8 (ACH70651; *Salmo salar*)	GO:0005524 GO:0006950	ATP binding (MF) response to stress (BP)	711	236/236 (100%)	3e-104	GT128536 GT128537 GT128538
22.1 22.2 22.3	3	Ig-superfamily protein (BAA03716; *Gallus gallus*)			805	46/124 (37%)	1e-07	GT128545 GT128546 GT128547
41.1	3	poly A binding protein,	GO:0003723	RNA binding (MF)	573	114/124	4e-55	GT128604

41.2 41.3		cytoplasmic 1 b (AAH59662.1; *Danio rerio*)	GO:0000166	nucleotide binding (MF)		(91%)		GT128605 GT128606
3.1 3.2 3.3	3	BAC CHORI214-8I14, complete sequence (EF427379; *Salmo salar*)			998	939/1020 (92%)	0.0	GT128488 GT128489 GT128490
15.1 15.2 15.3	3	Beta-2-microglobulin precursor (BT046530; *Salmo salar*)	GO:0002474 GO:0006955 GO:0042612 GO:0005576	antigen processing and presentation of peptide antigen via MHC class I (BP) immune response (BP) MHC class I protein complex (CC) extracellular region (CC) BA11 beta-2 microglobulin (B2m) (AF180479; *Salmo salar*) 2e-19	740	714/720 (99%)	0.0	GT128523 GT128524 GT128525
20.1 20.2 20.3	3	eukaryotic translation initiation factor 3 subunit A (ACN10789; *Salmo salar*)		Keywords: Protein biosynthesis Initiation factor	695	150/186 (80%)	5e-19	GT128539 GT128540 GT128541
26.1 26.2 26.3	3	SPARC (BT045906; *Salmo* *salar*)			669	650/652 (99%)	0.0	GT128554 GT128555 GT128556
47.1 47.2 47.3	3	cytosolic non-specific dipeptidase (BT046056; *Salmo salar*)			435	394/439 (89%)	9e-150	GT128624 GT128625 GT128626
50.1 50.2 50.3	3	voltage-gated sodium channel alpha type IV (ABP98938 ;*Oncorhynchus* *mykiss*)	GO:0006814 GO:0001518 GO:0005248	sodium ion transport (BP) voltage-gated sodium channel complex (CC) voltage-gated sodium channel activity (MF)	344	192/259 (74%)	5e-80	GT128633 GT128634 GT128635
21.1 21.2 21.3	3	platelet-derived growth factor receptor-like protein precursor (ACN10790; *Salmo salar*) platelet-derived growth factor receptor-like protein precursor (BT059077; *Salmo* *salar*)		Keywords Receptor (MF)	691	52/52 (100%) 666/700 (95%)	2e-21 0.0	GT128542 GT128543 GT128544
1.1 1.2	2	cytochrome oxidase subunit I (AAD04735; *Salmo salar*)	GO:0009060 GO:0022900 GO:0006810 GO:0016021 GO:0005743 GO:0070469 GO:0005507 GO:0004129 GO:0009055 GO:0020037	aerobic respiration (BP) electron transport chain (BP) transport (BP) integral to membrane (CC) mitochondrial inner membrane (CC) respiratory chain (CC) copper ion binding (MF) cytochrome-c oxidase activity (MF) electron carrier activity (MF) heme binding (MF)	220	66/72 (91%)	3e-29	GT128484 GT128485
5.1	2	dynein cytoplasmic 1 heavy	GO:0030286	dynein complex (CC)	982	267/315	7e-147	GT128495

5.2		chain 1 (ABC59849;*Danio rerio*)	GO:0005524 GO:0016887 GO:0003777 GO:0007018	ATP binding (MF) ATPase activity (MF) microtubule motor activity (MF) microtubule -based movement (BP)		(84%)		GT128496
7.1 7.2	2	transposable element Tc1 transposase (ACI68988; *Salmo salar*)	GO:0016787	hydrolase activity (MF) guanine deaminase (CAI16262; *Homo sapiens*) 6e-27	1074	88/108 (81%)	1e-46	GT128499 GT128500
9.1 9.2	2	receptor expression-enhancing protein 5 (ACI67469; *Salmo salar*)	GO:0004872	receptor activity (MF) FUNCTION: May promote functional cell surface expression of olfactory receptors. Receptor accessory protein 5 (AAH65926; *Homo sapiens*) 2e-61	818	188/189 (99%)	4e-83	GT128508 GT128509
11.1 11.2	2	optineurin (ACI33853; *Salmo salar*)	GO:0005794 GO:0008022 GO:0008219 GO:0007030 GO:0043001 GO:0000042 GO:0007165	golgi apparatus (CC) protein C-terminus binding (MF) cell death (BP) golgi (BP) golgi to plasma membrane protein transport (BP) protein targeting to Golgi (BP) signal transduction (BP) FUNCTION: Plays a neuroprotective role in the eye and optic nerve. Probably part of the TNF-alpha signaling pathway that can shift the equilibrium toward induction of cell death. According to some authors (Vittitow and Borrás, 2002) its expression is regulated by intraocular pressure, suggesting a protective role in case of high pressure, while according to other authors (Kamphuis and Schneemann, 2003), it is not up-regulated in response to pressure elevation.	800	244/261 (93%)	2e-122	GT128512 GT128513
33.1 33.2	2	tubulin alpha chain (ACI33964; *Salmo salar*)	GO:0005874 GO:0043234 GO:0005525 GO:0003924 GO:0005198 GO:0007018 GO:0051258	microtubule (CC) protein complex (CC) GTP binding (MF) GTPase activity (MF) structural molecule activity (MF) microtubule -based movement (BP) protein polymerization (BP) Function: Tubulin is the major constituent of microtubules. It binds two moles of GTP,	602	107/111 (96%)	9e-57	GT128590 GT128591

				one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain.				
36.1 36.2	2	serine incorporator 1 (ACI32887; *Salmo salar*)	GO:0016020	membrane (CC)	595	47/47 (100%)	2e-20	GT128594 GT128595
37.1 37.2	2	tubulin alpha-1 (CAP72044; *Fasciola hepatica*)	GO:0005874 GO:0043234 GO:0005525 GO:0003924 GO:0005198 GO:0007018 GO:0051258	microtubule (CC) protein complex (CC) GTP binding (MF) GTPase activity (MF) structural molecule (MF) microtubule -based movement (BP) protein polymerization (BP)	615	119/120 (99%)	9e-65	GT128596 GT128597
38.1 38.2	2	SPARC precursor (ACI34168; *Salmo salar*)	GO:0005509	calcium ion binding (MF)	583	169/174 (97%)	2e-86	GT128598 GT128599
39.1 39.2	2	60S ribosomal protein L8 (ACI68574; *Salmo salar*)	GO:0030529	ribonucleoprotein complex (CC)	579	188/189 (99%)	2e-98	GT128600 GT128601
40.1 40.2	2	glyceraldehyde-3-phosphate dehydrogenase (ACI33883; *Salmo salar*)	GO:0006006 GO:0051287 GO:0004365	glucose metabolic process (BP) NAD or NADH binding (MF) glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity (MF)	578	184/192 (95%)	1e-95	GT128602 GT128603
43.1 43.2	2	cathepsin L1 precursor (ACN10121; *Salmo salar*)	GO:0006508 GO:0004197	proteolysis (BP) cysteine-type endopeptidase activity (MF) cathepsin L1 (CAI16307; *Homo sapiens*) 1e-65	564	171/172 (99%)	4e-102	GT128609 GT128610
46.1 46.2	2	glutathione S-transferase A (ACM08643; *Salmo salar*)	GO:0016740	transferase activity (MF) Glutathione S-transferase A (ACI69668; *Salmo salar*) 9e-68	478	126/126 (100%)	3e-68	GT128622 GT128623
2.1 2.2	2	uncharacterized			1086			GT128486 GT128487
10.1 10.2	2	novel cds (BT072520; Salmo salar)			813	387/430 (90%)	1e-147	GT128510 GT128511
16.1 16.2	2	nucleosome assembly protein 1-like 1 (BT045229; *Salmo salar*)	GO:0006334 GO:0005634	nucleosome assembly (BP) nucleus (CC)	720	698/744 (93%)	0.0	GT128526 GT128527
24.1 24.2	2	mitochondrion complete genome (AF133701; *Salmo salar*)			695	658/664 (99%)	0.0	GT128550 GT128551
31.1 31.2	2	microfibrillar-associated protein 2 precursor (BT049030; *Salmo salar*)	GO:0001527	microfibril (CC)	616	602/605 (99%)	0.0	GT128586 GT128587
32.1	2	endothelial differentiation-	GO:0043565	sequence-specific DNA binding (MF)	615	615/615	0.0	GT128588

32.2		related factor 1 homolog (BT049693; *Salmo salar*)				(100%)		GT128589
34.1 34.2	2	sulfate transporter (BT059146; *Salmo salar*)			599	154/177 (87%)	9e-47	GT128592 GT128593
23.1 23.2	2	myelin basic protein (AAH65248; *Homo sapiens*) * unknown large open reading frame (BT071909; Salmo salar)	GO:0007417 GO:0006955 GO:0007268 GO:0043209 GO:0019911	central nervous system development (BP) immune response (BP) synaptic transmission (BP) myelin sheath (CC) structural constituent of myelin sheath (MF)	715	70/136 (51%) 700/716 (97%)	2e-22 0.0	GT128548 GT128549
51.1 51.2	2	collagen alpha-2l chain precursor (BT071961; *Salmo salar*)			257	226/272 (83%)	5e-54	GT128636 GT128637
13.1 13.2	2	selenoprotein Pa precursor (BT072678; *Salmo salar*)			779	777/779 (99%)	0.0	GT128517 GT128518
25.1 25.2	2	claudin domain-containing protein 1 (BT058944; *Salmo salar*)			655	652/656 (99%)	0.0	GT128552 GT128553
27.1 27.2	2	uncharacterized			641			GT128557 GT128558
6.1 6.2	2	uncharacterized			836			GT128497 GT128498
17.1 17.2	2	uncharacterized			718			GT128528 GT128529
28.1 28.2	2	uncharacterized			640			GT128559 GT128560
42.1 42.2	2	uncharacterized			587			GT128607 GT128608
45.1 45.2	2	uncharacterized			485			GT128620 GT128621
49.1 49.2	2	uncharacterized			362			GT128631 GT128632
A1p8	1	main olfactory receptor-like protein (ABD33498; *Salmo salar*)	GO:0007186 GO:0016021 GO:0005886 GO:0004984	G-protein coupled receptor protein signaling pathway (BP) integral to membrane (CC) plasma membrane (CC) olfactory receptor activity (MF)	259	54/64 (84%)	8e-23	GT128638
A2p7	1	NADH dehydrogenase subunit 1 (AAD43230; *Salmo salar*)	GO:0055114 GO:0016021 GO:0005739 GO:0008137	oxidation reduction (BP) integral to membrane (CC) mitochondrion (CC) NADH dehydrogenase (ubiquinone) activity (MF)	304	92/96 (95%)	3e-44	GT128639
A3p5	1	oligoribonuclease, mitochondrial precursor (ACI66405; *Salmo salar*)	GO:0006139 GO:0005739	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process (BP) mitochondrion (CC)	640	38/38 (100%)	5e-14	GT128640

			GO:0008408 GO:0003676	3'-5' exonuclease activity (MF) nucleic acid binding (MF) Rexo2 protein (AAH03445;*Mus musculus*) 4e-06				
A3p6	1	26S protease regulatory subunit 4 (ACI68908; *Salmo salar*)	GO:0005524 GO:0008233	ATP binding (MF) peptidase activity (MF)	574	157/158 (99%)	4e-85	GT128641
A3p8	1	Uncharacterized			464			GT128642
A4p8	1	NADH dehydrogenase iron-sulfur protein 2, mitochondrial precursor (ACI33290; *Salmo salar*)	GO:0051287 GO:0009055 GO:0016651	NAD or NADH binding (MF) electron carrier activity (MF) oxidoreductase activity, acting on NADH or NADPH (MF)	293	97/97 (100%)	1e-52	GT128643
A5p8	1	VMRC26-21K14 (AC187703; *Gasterosteus aculeatus*)			862	183/200 (91%)	2e-69	GT128644
A6p5	1	26S protease regulatory subunit 7 (ACH70710; *Salmo salar*)	GO:0030163 GO:0005737 GO:0005634 GO:0005524 GO:0017111 GO:0008233	protein catabolic process (BP) cytoplasm (CC) nucleus (CC) ATP binding (MF) nucleoside-triphosphatase activity (MF) peptidase activity (MF)	645	197/199 (98%)	2e-99	GT128645
A6p7	1	uncharacterized			402			GT128646
A7p5	1	Alkaline phosphatase (BT044992; *Salmo salar*)	GO:0008152 GO:0016791	metabolic process (BP) phosphatase activity (MF)	845	274/328 (83%)	6e-75	GT128647
A9p8	1	Serum amyloid A-5 protein (ACM09349; *Salmo salar*)	GO:0006953 GO:0005576 GO:0005319	acute-phase response (BP) extracellular region (CC) lipid transporter activity (MF) Serum amyloid A (CAM12347; *Oncorhynchus mykiss*) 6e-13	255	37/38 (97%)	7e-16	GT128648
A10p5	1	uncharacterized			849			GT128649
A12p6	1	Selenoprotein Pa precursor (BT072678; *Salmo salar*)	GO:0006979 GO:0005576 GO:0008430	response to oxidative stress (BP) extracellular region (CC) selenium binding (MF)	883	837/855 (97%)	0.0	GT128650
A12p8	1	uncharacterized			540			GT128651
B1p5	1	elongation factor 1 gamma (ACH85371; *Salmo salar*)	GO:0003746	translation elongation factor activity (MF)	363	92/107 (85%)	2e-47	GT128652
B1p7	1	proteasome subunit beta type-4 precursor (ACI67450; *Salmo salar*)	GO:0006511 GO:0005829 GO:0005839 GO:0004298	ubiquitin-dependent protein catabolic process (BP) cytosol (CC) proteasome core complex (CC) threonine-type endopeptidase activity (MF)	387	122/122 (100%)	3e-65	GT128653
B1p8	1	Barrier-to-autointegration factor (ACI66039; *Salmo*	GO:0003677	DNA binding (MF)	298	40/40 (100%)	6e-16	GT128654

