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OPEN Comparative transcriptome profiling of selected osmotic