		salar)						
B2p8	1	uncharacterized			555			GT128655
B3p5	1	CA protein (AF515834; *Schistosoma japonicum*)			628	84/100 (84%)	7e-16	GT128656
B3p6	1	uncharacterized			597			GT128657
B3p7	1	novel cds (BT071844.1; *Salmo salar*)			371	333/357 (93%)	1e-142	GT128658
B3p8	1	NF-kappa-B inhibitor alpha (ACI67986; *Salmo salar*)	GO:0006915 GO:0007253 GO:0044419 GO:0043392 GO:0010745 GO:0010888 GO:0032270 GO:0010875 GO:0010552 GO:0033256 GO:0005829 GO:0005634 GO:0051059 GO:0042802 GO:0008139 GO:0031625	apoptosis (BP) cytoplasmic sequestering of NF-kappaB (BP) interspecies interaction between organisms (BP) negative regulation of DNA binding (BP) negative regulation of foam cell differentiation (BP) negative regulation of lipid storage (BP) positive regulation of cellular protein metabolic process (BP) positive regulation of cholesterol efflux (BP) positive regulation of specific transcription from RNA polymerase II promoter (BP) I-kappaB/NF-kappaB complex (CC) cytosol (CC) nucleus (CC) NF-kappaB binding (MF) identical protein binding (MF) nuclear localization sequence binding (MF) ubiquitin protein ligase binding (MF) NF-kappa-B inhibitor alpha (P25963; *Homo sapiens*) 9e-24	738	119/119 (100%)	1e-53	GT128659
B5p5	1	uncharacterized			762			GT128660
B5p6	1	glutamine synthetase (AAM73660; *Oncorhynchus mykiss*)	GO:0006542 GO:0004356	glutamine biosynthetic process (BP) glutamate-ammonia ligase activity (MF)	795	230/234 (98%)	8e-140	GT128661
B5p7	1	uncharacterized			390			GT128662
B5p8	1	unknown large open reading frame mRNA, novel cds (BT072783; *Salmo salar*)			595	99/116 (85%)	2e-21	GT128663
B6p7	1	Translocon-associated protein subunit alpha precursor (ACM08230; *Salmo salar*)	GO:0006613 GO:0008284 GO:0005789 GO:0016021 GO:0005509	cotranslational protein targeting to membrane (BP) positive regulation of cell proliferation (BP) endoplasmic reticulum membrane (CC) integral to membrane (CC) calcium ion binding (MF)	446	148/148 (100%)	1e-65	GT128664

			GO:0005048	signal sequence binding (MF) signal sequence receptor, alpha variant (BAD96529; *Homo sapiens*) 8e-31				
B6p8	1	Cold-inducible RNA-binding protein (BT057118; *Salmo salar*)	GO:0003676 GO:0000166	nucleic acid binding (MF) nucleotide binding (MF) hyperosmotic glycine rich protein (AF533016; *Salmo salar*) 3e-134	295	281/284 (98%)	1e-140	GT128665
B7p6	1	matrix metalloproteinase-2 (BAA78479; *Oncorhynchus mykiss*)	GO:0006508 GO:0005578 GO:0005509 GO:0004222 GO:0008270	proteolysis (BP) proteinaceous extracellular matrix (CC) calcium ion binding (MF): metalloendopeptidase activity (MF) zinc ion binding (MF)	538	163/169 (96%)	2e-95	GT128666
B8p5	1	grancalcin (ACI66013; *Salmo salar*)	GO:0006944 GO:0005737 GO:0005886 GO:0005509	membrane fusion (BP) cytoplasm (CC) plasma membrane (CC) calcium ion binding (MF) Grancalcin, EF-hand calcium binding protein (AAH05214; *Homo sapiens*) 6e-65	694	159/160 (99%)	5e-87	GT128667
B10p5	1	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-3 (BT048157; Salmo salar)			576	542/567 (95%)	0.0	GT128668
B10p7	1	eukaryotic translation initiation factor 3, subunit 5 epsilon, 47kDa (BAF62356; *Pan troglodytes verus*)	GO:0006412 GO:0003743	translation (BP) translation initiation factor activity (MF)	592	153/195 (78%)	8e-87	GT128669
C1p6	1	uncharacterized			777			GT128670
C4p6	1	tripartite motif-containing protein 16 (BT045784; *Salmo salar*)	GO:0005622 GO:0005515 GO:0008270	Intracellular (CC) protein binding (MF) zinc ion binding (MF)	673	617/628 (98%)	0.0	GT128671
C4p8		iron-sulfur cluster assembly enzyme ISCU, mitochondrial precursor (BT049908; *Salmo salar*)	GO:0016226 GO:0005506 GO:0051536	iron-sulfur cluster assembly (BP) iron ion binding (MF) iron-sulfur cluster binding (MF)	437	380/384 (98%)	0.0	GT128672
C5p8	1	alpha tubulin 2 (AAM09674; *Aplysia californica*)	GO:0007018 GO:0051258 GO:0005874 GO:0043234 GO:0005525 GO:0003924 GO:0005198	microtubule -based movement (BP) protein polymerization (BP) microtubule (CC) protein complex (CC) GTP binding (MF) GTPase activity (MF) structural molecule activity (MF)	231	63/71 (88%)	5e-27	GT128673
C6p5	1	septin-2 (BT071903; *Salmo salar*)			789	786/790 (99%)	0.0	GT128674

C6p6	1	insulin-like growth factor binding protein 2 (DQ146968; *Oncorhynchus mykiss*)	GO:0001558 GO:0005576 GO:0005520	regulation of cell growth (BP) extracellular region (CC) insulin-like growth factor binding (MF)	637	469/513 (91%)	0.0	GT128675
C7p6	1	neuroblast differentiation-associated protein AHNAK (ACN60226; *Salmo salar*) Neuroblast differentiation-associated protein AHNAK (BT072112; *Salmo salar*)			901	85/86 (98%) (aa) 881/901 (97%)	1e-10 0.0	GT128676
C7p7	1	uncharacterized			485			GT128677
C8p8	1	LWamide neuropeptide precursor protein (CAA61886; *Hydractinia echinata*) Heme-binding protein 2 (BT059063.1; *Salmo salar*)	GO:0007218 GO:0005576	neuropeptide signaling pathway (BP) extracellular region (CC)	601	36/108 (33%) (aa) 502/540 (92%) (nt)	3e-08 0.0	GT128678
C9p5	1	phosphoglycerate mutase 1 (BT044637; *Salmo salar*)	GO:0006096 GO:0016868	glycolysis (BP) intramolecular transferase activity, phosphotransferases (MF)	397	360/360 (100%)	0.0	GT128679
C9p6	1	Zinc finger protein 503 (ACI33520; *Salmo salar*)	GO:0005622 GO:0008270	intracellular (CC) zinc ion binding (MF)	632	209/210 (99%)	4e-85	GT128680
C10p5	1	origin recognition complex subunit 2 (BT059550; *Salmo salar*)			487	487/487 (100%)	0.0	GT128681
C10p8	1	uncharacterized			342			GT128682
C11p5	1	nuclear factor erythroid derived 2-like protein 1 (ABW77494; *Salmo salar*)	GO:0006355 GO:0043565 GO:0003700	regulation of transcription, DNA-dependent (BP) sequence-specific DNA binding (MF) transcription factor activity (MF)	812	233/235 (99%)	3e-77	GT128683
C11p8	1	uncharacterized			412			GT128684
C12p7	1	Splicing factor, arginine /serine-rich 7 (BT044936; *Salmo salar*)	GO:0003676 GO:0000166 GO:0008270	nucleic acid binding (MF) nucleotide binding (MF) zinc ion binding (MF)	787	784/787 (99%)	0.0	GT128685
D1p7	1	uncharacterized			216			GT128686
D1p8	1	uncharacterized			249			GT128687
D2p6	1	uncharacterized			565			GT128688
D3p5	1	uncharacterized			648			GT128689
D3p6	1	1-phosphatidylinositol-4,5-bisphosphate			831	187/209 (89%)	6e-65	GT128690

		phosphodiesterase beta-3 (BT048157; *Salmo salar*)						
D4p5	1	protein phosphatase 1, regulatory (inhibitor) subunit 1b-like (BT043676; *Salmo salar*)	GO:0007165 GO:0004864	signal transduction (BP) phosphoprotein phosphatase inhibitor activity (MF)	690	671/692 (96%)	0.0	GT128691
D4p6	1	peripheral myelin protein 22 (BT045468; *Salmo salar*)	GO:0016020	membrane (CC)	851	842/863 (97%)	0.0	GT128692
D4p8	1	novel cds (BT072673; *Salmo salar*)			389	364/365 (99%)	0.0	GT128693
D5p5	1	cytoplasmic FMR1-interacting protein 2 (ACI69833; *Salmo salar*) L-xylulose reductase (BT059117; Salmo salar)	 GO:0008152 GO:0016491	 metabolic process (BP) oxidoreductase activity (MF)	776	30/38 (78%) 564/602 (93%) (nt)	1e-07 0.0	GT128694
D5p8	1	beta thymosin (AJ250180; *Oncorhynchus mykiss*)	GO:0042989 GO:0005737 GO:0003779	sequestering of actin monomers (BP) cytoplasm (CC) actin binding (MF)	290	255/262 (97%)	2e-120	GT128695
D6p8	1	uncharacterized			626			GT128696
D7p6	1	uncharacterized			966			GT128697
D7p8	1	abhydrolase domain-containing protein 10, mitochondrial precursor (BT045396; *Salmo salar*)	GO:0016787	hydrolase activity (MF)	661	657/662 (99%)	0.0	GT128698
D8p8	1	unknown large open reading frame (BT071909; *Salmo salar*)			243	179/181 (98%)	2e-84	GT128699
D9p5	1	BAC CH214-397C7 complete sequence (AC203456; *Salmo salar*)			771	766/771 (99%)	0.0	GT128700
D9p7	1	gametogenetin-binding protein 2 (BT056828; *Salmo salar*)			400	166/180 (92%)	8e-64	GT128701
D10p5	1	glucose transporter 1A (AAF75681; *Oncorhynchus mykiss*)	GO:0008643 GO:0055085 GO:0016021 GO:0022891 GO:0005351	carbohydrate transport (BP) transmembrane transport (BP) integral to membrane (CC) glucose transmembrane transporter activity (MF) sugar:hydrogen symporter activity (MF)	953	63/100 (63%)	6e-24	GT128702
D10p6	1	uncharacterized			586			GT128703
D10p7	1	uncharacterized			302			GT128704
D11p7	1	Tc1-like transporase (BAF37936; *Oncorhynchus*	GO:0015074 GO:0006313	DNA integration (BP) transposition, DNA-mediated (BP)	299	53/80 (66%)	2e-25	GT128705

		mykiss)	GO:0005634 GO:0003677 GO:0004803	Nucleus (CC) DNA binding (MF) transposase activity (MF)				
D12p5	1	serine/threonine-protein phosphatase PP1-beta catalytic subunit (BT072371; *Salmo salar)*			247	247/247 (100%)	6e-125	GT128706
D12p7	1	hypoxia-inducbile gene1 domain family member 1A (ACQ57876; *Anoplopoma fimbria*)	GO:0006950 GO:0016021 GO:0043234 GO:0005515	response to stress (BP) integral to membrane (CC) protein complex (CC) protein binding (MF) hypoxia-inducbile gene 1 (AAD33954; *Homo sapiens*) 1e-20	334	62/74 (83%)	3e-28	GT128707
E1p6	1	uncharacterized			933			GT128708
E1p8	1	ubiquitin-conjugating enzyme 16 (AAY24555; *Homo sapiens*)	GO:0043687 GO:0051246 GO:0019787	post-translational protein modification (BP) regulation of protein metabolic process (BP) small conjugating protein ligase activity (MF)	516	103/106 (97%)	2e-46	GT128709
E1p7	1	annexin A11 (BT072167; Salmo salar)			294	293/294 (99%)	6e-150	GT128710
E4p5	1	60S ribosomal protein L7a (ACH70989; *Salmo salar*)	GO:0042254 GO:0005840	ribosome biogenesis (BP) ribosome (CC)	468	51/60 (85%)	2e-24	GT128711
E4p6	1	unclassified			681			GT128712
E4p8	1	cathepsin A (AAQ18146; *Branchiostoma belcheri tsingtaunese*)	GO:0006508 GO:0004185	proteolysis (BP) serine-type carboxypeptidase activity (MF)	351	29/47 (61%)	1e-09	GT128713
E5p7	1	uncharacterized			330			GT128714
E6p6	1	fibulin-1 (ACN11181; *Salmo salar*)	GO:0005737 GO:0005576 GO:0005509	cytoplasm (CC) extracellular region (CC) calcium ion binding (MF) fibulin 1 (CAQ10153; *Homo sapiens*) 1e-14	535	124/124 (100%)	2e-27	GT128715
E7p6	1	GDP-mannose 4,6 dehydratase (BT049328; *Salmo salar*)	GO:0044237 GO:0003824 GO:0050662	cellular metabolic process (BP) catalytic activity (MF) coenzyme binding (MF)	676	675/676 (99%)	0.0	GT128716
E7p7	1	C7orf57 (ACO13926; *Esox lucius*) C7orf57 homolog (BT045484; *Salmo salar)*			724	135/161 (83%) (aa) 393/393 (100%)	4e-71 0.0	GT128717
E8p5	1	twinfilin-1 (BT059739; *Salmo salar*)			688	57/63 (90%) (nt)	4e-12	GT128718
E8p7		ubiquitin-conjugating enzyme E2 L3 putative mRNA, complete cds (BT056382;		Keywords Ubl conjugation pathway (BP) Ligase (MF)	613	572/590 (96%)	0.0	GT128719

		Salmo salar)						
E8p8	1	uncharacterized			386			GT128720
E9p7	1	iduronate 2-sulfatase (ABK59973; *Danio rerio*)	GO:0008152 GO:0008484	metabolic process (BP) sulfuric ester hydrolase activity (MF)	456	68/110 (61%)	1e-27	GT128721
E10p5	1	syntaxin binding protein 1 (AAI21968; *Xenopus tropicalis*) Syntaxin-binding protein 1 (BT072082; *Salmo salar*)	GO:0006904	vesicle docking during exocytosis (BP) Function: located in the pre-synaptic terminals and play central roles in Ca(2+)-triggered neurotransmitter release (Caoa et. al., 2009).	527	23/33 (69%) (aa) 353/354 (99%)	6e-06 0.0	GT128722
E10p7	1	serine/threonine-protein phosphatase PP1-beta catalytic subunit (ACI69393; *Salmo salar*)	GO:0016787	hydrolase activity (MF)	703	195/198 (98%)	2e-58	GT128723
E11p6	1	uncharacterized			591			GT128724
E12p7	1	growth hormone 1 gene, intron 5 (EF564440; *Oncorhynchus masou formosanus*)			190	76/82 (92%)	1e-22	GT128725
E12p8	1	uncharacterized			501			GT128726
A7p2	1	eukaryotic initiation factor 2 alpha subunit (AF338347; *Oncorhynchus mykiss*)	GO:0006412 GO:0005850 GO:0003723 GO:0003743	Translation (BP) eukaryotic translation initiation factor 2 complex (CC) RNA binding (MF) translation initiation factor activity (MF)	777	750/786 (95%)	0.0	GT128727
B3p1	1	uncharacterized			65			GT128728
B7p2	1	pleiotrophic factor-alpha-2 precursor (BT057895; Salmo salar)			761	760/764 (99%)	0.0	GT128729
C7p2	1	ES1 protein homolog, mitochondrial precursor (ACM08279; *Salmo salar*)	GO:0005739	mitochondrion (CC) HES1 (CAA68857; *Homo sapiens*) 4e-27	593	62/114 (54%)	2e-29	GT128730
F3p6	1	vacuolar ATP synthase subunit e 2 (BT057637; *Salmo salar*)			574	153/168 (91%)	5e-55	GT128731
F3p7	1	uncharacterized			580			GT128732
F4p5	1	uncharacterized			750			GT128733
F4p7	1	uncharacterized			495			GT128734
D3p1	1	chondromodulin-I (AAA30445; *Bos taurus*)	GO:0051216 GO:0030154 GO:0005576	cartilage development (BP) cell differentiation (BP) extracellular region (CC)	533	39/50 (78%)	3e-20	GT128735

			GO:0016021	integral to membrane (CC) Function: Bifunctional growth regulator that stimulates the growth of cultured chondrocytes in the presence of basic fibroblast growth factor (FGF) but inhibits the growth of cultured vascular endothelial cells. May contribute to the rapid growth of cartilage and vascular invasion prior to the replacement of cartilage by bone during endochondral bone development. Otolith development (Nemoto *et al*, 2007)				
E7p2	1	brain protein 44-like protein (ACI66404; *Salmo salar*)	GO:0005739	mitochondrion (CC) CGI-129 protein (gb|AAD34124.1; *Homo sapiens*) 9e-36	642	84/87 (96%)	1e-42	GT128736
F5p7	1	DNA-directed RNA polymerase II subunit RPB2 (ACN10943; *Salmo salar*)	GO:0006368 GO:0000398 GO:0006367 GO:0005665 GO:0003677 GO:0003899 GO:0000287 GO:0005515 GO:0008270	RNA elongation from RNA polymerase II promoter (BP) nuclear mRNA splicing, via spliceosome (BP) transcription initiation from RNA polymerase II promoter (BP) DNA-directed RNA polymerase II, core complex (CC) DNA binding (MF) DNA-directed RNA polymerase activity (MF) magnesium ion binding (MF) protein binding (MF) zinc ion binding (MF) RNA polymerase II 140 kDa subunit (*AAC09367; Homo* sapiens) 6e-66	372	122/123 (99%)	2e-66	GT128737
F5p8	1	uncharacterized			240			GT128738
E3p1	1	integrin beta-1 precursor (ACN11424; *Salmo salar*)	GO:0030183 GO:0033631 GO:0007160 GO:0006968 GO:0007156 GO:0007229 GO:0044419 GO:0007159 GO:0009986 GO:0008305 GO:0042470	B cell differentiation (BP) cell-cell adhesion mediated by integrin (BP) cell-matrix adhesion (BP) cellular defense response (BP) homophilic cell adhesion (BP) integrin-mediated signaling pathway (BP) interspecies interaction between organisms (BP) leukocyte adhesion (BP) cell surface (CC) integrin complex (CC) melanosome (CC)	600	142/146 (97%)	3e-83	GT128739

			GO:0031594 GO:0001726 GO:0042383 GO:0042802 GO:0046982 GO:0004872	neuromuscular junction (CC) ruffle (CC) sarcolemma (CC) identical protein binding (MF) protein heterodimerization activity (MF) receptor activity (MF) Integrin beta-1 (P05556; Homo sapiens) 9e-57				
F6p6	1	peripheral myelin protein 22 (ACI33730; *Salmo salar*)	GO:0007638 GO:0007422 GO:0007605 GO:0007268 GO:0016021	mechanosensory behavior (BP) peripheral nervous system development (BP) sensory perception of sound (BP) synaptic transmission (BP) integral to membrane (CC) peripheral myelin protein (AAB26811; *Homo sapiens)* 1e-22	517	74/82 (90%)	2e-34	GT128740
F3p1	1	40S ribosomal protein S27 (ACI67409; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF)	335	59/59 (100%)	1e-28	GT128741
G7p2	1	collagen alpha-1I chain precursor (BT072251; *Salmo salar*)			680	599/704 (85%)	0.0	GT128742
F7p6	1	Catenin beta-1 (ACN58608; *Salmo salar*)	GO:0060070 GO:0006915 GO:0045453 GO:0048593 GO:0016337 GO:0007160 GO:0032331 GO:0045671 GO:0042475 GO:0045669 GO:0050808 GO:0007268 GO:0048489 GO:0016328 GO:0030018 GO:0045296 GO:0003713	Wnt receptor signaling pathway through beta-catenin (BP) apoptosis (BP) bone resorption (BP) camera-type eye morphogenesis (BP) cell-cell adhesion (BP) cell-matrix adhesion (BP) negative regulation of chondrocyte differentiation (BP) negative regulation of osteoclast differentiation (BP) odontogenesis of dentine-containing tooth (BP) positive regulation of osteoblast differentiation (BP) synapse organization (BP) synaptic transmission (BP) synaptic vesicle transport (BP) lateral plasma membrane (CC) Z disc (CC) cadherin binding (MF) transcription coactivator activity (MF)	764	144/144 (100%)	8e-66	GT128743

				catenin beta-1 (BAG70078; *Homo sapiens*) 5e-61				
F7p7	1	uncharacterized			322			GT128744
F7p8	1	ribosomal protein L7-1 (ACH70988; *Salmo salar*)	GO:0006412 GO:0015934 GO:0003735 GO:0030528	translation (BP) large ribosomal subunit (CC) structural constituent of ribosome MF) transcription regulator activity (MF)	604	182/183 (99%)	2e-100	GT128745
F7p1	1	uncharacterized			310			GT128746
F8p5	1	uncharacterized			302			GT128747
F8p6	1	cytoskeleton-associated protein 5 (ACN58609; *Salmo salar*)	GO:0050658 GO:0051301 GO:0051297 GO:0030951 GO:0007067 GO:0051300 GO:0005813 GO:0005515	RNA transport (BP) cell division (BP) centrosome organization (BP) establishment or maintenance of microtubule cytoskeleton polarity (BP) mitosis (BP) spindle pole body organization (BP) centrosome (CC) protein binding (MF) cytoskeleton-associated protein 5 (BAA07892; *Homo sapiens*) 6e-91	889	253/254 (99%)	3e-134	GT128748
F8p7	1	uncharacterized			530			GT128749
F9p6	1	keratin associated protein 4.12 (CAC27582; *Homo sapiens*)	GO:0045095	keratin filament (CC)	696	85/232 (36%)	4e-23	GT128750
F9p7	1	uncharacterized			266			GT128751
F10p7	1	uncharacterized			374			GT128752
C8p2	1	chaperonin containing TCP1, subunit 7 (AAI63866; *Danio rerio*)	GO:0006457 GO:0005524 GO:0051082	protein folding (BP) ATP binding (MF) unfolded protein binding (MF)	567	168/188 (89%)	2e-88	GT128753
F11p6	1	serine/threonine kinase receptor (AAM53074; *Danio rerio*)	GO:0048514 GO:0048264	blood vessel morphogenesis (BP) determination of ventral identity (BP)	895	73/86 (84%)	5e-37	GT128754
F11p7	1	ependymin precursor (ACI33774; *Salmo salar*)	GO:0007160 GO:0007160 GO:0005509	cell-matrix adhesion (BP) extracellular region (CC) calcium ion binding (MF)	375	69/70 (98%)	1e-34	GT128755
F12p6	1	uncharacterized			648			GT128756
F12p7	1	uncharacterized			324			GT128757
D8p2	1	ubiquitin-conjugating enzyme E2 R1 (BT058795; *Salmo salar*)			623	205/222 (92%)	8e-83	GT128758
B9p1	1	Parvalbumin, thymic CPV3 (ACM09534; *Salmo salar*)	GO:0005509	calcium ion binding (MF) oncomodulin (AAA02869; *Homo sapiens*)	369	94/94 (100%)	3e-47	GT128759

				2e-25				
E8p2	1	uncharacterized			702			GT128760
F8p2	1	TAR DNA-binding protein 43 (ACI33676; *Salmo salar*)	GO:0003677 GO:0000166	DNA binding (MF) nucleotide binding (MF)	607	198/201 (98%)	2e-75	GT128761
D9p1	1	ribosomal protein L6 (ABY28366; *Oncorhynchus masou formosanus*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF)	247	62/62 (100%)	1e-28	GT128762
G8p2	1	growth factor receptor-bound protein 2 (BT058722; *Salmo salar*)		Keywords receptor (MF)	625	605/654 (92%)	0.0	GT128763
E9p1	1	uncharacterized			498			GT128764
H8p2	1	uncharacterized			1111			GT128765
A9p2	1	uncharacterized			728			GT128766
B9p2	1	uncharacterized			1223			GT128767
F9p1	1	SPARC precursor (ACI34168; *Salmo salar*)	GO:0001503 GO:0007169 GO:0005604 GO:0031093 GO:0005509 GO:0005518 GO:0005507	Ossification (BP) transmembrane receptor protein tyrosine kinase signaling pathway (BP) basement membrane (CC) platelet alpha granule lumen (CC) calcium ion binding (MF) collagen binding (MF) copper ion binding (MF) osteonectin (AAA60570; *Homo sapiens*) 3e-14	144	47/47 (100%)	6e-19	GT128768
C9p2	1	Na/K ATPase alpha subunit isoform 2 (AY319387; *Oncorhynchus mykiss*)	GO:0008152 GO:0006813 GO:0016021 GO:0005524 GO:0015662 GO:0015077 GO:0030955	metabolic process (BP) potassium ion transport (BP) integral to membrane (CC) ATP binding (MF) ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism (MF) monovalent inorganic cation transmembrane transporter activity (MF) potassium ion binding (MF)	777	194/205 (94%)	3e-82	GT128769
D9p2	1	Elongation factor 2 (BT071866; *Salmo salar*)		Keywords protein biosynthesis (BP) elongation factor (MF)	741	62/62 (100%)	3e-22	GT128770
F9p2	1	uncharacterized			891			GT128771
A10p2	1	carboxypeptidase X, member 1 (AAI33528; *Bos taurus*)	GO:0007155 GO:0006508 GO:0004181 GO:0008270	cell adhesion (BP) proteolysis (BP) metallocarboxypeptidase activity (MF) zinc ion binding (MF)	577	135/193 (69%)	7e-71	GT128772
C10p	1	apolipoprotein B mRNA			835	122/139	2e-34	GT128773

2		(X81856; *Salmo salar*)				(87%)		
D10p2	1	uncharacterized			527			GT128774
C11p2	1	simple type II keratin K8a (S1) (CAC45060; *Oncorhynchus mykiss*)	GO:0005882 GO:0005198	intermediate filament (CC) structural molecule activity (MF)	611	118/121 (97%)	1e-25	GT128775
E11p2	1	CD9 antigen (ACI66221; *Salmo salar*)	GO:0016021	integral to membrane (CC)	701	42/58 (72%)	4e-19	GT128776
H11p2	1	uncharacterized			667			GT128777
G1p7	1	uncharacterized			414			GT128778
G2p8	1	uncharacterized			453			GT128779
G3p5	1	PDZ and LIM domain protein 2 (BT044685; *Salmo salar*)	GO:0005515 GO:0008270	protein binding (MF) zinc ion binding (MF)	602	180/195 (92%)	5e-71	GT128780
G3p6	1	transposable element Tcb2 transposase (AAA28149; *Caenorhabditis briggsae*)	GO:0015074 GO:0006313 GO:0005634 GO:0003677 GO:0004803	DNA integration (BP) transposition, DNA-mediated (BP) nucleus (CC) DNA binding (MF) transposase activity (MF)	570	47/127 (37%)	3e-18	GT128781
G3p8	1	cytochrome oxidase subunit 1 (ABK06129 ; *Parupeneus indicus*)	GO:0009060 GO:0055114 GO:0006810 GO:0016021 GO:0005746 GO:0005507 GO:0004129 GO:0009055 GO:0020037 GO:0005506	aerobic respiration (BP) oxidation reduction (BP) transport (BP) integral to membrane (CC) mitochondrial respiratory chain (CC) copper ion binding (MF) cytochrome-c oxidase activity (MF) electron carrier activity (MF) heme binding (MF) iron ion binding (MF)	571	174/190 (91%)	2e-51	GT128782
G4p7	1	uncharacterized			472			GT128783
G4p8	1	60S ribosomal protein L17 (ACI33750; *Salmo salar*)	GO:0006412 GO:0015934 GO:0003735	translation (BP) large ribosomal subunit (CC) structural constituent of ribosome (MF)	440	136/137 (99%)	8e-65	GT128784
G7p7	1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1 (ACH70848; *Salmo salar*)	GO:0015986 GO:0000276 GO:0015078	ATP synthesis coupled proton transport (BP) mitochondrial proton-transporting ATP synthase complex, coupling factor F(o) (CC) hydrogen ion transmembrane transporter activity (MF)	464	150/150 (100%)	4e-78	GT128816
G7p8	1	chloride intracellular channel 4 (AAH51622; *Danio rerio*)	GO:0006821 GO:0016020 GO:0005247	chloride transport (BP) membrane (CC) voltage-gated chloride channel activity (MF)	405	34/46 (73%)	2e-14	GT128785
G8p6	1	uncharacterized			612			GT128786
G9p7	1	OclMSU21 sequence microsatellite (GQ249048; *Oncorhynchus clarkia*)			221	112/135 (82%)	4e-22	GT128787

G9p8	1	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2, 3-sialyltransferase (BT072382; *Salmo salar*)			280	279/280 (99%)	1e-141	GT128788
G10p 6	1	uncharacterized			891			GT128789
G10p 7	1	uncharacterized			279			GT128790
G10p 8	1	guanine nucleotide binding protein beta 2 (ABC75581; *Ictalurus punctatus*)	GO:0005886 GO:0019903 GO:0005102	plasma membrane (CC) protein phosphatase binding (MF) receptor binding (MF) guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1 variant (BAD96208; *Homo sapiens*) 2e-35	306	64/70 (91%)	8e-38	GT128791
G11p 8	1	uncharacterized			301			GT128792
G12p 6	1	Cyclin-dependent kinase 2-associated protein 2 (BT044623; *Salmo salar*)	GO:0016301	kinase activity (MF)	577	85/102 (83%)	3e-14	GT128793
G12p 8	1	cAMP-responsive element-binding protein-like 2 (BT058965; *Salmo salar*)	GO:0006355 GO:0005634 GO:0046983 GO:0043565 GO:0003700	regulation of transcription, DNA-dependent (BP) nucleus (CC) protein dimerization activity (MF) sequence-specific DNA binding (MF) transcription factor activity (MF)	211	73/76 (96%)	7e-25	GT128794
H1p5	1	salarin gene for cysteine proteinase inhibitor protein, exons 1-13 (AJ550228; *Salmo salar*)	GO:0008234	cysteine-type peptidase activity (MF)	511	35/35 (100%)	2e-07	GT128795
H1p6	1	uncharacterized			687			GT128796
H2p6	1	tubulin beta chain (BT044664; *Salmo salar*)	GO:0005874 GO:0043234 GO:0005525 GO:0003924 GO:0005198 GO:0007018 GO:0051258	microtubule (CC) protein complex (CC) GTP binding (MF) GTPase activity (MF) structural molecule activity (MF) microtubule -based movement (BP) protein polymerization (BP)	937	97/110 (88%)	3e-26	GT128797
H3p7	1	uncharacterized			500			GT128798
H3p8	1	uncharacterized			267			GT128799
H4p6	1	guanine nucleotide binding protein beta 2 (ABC75581; *Ictalurus punctatus*) (same as H03p7)	GO:0005886 GO:0019903 GO:0005102	plasma membrane (CC) protein phosphatase binding (MF) receptor binding (MF) guanine nucleotide binding protein (G	566	137/153 (89%)	1e-75	GT128800

				protein), beta polypeptide 2-like 1 variant (BAD96208; *Homo sapiens*) 2e-35				
H4p7	1	uncharacterized			496			GT128801
H5p8	1	ubiquitin-conjugating enzyme E2 L3 (BT072278; *Salmo salar*)			893	68/77 (88%)	1e-15	GT128802
H6p6	1	uncharacterized			571			GT128803
H6p8	1	uncharacterized			308			GT128804
H7p7	1	high affinity copper uptake protein 1 (BT059358; *Salmo salar*)			479	192/216 (88%)	1e-64	GT128805
H7p8	1	calmodulin (BT060344; *Salmo salar*)	GO:0005509 GO:0016301	calcium ion binding (MF) kinase activity (MF)	240	81/82 (98%)	3e-31	GT128806
H8p5	1	FK506-binding protein 2 precursor (ACI68911; *Salmo salar*)	GO:0006457	protein folding (BP)	534	137/138 (99%)	4e-66	GT128807
H8p6	1	uncharacterized			565			GT128808
H8p8	1	active breakpoint cluster region-related protein (BT071954; *Salmo salar*)			494	429/500 (85%)	5e-138	GT128809
H9p5	1	integral membrane protein 2B (ACI33031; *Salmo salar*)	GO:0007399 GO:0007605 GO:0000139 GO:0016021	nervous system development (BP) sensory perception of sound (BP) golgi membrane (CC) integral to membrane (CC) transmembrane protein BRI (AAF66130; *Homo sapiens*) 9e-26	359	98/100 (98%)	6e-49	GT128810
H9p6	1	myostatin 2a gene, promoter region (DQ138301; *Oncorhynchus mykiss*)	GO:0040007 GO:0005576 GO:0008083	growth (BP) extracellular region (CC) growth factor activity (MF)	752	270/301 (89%)	3e-100	GT128811
H9p7	1	actin, cytoplasmic 1 (BT045109; *Salmo salar*)	GO:0005524 GO:0005515	ATP binding (MF) protein binding (MF)	365	363/368 (98%)	0.0	GT128812
H9p8	1	fructose-bisphosphate aldolase A (BT058987; *Salmo salar*)			482	236/267 (88%)	3e-81	GT128813
H11p7	1	calumenin precursor (BT044805; *Salmo salar*)	GO:0005576 GO:0042470 GO:0033018 GO:0005509	extracellular region (CC) melanosome (CC) sarcoplasmic reticulum lumen (CC) calcium ion binding (MF)	340	312/325 (96%)	3e-145	GT128814
H12p8	1	orphan nuclear receptor NR1D2 variant (BAD92328; *Homo sapiens*)	GO:0006355 GO:0005634 GO:0043565 GO:0003707 GO:0003700	regulation of transcription, DNA-dependent (BP) nucleus (CC) sequence-specific DNA binding (MF) steroid hormone receptor activity (MF) transcription factor activity (MF)	446	59/71 (83%)	4e-18	GT128815

			GO:0008270	zinc ion binding (MF)				
ESTs from Forward SSH cDNA Library from salmon ear designed to be enriched for seismic exposed genes. Out of 50 contigs: 29 have a significant BLASTX hit (E<1e-05); 13 have a significant BLASTN hit. (E<1e-05) highlighted in blue font; and 8 have no significant BLASTX or BLASTN hit (E<1e-05). Out of 179 singletons: 60 have a significant BLASTX hit (E<1e-05); 58 have a significant BLASTN hit. (E<1e-05) highlighted in blue font; 61 have no significant BLASTX or BLASTN hit (E<1e-05). EST ID if reported as Contig # for contigs and well ID plus plate number, for singletons. Forward Library name is ssal_ear_SSH_seismicF. BLAST results were up-dated August 2010.								

7.4 Supplemental Table S4 ESTs from reverse SSH cDNA Atlantic salmon ear library (ssal_ear_SSH_seismicR)

contig #	# ESTs	BLAST identification of salmon cDNA (gene name, acc# of hit; species name)	GO term	GO description	Seq length (bp)	% ID align	E-value	Accession number
48.1 48.2 48.3 48.4 48.5 48.6 48.7 48.8 48.9 48.10 48.11 48.12 48.13 48.14 48.15 48.16	16	senescence-associated protein (ACA04850; *Picea abies*)			1389	69/90 (76%)	3e-31	GT128952 GT128953 GT128954 GT128955 GT128956 GT128957 GT128958 GT128959 GT128960 GT128961 GT128962 GT128963 GT128964 GT128965 GT128966 GT128967
31.1 31.2 31.3 31.4 31.5 31.6 31.7 31.8 31.9 31.10 31.11 31.12 31.13	13	cytochrome oxidase subunit II (AAD04736; *Salmo salar*)	GO:0022904 GO:0006810 GO:0016021 GO:0005746 GO:0005507 GO:0004129 GO:0009055 GO:0020037	respiratory electron transport chain (BP) transport (BP) integral to membrane (CC) mitochondrial respiratory chain (CC) copper ion binding (MF) cytochrome-c oxidase activity (MF) electron carrier activity (MF) heme binding (MF)	640	201/212 (94%)	3e-95	GT128898 GT128899 GT128900 GT128901 GT128902 GT128903 GT128904 GT128905 GT128906 GT128907 GT128908 GT128909 GT128910
12.1 12.2 12.3 12.4 12.5 12.6 12.7 12.8 12.9 12.10	10	28S ribosomal RNA (U34341; *Oncorhynchus mykiss*)			901	852/860 (99%)	0.0	GT128842 GT128843 GT128844 GT128845 GT128846 GT128847 GT128848 GT128849 GT128850 GT128851
15.1 15.2 15.3	6	matn4 protein (AAI63867; *Danio rerio*)		cytoskeletal structural protein in inner ear (Klockars et. al., 2002)	1234	154/188 (81%)	3e-80	GT128856 GT128857 GT128858

15.4 15.5 15.6								GT128859 GT128860 GT128861
36.1 36.2 36.3 36.4 36.5 36.6	6	transposable element Tc1 transposase (ACI68988; *Salmo salar*)			1137	69/80 (86%)	2e-57	GT128919 GT128920 GT128921 GT128922 GT128923 GT128924
6.1 6.2 6.3 6.4	4	growth hormone 1 (EU621898; *Salmo salar*)	GO:0005576 GO:0005179	extracellular region (CC) hormone activity hormone activity (MF)	1031	1028/1032 (99%)	0.0	GT128826 GT128827 GT128828 GT128829
8.1 8.2 8.3	3	integral membrane protein 2B (ACI33031; *Salmo salar*)	GO:0007399 GO:0007605 GO:0000139 GO:0016021	nervous system development (BP) sensory perception of sound (BP) golgi membrane (CC) integral to membrane (CC) transmembrane protein BRI (AAF66130; *Homo sapiens*) 8e-85	924	256/256 (100%)	2e-145	GT128832 GT128833 GT128834
9.1 9.2 9.3	3	heme-binding protein 2 (ACI66639; *Salmo salar*)			907	183/183 (100%)	2e-105	GT128835 GT128836 GT128837
18.1 18.2 18.3	3	follistatin-related protein 1 precursor (ACI33002; *Salmo salar*)	GO:0030509 GO:0030509 GO:0005509 GO:0008201	BMP signaling pathway (BP) extracellular space (CC) calcium ion binding (MF) heparin binding (MF) Follistatin-like 1 (AAH00055; *Homo sapiens*) 4e-96	803	222/236 (94%)	2e-131	GT128866 GT128867 GT128868
20.1 20.2 20.3	3	ribosomal protein L6 (ACH70868; *Salmo salar*)	GO:0006355 GO:0006414 GO:0022625 GO:0003677 GO:0003723 GO:0003735	regulation of transcription, DNA-dependent (BP) translational elongation (BP) cytosolic large ribosomal subunit (CC) DNA binding (MF) RNA binding (MF) structural constituent of ribosome (MF) Ribosomal protein L6 (AAH04138; *Homo sapiens*) 6e-96	791	252/256 (98%)	1e-118	GT128871 GT128872 GT128873
23	3	sodium/potassium-transporting ATPase subunit beta-233 (ACN11046; *Salmo salar*)	GO:0006813 GO:0006814 GO:0016021 GO:0030955 GO:0031402 GO:0005391	potassium ion transport (BP) sodium ion transport (BP) integral to membrane (CC) potassium ion binding (MF) sodium ion binding (MF) sodium:potassium-exchanging ATPase activity (MF)	777	73/74 (98%)	1e-36	GT128878 GT128879 GT128880

				Sodium/potassium ATPase beta subunit (AAT48994; *Rhabdosargus sarba*)				
25.1 25.2 25.3	3	epididymal secretory protein E1 precursor (ACM09246; *Salmo salar*)	GO:0042632 GO:0046836 GO:0032367 GO:0015914 GO:0019747 GO:0005576 GO:0005764 GO:0005634 GO:0015485 GO:0019899	cholesterol homeostasis (BP) glycolipid transport (BP) intracellular cholesterol transport (BP) phospholipid transport (BP) regulation of isoprenoid metabolic process (BP) extracellular region (CC) lysosome (CC) nucleus (CC) cholesterol binding (MF) enzyme binding (MF) Niemann-Pick disease, type C2 (AAH02532; *Homo sapiens*) 8e-48	1057	123/123 (100%)	4e-49	GT128883 GT128884 GT128885
26.1 26.2 26.3	3	beta-tubulin (ABO28786; *Mesenchytraeus solifugus*)	GO:0007018 GO:0051258 GO:0005874 GO:0043234 GO:0005525 GO:0003924 GO:0005198	microtubule-based movement (BP) protein polymerization (BP) microtubule (CC) protein complex (CC) GTP binding (MF) GTPase activity (MF) structural molecule activity (MF)	736	241/245 (98%)	3e-48	GT128886 GT128887 GT128888
29.1 29.2 29.3	3	cytochrome oxidase subunit I (AAD04735; *Salmo salar*)	GO:0009060 GO:0022900 GO:0006810 GO:0016021 GO:0005746 GO:0005507 GO:0004129 GO:0009055 GO:0020037 GO:0005506	aerobic respiration (BP) electron transport chain (BP) transport (BP) integral to membrane (CC) mitochondrial respiratory chain (CC) copper ion binding (MF) cytochrome-c oxidase activity (MF) electron carrier activity (MF) heme binding (MF) iron ion binding (MF)	791	221/244 (90%)	1e-123	GT128893 GT128894 GT128895
40.1 40.2 40.3	3	hemoglobin subunit alpha-4 (BT059981; Salmo salar)	GO:0020037 GO:0005506	heme binding (MF) iron ion binding (MF)	670	666/668 (99%) (nt)	0.0	GT128932 GT128933 GT128934
41.1 41.2 41.3	3	poly synthetase 3 (ACI33214; *Salmo salar*)	GO:0006471 GO:0005634 GO:0003950	protein amino acid ADP-ribosylation (BP) nucleus (CC) NAD+ ADP-ribosyltransferase activity (MF)	581	143/144 (99%)	2e-75	GT128935 GT128936 GT128937
45.1 45.2 45.3	3	BAC S0188I22, partial sequence (DQ156150; *Salmo salar*)			510	451/499 (90%)	0.0	GT128945 GT128946 GT128947
2.1 2.2	3	10 kDa heat shock protein, mitochondrial (ACM09432;	GO:0006457 GO:0006950	protein folding (BP) response to stress (BP)	1198	99/99 (100%)	8e-48	GT128819 GT128820

2.3		*Salmo salar*) * mitochondrion, complete genome (AF133701; *Salmo salar*)	GO:0005524	ATP binding (MF) heat shock protein 10 (ACH70787; *Salmo salar*) 5e-47		1102/1107 (99%)	0.0	GT128821
39.1 39.2 39.3	3	glycogen phosphorylase, brain form (BT072327; *Salmo salar*)			591	590/591 (99%)	0.0	GT128929 GT128930 GT128931
44.1 44.2 44.3	3	uncharacterized			556			GT128942 GT128943 GT128944
1.1 1.2	2	neighbor of COX4 (ACI69932; *Salmo salar*)			1223	191/191 (100%)	3e-109	GT128817 GT128818
4.1 4.2	2	poly A binding protein, cytoplasmic 1 b (AAH59662; *Danio rerio*)	GO:0003723 GO:0000166	RNA binding (MF) nucleotide binding (MF)	1368	275/316 (87%)	6e-148	GT128824 GT128825
13.1 13.2	2	heat shock protein hsp90 beta (AAD30275; *Salmo salar*)	GO:0006457 GO:0006950 GO:0005524 GO:0051082	protein folding (BP) response to stress (BP) ATP binding (MF) unfolded protein binding (MF)	866	201/205 (98%)	7e-97	GT128852 GT128853
16.1 16.2	2	proteasome subunit alpha type-3 (ACI66058; *Salmo salar*)	GO:0006511 GO:0005839 GO:0004298	ubiquitin-dependent protein catabolic process (BP) proteasome core complex (CC) threonine-type endopeptidase activity (MF) PSMA3 (CAG33214; *Homo sapiens*) 8e-114	814	195/196 (99%)	1e-123	GT128862 GT128863
17.1 17.2	2	glutamine synthetase (AAM73660; *Oncorhynchus mykiss*)	GO:0006542 GO:0004356	glutamine biosynthetic process (BP) glutamate-ammonia ligase activity (MF)	809	232/234 (99%)	2e-125	GT128864 GT128865
21.1 21.2	2	ATPase subunit 6 (AAF61383; *Salmo salar*)	GO:0015986 GO:0016021 GO:0005743 GO:0045263 GO:0015078 GO:0046872	ATP synthesis coupled proton transport (BP) integral to membrane (CC) mitochondrial inner membrane (CC) proton-transporting ATP synthase complex, coupling factor F(o) (CC) hydrogen ion transmembrane transporter activity (MF) metal ion binding (MF)	788	204/216 (94%)	1e-79	GT128874 GT128875
22.1 22.2	2	DEAD-box RNA-dependent helicase p68 (AAX22124; *Carassius auratus*)	GO:0005524 GO:0008026 GO:0003676	ATP binding (MF) ATP-dependent helicase activity (MF) nucleic acid binding (MF)	809	237/269 (88%)	3e-124	GT128876 GT128877
27.1 27.2	2	kainate receptor beta subunit (AAA21577; *Carassius auratus*)	GO:0006811 GO:0030054 GO:0016021 GO:0045211 GO:0005234	ion transport (BP) cell junction (CC) integral to membrane (CC) postsynaptic membrane (CC) extracellular-glutamate-gated ion channel activity (MF)	724	47/92 (51%)	3e-08	GT128889 GT128890

			GO:0004970	ionotropic glutamate receptor activity (MF)				
28.1 28.2	2	tubulin alpha-1A chain (ACI69569; *Salmo salar*)	GO:0051258 GO:0043234 GO:0005525 GO:0003924	protein polymerization (BP) protein complex (CC) GTP binding (MF) GTPase activity (MF)	677	196/197 (99%)	1e-94	GT128891 GT128892
30.1 30.2	2	ReO_6 (BAB83841; *Oryzias latipes*)	GO:0006278 GO:0003723 GO:0003964	RNA-dependent DNA replication (BP) RNA binding (MF) RNA-directed DNA polymerase activity (MF)	661	31/58 (53%)	5e-25	GT128896 GT128897
32.1 32.2	2	iron-sulfur cluster assembly enzyme ISCU, mitochondrial precursor (ACI69709; *Salmo salar*)	GO:0016226 GO:0009399 GO:0005829 GO:0005739 GO:0005634 GO:0005506 GO:0051536 GO:0032947	iron-sulfur cluster assembly (BP) nitrogen fixation (BP) cytosol (CC) mitochondrion (CC) nucleus (CC) iron ion binding (MF) iron-sulfur cluster binding (MF) protein complex scaffold (MF) Iron-sulfur cluster scaffold homolog (*E. coli*) (AAH61903; *Homo sapiens*) 2e-09	694	40/53 (75%)	3e-13	GT128911 GT128912
34.1 34.2	2	NADH dehydrogenase 1 alpha subcomplex subunit 8 (ACI67834; *Salmo salar*)	GO:0006120 GO:0006810 GO:0005747 GO:0008137	mitochondrial electron transport, NADH to ubiquinone (BP) transport (BP) mitochondrial respiratory chain complex I (CC) NADH dehydrogenase (ubiquinone) activity (MF) NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8 (P51970; *Homo sapiens*) 1e-72	622	170/172 (98%)	4e-99	GT128915 GT128916
42.1 42.2	2	cytochrome b (AAF06986; *Salmo salar*)	GO:0022904 GO:0006810 GO:0016021 GO:0005746 GO:0009055 GO:0005506 GO:0005506	respiratory electron transport chain (BP) transport (BP) integral to membrane (CC) mitochondrial respiratory chain (CC) electron carrier activity (MF) iron ion binding (MF) oxidoreductase activity (MF)	567	174/188 (92%)	6e-87	GT128938 GT128939

43.1 43.2	2	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 (ACH85363; *Salmo salar*)	GO:0005634 GO:0005524 GO:0004004 GO:0003723	nucleus (CC) ATP binding (MF) ATP-dependent RNA helicase activity (MF) RNA binding (MF)	558	181/185 (97%)	2e-71	GT128940 GT128941
47.1 47.2	2	reticulon 4 (DAA05186; *Salmo salar*)	GO:0005783	endoplasmic reticulum (CC)	453	148/148 (100%)	1e-66	GT128950 GT128951
7.1 7.2	2	insulin-like growth factor binding protein 2 (DQ146968; *Oncorhynchus mykiss*)	GO:0001558 GO:0005576 GO:0005520	regulation of cell growth (BP) extracellular region (CC) insulin-like growth factor binding (MF)	1002	717/770 (93%)	0.0	GT128830 GT128831
14.1 14.2	2	uncharacterized			857			GT128854 GT128855
33.1 33.2	2	DNA, dispersed repeat HpaI (D32149; *Thymallus thymallus*)			634	173/187 (92%)	7e-68	GT128913 GT128914
35.1 35.2	2	uncharacterized			615			GT128917 GT128918
38.1 38.2	2	uncharacterized			609			GT128927 GT128928
49.1 49.2	2	voltage-dependent anion-selective channel protein 2 (BT045741; *Salmo salar*)	GO:0006820 GO:0005741 GO:0008308	anion transport (BP) mitochondrial outer membrane (CC) voltage-gated anion channel activity (MF)	374	374/374 (100%)	0.0	GT128968 GT128969
51.1 51.2	2	follistatin-related protein 1 precursor (BT046049; *Salmo salar*)			298	116/125 (92%)	2e-42	GT128972 GT128973
11.1 11.2	2	unknown large open reading frame (BT072322; *Salmo salar*)			892	891/896 (99%)	0.0	GT128840 GT128841
50.1 50.2	2	poliovirus receptor-related protein 1 precursor (ACM09386; Salmo salar)		Keyword: Receptor	357	64/73 (87%)	1e-29	GT128970 GT128971
3.1 3.2	2	nanos homolog 1 (ACI70036; *Salmo salar*) * (87% identity at nt level to ssal_evf_508_302_f)	GO:0003723 GO:0008270	RNA binding (MF) zinc ion binding (MF)	1168	26/27 (96%) 225/226 (99%)	1e-06 2e-122	GT128822 GT128823
10.1 10.2	2	uncharacterized			949			GT128838 GT128839
19.1 19.2	2	uncharacterized			791			GT128869 GT128870
24.1 24.2	2	uncharacterized			752			GT128881 GT128882
37.1 37.2	2	uncharacterized			613			GT128925 GT128926

46.1 46.2	2	uncharacterized			485			GT128948 GT128949
A1p1	1	glutamate receptor U1 precursor (BT059539; *Salmo salar*)		Keywords: ion transport (BP) transport (BP) cell junction (CC) cell membrane (CC) membrane (CC) postsynaptic cell membrane (CC) synapse (CC) ionic channel (MF) receptor (MF)	470	458/464 (98%)	0.0	GT128974
A5p1	1	uncharacterized			386			GT128975
A5p2	1	uncharacterized			807			GT128976
A5p4	1	transcription factor BTF3 homolog 4 (ACI69060; *Salmo salar*)			655	138/139 (99%)	6e-53	GT128977
A6p2	1	unclassified			765			GT128978
A7p4	1	thyroid hormone receptor interactor 12 (EAW70907; *Homo sapiens*)			427	99/104 (95%)	2e-50	GT128979
A9p4	1	phosphodiesterase 5A, cGMP-specific (CAQ14723; *Danio rerio*)	GO:0007165 GO:0004114	signal transduction (BP) 3',5'-cyclic-nucleotide phosphodiesterase activity (MF)	729	201/227 (88%)	1e-115	GT128980
A9p10	1	Gnl3 protein (AAH93001; *Danio rerio*)	GO:0005622 GO:0005525	intracellular (CC) GTP binding (MF)	286	63/83 (75%)	2e-28	GT128981
A12p9	1	GTP-binding protein SAR1a (ACI33991; *Salmo salar*)	GO:0006888 GO:0006886 GO:0005794 GO:0005525 GO:0003924	ER to Golgi vesicle-mediated transport (BP) intracellular protein transport (BP) Golgi apparatus (CC) GTP binding (MF) GTPase activity (MF) GTP-binding protein SAR1 (AAG16638; *Homo sapiens*) 1e-92	662	183/183 (100%)	3e-101	GT128982
A12p4	1	uncharacterized			568			GT128983
A3p3	1	uncharacterized			763			GT128984
A5p3	1	uncharacterized			767			GT128985
A5p10	1	uncharacterized			465			GT128986
A6p4	1	probable peptidyl-tRNA hydrolase 2 (BT072330; *Salmo salar*)			662	561/576 (97%)	0.0	GT128987
A7p10	1	uncharacterized			689			GT128988
A8p4	1	uncharacterized			647			GT128989
A8p10	1	uncharacterized			946			GT128990
A8p9	1	Sushi, von Willebrand factor			411	139/139	4e-65	GT128991

		type A, EGF and pentraxin domain-containing protein 1 precursor (BT072024; *Salmo salar*)				(100%)		
B1p2	1	transposase (CAB51372; *Pleuronectes platessa*)	GO:0015074 GO:0006313 GO:0005634 GO:0003677 GO:0004803	DNA integration (BP) transposition, DNA-mediated (BP) nucleus (CC) DNA binding (MF) transposase activity (MF)	639	83/120 (69%)	1e-59	GT128992
B1p3	1	signalosome complex subunit COP94 (ACN11367; *Salmo salar*)	GO:0005737 GO:0008180 GO:0005515	cytoplasm (CC) signalosome (CC) protein binding (MF) Function: Involved at multiple steps in cell cycle control (Ullah et. al., 2007) COP9 complex subunit 4 (AAD43021; *Homo sapiens*) 4e-92	808	182/198 (91%)	4e-97	GT128993
B2p3	1	4F2 cell-surface antigen heavy chain (ACI33885; *Salmo salar*)	GO:0005975 GO:0003824 GO:0043169	carbohydrate metabolic process (BP) catalytic activity (MF) cation binding (MF) Slc3a2 protein (AAI59203; *Danio rerio*) 2e-08	724	62/63 (98%)	5e-26	GT128994
B2p4	1	uncharacterized			956			GT128995
B2p10	1	deoxyribonuclease gamma precursor (ACM08762; *Salmo salar*)	GO:0006308 GO:0004536	DNA catabolic process (BP) deoxyribonuclease activity (MF)	1119	28/40 (70%)	6e-07	GT128996
B3p2	1	anterior gradient protein 2 homolog precursor (ACI68219; *Salmo salar*)		Function: metastasis-inducing protein (Myung et. al., 2008).	594	70/92 (76%)	1e-30	GT128997
B3p3	1	isocitrate dehydrogenase 2-2 (NADP+), mitochondrial (ACH70947; *Salmo salar*)	GO:0006102 GO:0004450	isocitrate metabolic process (BP) isocitrate dehydrogenase (NADP+) activity (MF)	693	213/213 (100%)	7e-123	GT128998
B3p4	1	HSC71 (AAB21658; *Oncorhynchus mykiss*)	GO:0006950 GO:0005524	response to stress (BP) ATP binding (MF)	889	263/288 (91%)	3e-117	GT128999
B3p9	1	U4/U6 small nuclear ribonucleoprotein Prp31 (ACN11428; Salmo salar)	GO:0008380 GO:0006397 GO:0005681	RNA splicing (BP) mRNA processing (BP) spliceosome (CC) U4/U6 small nuclear ribonucleoprotein Prp31 (Q7SXM7; *Danio rerio*) 2e-16	261	47/47 (100%)	5e-18	GT129000
B4p3	1	potassium channel subunit (AAM18770; *Gallus gallus*)	GO:0008152 GO:0006813 GO:0016021 GO:0005886 GO:0005509	metabolic process (BP) potassium ion transport (BP) integral to membrane (CC) plasma membrane (CC) calcium ion binding (MF)	552	115/133 (86%)	1e-58	GT129001

			GO:0015269 GO:0003824 GO:0030955	calcium-activated potassium channel activity (MF) catalytic activity (MF) potassium ion binding (MF)				
B4p4	1	ATP synthase subunit alpha (ACH70752; *Salmo salar*)	GO:0015986 GO:0045261 GO:0046933 GO:0046961	ATP synthesis coupled proton transport (BP) proton-transporting ATP synthase complex, catalytic core F(1) (CC) hydrogen ion transporting ATP synthase activity, rotational mechanism (MF) proton transporting ATPase activity, rotational mechanism (MF)	567	188/188 (100%)	6e-101	GT129002
B4p9	1	40S ribosomal protein S14 (ACN09966; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF) ribosomal protein S14 (ABY28375; *Oncorhynchus masou formosanus*) 1e-66	532	128/134 (95%)	1e-66	GT129003
B5p2	1	uncharacterized			577			GT129004
B5p4	1	uncharacterized			891			GT129005
B5p10	1	simple type II keratin K8a (S1) (CAC45060; *Oncorhynchus mykiss*)	GO:0005882 GO:0005198	intermediate filament (CC) structural molecule activity (MF)	1167	286/294 (97%)	2e-158	GT129006
B6p9	1	60S ribosomal protein L13 (ACI68750; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF)	283	64/64 (100%)	1e-18	GT129007
B6p10	1	uncharacterized			509			GT129008
B7p3	1	Na/K ATPase alpha subunit isoform 1b (AAQ82789; *Oncorhynchus mykiss)*	GO:0008152 GO:0006813 GO:0016021 GO:0005524 GO:0015662 GO:0015077 GO:0030955	metabolic process (BP) potassium ion transport (BP) integral to membrane (CC) ATP binding (MF) ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism (MF) monovalent inorganic cation transmembrane transporter activity (MF) potassium ion binding (MF)	543	181/181 (100%)	6e-90	GT129009
B7p4	1	uncharacterized			772			GT129010
B7p10	1	uncharacterized			344			GT129011
B8p10	1	uncharacterized			276			GT129012
B9p3	1	stereocilin (AAL35321; *Mus musculus*)	GO:0009986	cell surface (CC) Keyword: Hearing (BP) Expressed only in the sensory hair cells and is associated with the stereocilia, the stiff microvilli forming the structure for mechanoreception of sound stimulation (Verpy	661	46/96 (47%)	1e-16	GT129013

				et. al., 2001).				
B9p10	1	cytochrome oxidase subunit III (ABZ01931; *Salmo salar*)	GO:0006123 GO:0016021 GO:0005739 GO:0004129	mitochondrial electron transport, cytochrome c to oxygen (BP) integral to membrane (CC) mitochondrion (CC) cytochrome-c oxidase activity (MF)	191	60/63 (95%)	8e-23	GT129014
B10p4	1	PKC-delta1 protein (AAH78019; *Xenopus laevis*)	GO:0007242 GO:0006468 GO:0005524 GO:0019992 GO:0004697 GO:0008270	intracellular signaling cascade (BP) protein amino acid phosphorylation (BP) ATP binding (MF) diacylglycerol binding (MF) protein kinase C activity (MF) zinc ion binding (MF)	729	102/132 (77%)	3e-55	GT129015
B11p3	1	uncharacterized			582			GT129016
B11p4	1	uncharacterized			197			GT129017
B12p3	1	uncharacterized			660			GT129018
B12p4	1	uncharacterized			359			GT129019
B12p9	1	hemoglobin subunit beta (ACI68762; *Salmo salar*)	GO:0020037 GO:0005506	heme binding (MF) iron ion binding (MF)	452	124/125 (99%)	1e-64	GT129020
C1p2	1	transmembrane protein 43 (ACN11108; *Salmo salar*)	GO:0005783 GO:0016021 GO:0005637 GO:0005794	endoplasmic reticulum (CC) integral to membrane (CC) nuclear inner membrane (CC) golgi apparatus (CC) TMEM43 protein (AAH08054; *Homo sapiens*) 2e-49	912	190/194 (97%)	1e-94	GT129021
C2p3	1	NOD-like receptor C (ABU89560; *Danio rerio*)	GO:0004872	receptor activity (MF) Immune response (Lamkanfi and Kannegantic, 2010).	646	58/117 (49%)	2e-18	GT129022
C3p2	1	follistatin-related protein 1 precursor (BT072046; *Salmo salar*)			634	188/228 (82%)	9e-42	GT129023
C3p3	1	uncharacterized			745			GT129024
C3p9	1	uncharacterized			628			GT129025
C3p10	1	uncharacterized			421			GT129026
C4p2	1	K-Cl cotransporter (BAD86529; Canis familiaris)	GO:0006821 GO:0006814 GO:0016021 GO:0015377	chloride transport (BP) sodium ion transport (BP) integral to membrane (CC) cation:chloride symporter activity (MF)	446	113/148 (76%)	2e-52	GT129027
C4p3	1	FK506-binding protein 1A (ACO07946; *Oncorhynchus mykiss*)	GO:0006457 GO:0003755	protein folding (BP) peptidyl-prolyl cis-trans isomerase activity (MF) FKBP1A protein (AAI19733; *Homo sapiens*) 7e-45	656	107/107 (100%)	2e-57	GT129028

C4p9	1	NADH dehydrogenase subunit 1 (AAD43230; *Salmo salar*)	GO:0055114 GO:0016021 GO:0005739 GO:0008137	oxidation reduction (BP) integral to membrane (CC) mitochondrion (CC) NADH dehydrogenase (ubiquinone) activity (MF)	513	149/155 (96%)	7e-63	GT129029
C4p10	1	60S ribosomal protein L3 (ACI68315; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF)	453	149/150 (99%)	2e-82	GT129030
C5p1	1	sodium/potassium-transporting ATPase subunit beta-233 (ACN11038; *Salmo salar*)	GO:0006754 GO:0006813 GO:0006814 GO:0016020 GO:0005391	ATP biosynthetic process (BP) potassium ion transport (BP) sodium ion transport (BP) membrane (CC) sodium:potassium-exchanging ATPase activity (MF) Sodium/potassium-transporting ATPase subunit beta-233 (ACI66117; *Salmo salar*) 5e-29	506	62/62 (100%)	2e-29	GT129031
C5p4	1	APEX nuclease 1 (ACH70702; *Salmo salar*)	GO:0006281 GO:0006281: GO:0003677 GO:0004519 GO:0016829	DNA repair (BP) intracellular (CC) DNA binding (MF) endonuclease activity (MF) lyase acyivity (MF)	869	211/223 (94%)	2e-121	GT129032
C6p10	1	insulin-like growth factor-binding protein 7 precursor (ACN11329; *Salmo salar*)	GO:0007155 GO:0008285 GO:0001558 GO:0005576 GO:0005520	cell adhesion (BP) negative regulation of cell proliferation (BP) regulation of cell growth (BP) extracellular region (CC) insulin-like growth factor binding (MF) Insulin-like growth factor binding protein 7 (Q16270; *Homo sapiens*) 1e-80	920	230/245 (93%)	1e-135	GT129033
C7p3	1	uncharacterized			679			GT129034
C7p4	1	eukaryotic translation initiation factor 3, subunit 5 epsilon (BAF62356; *Pan troglodytes verus*)	GO:0006412 GO:0003743	translation (BP) translation initiation factor activity (MF)	528	122/148 (82%)	3e-68	GT129035
C8p3	1	uncharacterized			732			GT129036
C8p9	1	UMP-CMP kinase (ACO14015; *Esox lucius*)	GO:0009220 GO:0005737 GO:0005634 GO:0005524 GO:0004127 GO:0016776 GO:0004849	pyrimidine ribonucleotide biosynthetic (BP) cytoplasm (CC) nucleus (CC) ATP binding (MF) cytidylate kinase activity (MF) phosphotransferase activity, phosphate group as acceptor (MF) uridine kinase activity (MF)	558	46/57 (80%)	5e-17	GT129037

				UMP-CMP kinase (AAF17709; Homo sapiens) 2e-15				
C8p10	1	FACT complex subunit SSRP1 (BT059584; *Salmo salar*)			591	543/547 (99%)	0.0	GT129038
C9p3	1	72 kDa type IV collagenase precursor (ACI33172; *Salmo salar*)	GO:0006508 GO:0005578 GO:0004222	proteolysis (BP) proteinaceous extracellular matrix (CC) metalloendopeptidase activity (MF)	617	68/69 (98%)	2e-34	GT129039
C9p9	1	cytokine-like protein 1 precursor (BT057798; Salmo salar)			513	513/513 (100%)	0.0	GT129040
C10p4	1	dolichyl-P-Man :Man7GlcNAc2-PP-dolichyl-alpha-1, 6-mannosyltransferase (ACI34126; *Salmo salar*)	GO:0000009 GO:0031227 GO:0016757	GPI anchor biosynthetic process (BP) intrinsic to endoplasmic reticulum membrane (CC) transferase activity, transferring glycosyl groups (MF)	667	221/221 (100%)	5e-114	GT129041
C11p4	1	uncharacterized			705			GT129042
C11p10	1	ependymin (AAA49551; *Salmo salar*)	GO:0007160 GO:0005576 GO:0005509	cell-matrix adhesion (BP) extracellular region (CC) calcium ion binding (MF) Function: Might have a function during axon regeneration. Are thought to play a role in neural plasticity. (Uniprot)	551	165/165 (100%)	2e-91	GT129043
C12p3	1	DNA-directed RNA polymerase III subunit RPC4 (ACI33723; *Salmo salar*)	GO:0006383 GO:0005666 GO:0003677 GO:0003899	transcription from RNA polymerase III promoter (BP) DNA-directed RNA polymerase III complex (CC) DNA binding (MF) DNA-directed RNA polymerase activity (MF)	574	168/169 (99%)	7e-74	GT129044
C12p10	1	pyruvate kinase (ACI34219; *Salmo salar*)	GO:0006096 GO:0000287 GO:0030955 GO:0004743	glycolysis (BP) magnesium ion binding (MF) potassium ion binding (MF) pyruvate kinase activity (MF)	516	82/82 (100%)	4e-42	GT129045
D1p1	1	uncharacterized			270			GT129046
D1p2	1	testis enhanced gene transcript (BAX inhibitor 1) (ACH70693; *Salmo salar*)	GO:0043066 GO:0005783 GO:0005626 GO:0005887 GO:0005634	negative regulation of apoptosis (BP) endoplasmic reticulum (CC) insoluble fraction (CC) integral to plasma membrane (CC) nucleus (CC) BAX inhibitor (AAU29521; *Homo sapiens*) 8e-20	627	55/57 (96%)	9e-24	GT129047
D2p4	1	matrix-remodeling-associated protein 8 precursor (ACN10981; *Salmo salar*)	GO:0016021	integral to membrane (CC) Function: physically and functionally associated with the blood-brain barrier (Yonezawa *et. al.*, 2003)	685	202/224 (90%)	4e-106	GT129048

				matrix-remodelling associated 8 (synonm: limitrin) (CAI23188; *Homo sapiens*) 9e-53				
D3p2	1	methionine aminopeptidase 2 (ACI33485; *Salmo salar*)	GO:0031365 GO:0018206 GO:0016485 GO:0006508 GO:0005737 GO:0004177 GO:0050897 GO:0008235	N-terminal protein amino acid modification (BP) peptidyl-methionine modification (BP) protein processing (BP) proteolysis (BP) cytoplasm (CC) aminopeptidase activity (MF) cobalt ion binding (MF) metalloexopeptidase activity (MF) Methionyl aminopeptidase 2 (AAH13782; *Homo sapiens*) 6e-62	722	109/155 (70%)	2e-64	GT129069
D3p3	1	tetraspanin-3 (ACI33238; *Salmo salar*)	GO:0016021	integral to membrane (CC)	708	127/127 (100%)	4e-72	GT129049
D3p4	1	RNA-binding protein 5 (BT072055; Salmo salar)			565	562/565 (99%)	0.0	GT129050
D3p9	1	cofilin-2 (ACI68104; *Salmo salar*)	GO:0005622 GO:0003779	Intracellular (CC) actin binding (MF) Function: Cofilin-mediated actin filament severing (McCullough *et. al.*, 2008).	805	104/105 (99%)	4e-54	GT129051
D4p2	1	leucine aminopeptidase (CAZ30678; *Schistosoma mansoni*)	GO:0006508 GO:0005622 GO:0004177	proteolysis (BP) intracellular (CC) aminopeptidase activity (MF)	726	93/247 (37%)	3e-31	GT129052
D4p3	1	fatty acid-binding protein, heart (ACI66142; *Salmo salar*)	GO:0005488 GO:0008289 GO:0005215	transport (BP) lipid binding (MF) transporter activity (MF)	578	135/135 (100%)	5e-72	GT129053
D4p4	1	development and differentiation-enhancing factor 2 (BT071907; *Salmo salar*)			970	46/46 (100%)	3e-13	GT129054
D4p10	1	membrane protein (BT049454; *Salmo salar*)			316	57/57 (100%)	2e-19	GT129055
D5p2	1	brain protein 44 (ACH70732; *Salmo salar*)			935	33/33 (100%)	3e-12	GT129056
D5p4	1	tensin-like C1 domain-containing phosphatase (BT072145; *Salmo salar*)			660	652/654 (99%)	0.0	GT129057
D5p9	1	hemoglobin subunit beta-1 (ACI66559; *Salmo salar*)	GO:0020037 GO:0005506	heme binding (MF) iron ion binding (MF)	357	84/87 (96%)	1e-46	GT129058
D5p10	1	heme-binding protein 2 (ACN10776; *Salmo salar*)			514	82/84 (97%)	9e-43	GT129059
D6p3	1	probable ATP-dependent			494	492/494	0.0	GT129060

		RNA helicase DDX5 (BT059556; *Salmo salar*)				(99%)		
D6p4	1	histidine-rich glycoprotein precursor, putative (EDP36098; *Brugia malayi*)			900	53/115 (46%)	4e-11	GT129061
D7p4	1	gap junction alpha-1 protein (BT059188; *Salmo salar*)		Keywords: cell junction (CC) cell membrane (CC) gap junction (CC) membrane (CC)	783	777/786 (98%)	0.0	GT129062
D8p3	1	retinol dehydrogenase 3 (ACI67927; *Salmo salar*)	GO:0008152 GO:0016491	metabolic process (BP) oxidoreductase activity (MF)	1044	297/298 (99%)	1e-177	GT129063
D9p4	1	Onmy-LDA gene for MHC class I antigen allele: Onmy-LDA*0101, and other genes complete cds (AB258536; *Oncorhynchus mykiss*),			685	198/233 (84%)	1e-52	GT129064
D9p10	1	piggyBac transposase Uribo1 (BAF82019; *Xenopus (Silurana) tropicalis*)			448	58/138 (42%)	6e-20	GT129065
D10p3	1	lysosome-associated membrane glycoprotein 1 precursor (BT058886; *Salmo salar*)			628	68/70 (97%)	3e-23	GT129066
D12p4	1	uncharacterized			689			GT129067
D12p9	1	ubiquitin and ribosomal protein S27a precursor (ABC75552; *Ictalurus punctatus*)	GO:0006464 GO:0005840	protein modification process (BP) ribosome (CC)	341	84/86 (97%)	4e-40	GT129068
E1p2	1	coagulation factor C homolog, cochlin (AAI62194; *Danio rerio*)	GO:0007605 GO:0005578	sensory perception of sound (BP) proteinaceous extracellular matrix (CC) coagulation factor C homolog, cochlin (AAW82432; *Homo sapiens*) 1e-113	1168	233/356 (65%)	4e-131	GT129070
E1p4	1	microsomal epoxide hydrolase (AAC41694; *Homo sapiens*)	GO:0019439 GO:0009636 GO:0005789 GO:0016021 GO:0005792 GO:0033961 GO:0004301	aromatic compound catabolic process (BP) response to toxin (BP) endoplasmic reticulum membrane (CC) integral to membrane (CC) microsome (CC) cis-stilbene-oxide hydrolase activity (MF) epoxide hydrolase activity (MF)	690	141/229 (61%)	3e-76	GT129071
E1p9	1	leucine rich repeat containing 40 (ACH70736; *Salmo salar*)	GO:0005515	protein binding (MF)	764	154/155 (99%)	3e-80	GT129072
E2p2	1	uncharacterized			646			GT129073

E2p3	1	Gmpr2 protein (AAH90559; *Xenopus tropicalis*)	GO:0009117 GO:0055114 GO:0003920 GO:0030955	nucleotide metabolic process (BP) oxidation reduction (BP) GMP reductase activity (MF) potassium ion binding (MF)	399	93/112 (83%)	2e-48	GT129074
E2p4	1	transposable element Tcb1 transposase (ACN11391; *Salmo salar*)	GO:0015074 GO:0006313 GO:0005634 GO:0003677 GO:0004803	DNA integration (BP) transposition, DNA-mediated (BP) nucleus (CC) DNA binding (MF) transposase activity (MF) Tc1-like transporase (BAF37936; *Oncorhynchus mykiss*) 2e-15	728	45/62 (72%)	6e-19	GT129075
E3p3	1	insulin-like growth factor-binding protein 7 precursor (BT059616; *Salmo salar*)			738	731/738 (99%)	0.0	GT129076
E3p9	1	heat shock 70 kDa protein (ACI34374; *Salmo salar*)	GO:0006950 GO:0005524	response to stress (BP) ATP binding (MF)	652	146/146 (100%)	1e-78	GT129077
E3p10	1	prothymosin (BT057594; *Salmo salar*)			398	337/363 (92%)	5e-145	GT129078
E4p4	1	uncharacterized			671			GT129079
E4p10	1	uncharacterized			287			GT129080
E5p1	1	uncharacterized			198			GT129081
E5p2	1	Alstrom syndrome protein 1 (Q8TCU4; *Homo sapiens*)	GO:0050896 GO:0007605 GO:0007601 GO:0005813 GO:0005929	response to stimulus (BP) sensory perception of sound (BP) visual perception (BP) centrosome (CC) cilium (CC)	588	78/193 (40%)	8e-26	GT129082
E5p10	1	uncharacterized			623			GT129083
E6p2	1	uncharacterized			610			GT129084
E6p10	1	uncharacterized			318			GT129085
E8p9	1	envelope polyprotein (ABB52640; *Cercopithecus aethiops*)	GO:0019028 GO:0019031 GO:0005198	viral capsid (CC) viral envelope (CC) structural molecule activity (MF)	707	71/138 (51%)	2e-26	GT129086
E8p10	1	transposable element Tcb1 transposase (ACN11475; *Salmo salar)*	GO:0015074 GO:0006313 GO:0005634 GO:0003677 GO:0004803	DNA integration (BP) transposition, DNA-mediated (BP) nucleus (CC) DNA binding (MF) transposase activity (MF) RCC2 homolog (ACI33792; *Salmo salar*) 6e-18	341	53/98 (54%)	1e-20	GT129087
E9p9	1	uncharacterized			600			GT129088
E11p3	1	uncharacterized			600			GT129089
E11p4	1	NDRG3 (ACN60253; *Salmo salar*)			837	59/60 (98%)	1e-10	GT129090

		NDRG3 (BT072295; *Salmo salar*)				835/839 (99%)	0.0	
E11p9	1	uncharacterized			709			GT129091
E12p3	1	myelin P0 protein (BT045194; *Salmo salar*)	GO:0016020	Membrane (CC)	559	559/559 (100%) (nt)	0.0	GT129092
E12p4	1	uncharacterized			713			GT129093
E12p9	1	tyrosine-protein kinase RYK (CAA49591; *Homo sapiens*)	GO:0006468 GO:0007165 GO:0005887 GO:0005524 GO:0004714	protein amino acid phosphorylation (BP) signal transduction (BP) integral to plasma membrane (CC) ATP binding (MF) transmembrane receptor protein tyrosine kinase activity (MF)	335	61/91 (67%)	2e-16	GT129094
F1p1	1	insulin-like growth factor binding protein 5a (BC066725; *Danio rerio*)			431	64/67 (95%)	4e-19	GT129095
F1p2	1	Zinc finger protein ZIC 2 (ACN11131; *Salmo salar*)	GO:0007420 GO:0030154 GO:0005634 GO:0003677 GO:0008270	brain development (BP) cell differentiation (BP) nucleus (CC) DNA binding (MF) zinc ion binding (MF) ZIC2 protein (AAC96325; *Homo sapiens*) 3e-39	1200	96/99 (96%)	5e-42	GT129096
F2p4	1	MHC class I (AAO14685; *Salmo salar*)	GO:0019882 GO:0006955 GO:0042612	antigen processing and presentation (BP) immune response (BP) MHC class I protein complex (CC)	607	91/95 (95%)	9e-47	GT129097
F2p9	1	ferritin, heavy subunit (ACI67950; *Salmo salar*)	GO:0006879 GO:0006826 GO:0008199 GO:0016491	cellular iron ion homeostasis (BP) iron ion transport (BP) ferric iron binding (MF) oxidoreductase activity (MF)	442	107/132 (81%)	4e-51	GT129098
F3p3	1	uncharacterized			671			GT129099
F3p4	1	uncharacterized			92			GT129100
F3p9	1	S-phase kinase-associated protein 1 (ACM09794; *Salmo salar*)	GO:0051437 GO:0006511 GO:0005829 GO:0005654 GO:0005515	positive regulation of ubiquitin-protein ligase activity during mitotic cell cycle (BP) ubiquitin-dependent protein catabolic process (BP) cytosol (CC) nucleoplasm (CC) protein binding (MF) S-phase kinase-associated protein 1 (AAA79202; *Homo sapiens*) 2e-41	590	99/100 (99%)	2e-41	GT129101
F3p10	1	uncharacterized			775			GT129102
F4p2	1	otolith matrix macromolecule-64			689	56/59 (94%)	3e-22	GT129103

		(BAG14384; *Oncorhynchus mykiss*)						
F4p4	1	uncharacterized			936			GT129104
F5p1	1	BNIP2 motif-containing molecule at the C-terminal region 1 (ACI33014; *Salmo salar*)	GO:0051318 GO:0006917 GO:0005737 GO:0016491 GO:0046914	G1 phase (BP) induction of apoptosis (BP) cytoplasm (CC) oxidoreductase activity (MF) transition metal ion binding (MF) BNIP2 motif-containing molecule at the C-terminal region 1(BAA20822; *Homo sapiens*) 3e-20	582	137/148 (92%)	5e-53	GT129105
F5p2	1	uncharacterized			757			GT129106
F5p9	1	uncharacterized			253			GT129107
F6p3	1	uncharacterized			683			GT129108
F6p4	1	testis enhanced gene transcript (BAX inhibitor 1) (BT043578; *Salmo salar*)			621	374/376 (99%) (nt)	0.0	GT129109
F7p3	1	integral membrane protein GPR177 precursor (BT059614; *Salmo salar*)			541	531/541 (98%)	0.0	GT129110
F7p10	1	uncharacterized			821			GT129111
F8p10	1	ependymin-2 precursor (ACI67122; *Salmo salar*)	GO:0007160 GO:0005576 GO:0005509	cell-matrix adhesion (BP) extracellular region (CC) calcium ion binding (MF) Function: Might have a function during axon regeneration and is thought to play a role in neural plasticity (Shashoua 1991).	484	51/51 (100%)	5e-21	GT129112
F9p4	1	cytochrome c oxidase subunit I (CAP40321; *Salmo trutta trutta*)	GO:0009060 GO:0016021 GO:0005743 GO:0004129 GO:0009055 GO:0020037 GO:0005506	aerobic respiration (BP) integral to membrane (CC) mitochondrial inner membrane (CC) cytochrome-c oxidase activity (MF) electron carrier activity (MF) heme binding (MF) iron ion binding (MF)	402	93/103 (90%)	7e-26	GT129113
F9p9	1	complement receptor-like protein 1 (AJ620466; *Oncorhynchus mykiss*)	GO:0004872	receptor activity (MF)	335	109/137 (79%)	2e-12	GT129114
F10p3	1	ATPase H+ transporting V1 subunit D (ACH70735; *Salmo salar*)	GO:0015986 GO:0033178 GO:0046961	ATP synthesis coupled proton transport (BP) proton-transporting two-sector ATPase complex, catalytic domain (CC) proton-transporting ATPase activity, rotational mechanism (MF)	751	66/66 (100%)	1e-21	GT129115
F11p10	1	uncharacterized			448			GT129116

F12p3	1	uncharacterized			565			GT129117
F12p9	1	actin, cytoplasmic 1 (ACI67042; *Salmo salar*)	GO:0005515	protein binding (MF)	392	130/130 (100%)	5e-69	GT129118
G2p9	1	60S ribosomal protein L35 (ACI66532; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF)	375	122/122 (100%)	7e-48	GT129119
G3p9	1	uncharacterized			427			GT129120
G3p10	1	otolin 1 (CAN88052; *Danio rerio*)	GO:0048840 GO:0005576	otolith development (BP) extracellular region (CC)	441	67/83 (80%)	2e-29	GT129121
G4p9	1	uncharacterized			604			GT129122
G4p10	1	NADH dehydrogenase subunit 1 (AAD43227; *Salmo salar*)	GO:0016020 GO:0005739 GO:0008137	membrane (CC) mitochondrion (CC) NADH dehydrogenase (ubiquinone) activity (MF)	316	98/100 (98%)	8e-33	GT129123
G5p2	1	C8orf4 homolog (ACI66959; *Salmo salar*) C8orf4 homolog (BT047158; *Salmo salar*)	GO:0006915	apoptosis (BP) Chromosome 8 open reading frame 4 (AAH21672; *Homo sapiens*) 3e-07	825	101/101 (100%) 825/825 (100%)	3e-23 0.0	GT129124
G5p3	1	uncharacterized			631			GT129125
G5p9	1	uncharacterized			376			GT129126
G6p3	1	transposase (ABB83608; *Siniperca chuatsi*)			859	38/50 (76%)	2e-23	GT129127
G6p10	1	uncharacterized			297			GT129128
G7p3	1	stathmin (ACI69595; *Salmo salar*)	GO:0007242	intracellular signaling cascade (BP) Function: enriched in the growth cones of developing neurons and plays a role in regulating neurite outgrowth (Burzynski et. al., 2009)	716	144/144 (100%)	2e-51	GT129129
G7p9	1	parvalbumin, thymic CPV3 (ACM09534; *Salmo salar*)	GO:0005509	calcium ion binding (MF) oncomodulin (AAA02869; *Homo sapiens*) 7e-29	640	106/109 (97%)	7e-53	GT129130
G8p4	1	FACT complex large subunit (Suppressor of Ty 16 homolog) (AAI63334; *Danio rerio*)	GO:0009987	cellular process (BP)	685	176/199 (88%)	1e-76	GT129131
G9p4	1	unknown large open reading frame mRNA (BT071900; *Salmo salar*)			700	696/700 (99%)	0.0	GT129132
G10p4	1	myotrophin (ACN10623; *Salmo salar*)	GO:0016049 GO:0016202 GO:0006417 GO:0005737	cell growth (BP) regulation of striated muscle development (BP) regulation of translation (BP) cytoplasm (CC)	1141	117/117 (100%)	4e-61	GT129133

			GO:0005515	protein binding (MF) myotrophin (P58546; *Homo sapiens*) 2e-45				
G10p9	1	ribosomal protein S12 (ACI66971; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF)	385	77/78 (98%)	7e-13	GT129134
G12p9	1	cold-inducible RNA-binding protein (ACI68319; *Salmo salar*)	GO:0003676	nucleic acid binding (MF) Function: Response to stress (DeLeeuw et. al., 2007)	501	81/83 (97%)	2e-39	GT129135
G11p10	1	CD209 antigen-like protein E (BT049669; *Salmo salar*)	GO:0005488	binding (MF)	625	611/614 (99%)	0.0	GT129136
H1p1	1	uncharacterized			410			GT129137
H1p2	1	creatine kinase B-type (ACI32901; *Salmo salar*)	GO:0016301	kinase activity (MF)	527	77/85 (90%)	2e-34	GT129138
H1p10	1	N-cadherin precursor (CAA47890; *Danio rerio*)	GO:0060088 GO:0048331 GO:0048331 GO:0010002 GO:0001502 GO:0060030 GO:0035142 GO:0007398 GO:0048048 GO:0035118 GO:0030902 GO:0007156 GO:0030901 GO:0021915 GO:0001764 GO:0007422 GO:0042478 GO:0060041 GO:0048752 GO:0001756 GO:0007416 GO:0035121 GO:0042246 GO:0016342 GO:0016021 GO:0042734 GO:0005509 GO:0005515	auditory receptor cell stereocilium organization (BP) axial mesoderm structural organization (BP) axonal fasciculation (BP) cardioblast differentiation (BP) cartilage condensation (BP) dorsal convergence (BP) dorsal fin morphogenesis (BP) ectoderm development (BP) embryonic eye morphogenesis (BP) embryonic pectoral fin morphogenesis (BP) hindbrain development (BP) homophilic cell adhesion (BP) midbrain development (BP) neural tube development (BP) neuron migration (BP) peripheral nervous system development (BP) regulation of eye photoreceptor cell development (BP) retina development in camera-type eye (BP) semicircular canal morphogenesis (BP) somitogenesis (BP) synaptogenesis (BP) tail morphogenesis (BP) tissue regeneration (BP) catenin complex (CC) integral to membrane (CC) presynaptic membrane (CC) calcium ion binding (MF) protein binding (MF)	556	153/185 (82%)	3e-72	GT129139
H2p3	1	transposase (ABV31711; *Salmo salar*)	GO:0015074 GO:0006313	DNA integration (BP) transposition, DNA-mediated (BP)	395	75/91 (82%)	1e-35	GT129140

			GO:0005634 GO:0003677 GO:0004803	nucleus (CC) DNA binding (MF) transposase activity (MF)				
H2p4	1	integral membrane protein 2B (BT045039; *Salmo salar*)			934	921/938 (98%)	0.0	GT129141
H2p9	1	60S ribosomal protein L35a (ACN10038; *Salmo salar*)	GO:0006412 GO:0005840 GO:0003735	translation (BP) ribosome (CC) structural constituent of ribosome (MF) ribosomal protein *L35a (*BAF98686; *Solea* senegalensis) 4e-24	390	110/110 (100%)	4e-27	GT129142
H2p10	1	uncharacterized			415			GT129143
H3p2	1	uncharacterized			441			GT129144
H3p3	1	myelin P0 protein precursor (BT058974; *Salmo salar*)			583	583/583 (100%)	0.0	GT129145
H3p4	1	peripheral myelin protein 22 (BT045468; Salmo salar)	GO:0016020	membrane (CC)	765	743/774 (95%)	0.0	GT129146
H3p9	1	active breakpoint cluster region-related protein (BT071954; *Salmo salar*)			545	428/500 (85%)	3e-136	GT129147
H4p3	1	eukaryotic translation initiation factor 3 subunit I (ACI32945; *Salmo salar*)	GO:0003743	translation initiation factor activity (MF)	834	213/217 (98%)	3e-69	GT129148
H4p9	1	FXYD domain containing ion transport regulator 5a (BK006253; *Salmo salar*)	GO:0016337 GO:0006811 GO:0016020 GO:0005216	cell-cell adhesion (BP) ion transport (BP) membrane (CC) ion channel activity (MF)	477	380/383 (99%)	0.0	GT129149
H5p1	1	collagen triple helix repeat-containing protein 1 precursor (ACI66329; *Salmo salar*)			445	77/77 (100%)	4e-07	GT129150
H5p3	1	glia-derived nexin precursor (ACI33531; *Salmo salar*)	GO:0004867	serine-type endopeptidase inhibitor activity (MF)	983	166/166 (100%)	8e-81	GT129151
H5p10	1	uncharacterized			462			GT129152
H6p4	1	lysostaphin (AAB53783; *Staphylococcus simulans*)	GO:0007047 GO:0006508 GO:0005576 GO:0004222 GO:0008270	cell wall organization (BP) proteolysis (BP) extracellular region (CC) metalloendopeptidase activity (MF) zinc ion binding (MF)	745	39/169 (23%)	1e-18	GT129153

H6p9	1	60S ribosomal protein L8 (ACN10341; *Salmo salar*)			580	188/188 (100%)	9e-98	GT129154
H6p10	1	extracellular matrix protein 1 precursor (ACN10906; Salmo salar)		Function: regulate endochondral bone formation, and to stimulate proliferation of endothelial cells and induce angiogenesis (Chan I, 2004).	501	144/144 (100%)	8e-56	GT129155
H7p9	1	Na/K ATPase alpha subunit isoform 3 (AY319388; *Oncorhynchus mykiss*)	GO:0006754 GO:0015672 GO:0016021 GO:0005524 GO:0015662 GO:0015077	ATP biosynthetic process (BP) monovalent inorganic cation transport (BP) integral to membrane (CC) ATP binding (MF) ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism (MF) monovalent inorganic cation transmembrane transporter activity (MF)	583	549/604 (90%)	0.0	GT129156
H8p3	1	transposase (AAP49009; *Rana pipiens*)	GO:0015074 GO:0006313 GO:0005634 GO:0003677 GO:0004803	DNA integration (BP) transposition, DNA-mediated (BP) nucleus (CC) DNA binding (MF) transposase activity (MF)	990	54/83 (65%)	2e-35	GT129157
H8p4	1	cold-inducible RNA-binding protein (BT058753; *Salmo salar*)			931	768/776 (98%)	0.0	GT129158
H8p10	1	uncharacterized			217			GT129159
H9p4	1	uncharacterized			773			GT129160
H9p9	1	uncharacterized			609			GT129161
H9p10	1	uncharacterized			201			GT129162
H10p4	1	uncharacterized			569			GT129163
H11p3	1	thymosin beta-12 (ACI66596; *Salmo salar*)	GO:0042989 GO:0005737 GO:0003779	sequestering of actin monomers (BP) cytoplasm (CC) actin binding (MF)	681	42/42 (100%)	2e-07	GT129164
H11p4	1	uncharacterized			396			GT129165
H11p10	1	uncharacterized			920			GT129166

ESTs from Reverse SSH cDNA Library from salmon ear designed to be enriched for seismic exposed genes. Out of 50 contigs: 30 have a significant BLASTX hit (E<1e-05); 11 have a significant BLASTN hit (E<1e-05) highlighted in blue font; and 9 have no significant BLASTX or BLASTN hit (E<1e-05). Out of 193 singletons: 100 have a significant BLASTX hit (E<1e-05); 28 have a significant BLASTN hit (E<1e-05) highlighted in blue font; 65 have no significant BLASTX or BLASTN hit (E<1e-05). EST ID is reported as Contig # for contigs and well ID plus plate number for singletons. Reverse Library name is ssal_ear_SSH_seismicR. BLAST Results were up-dated August 2009.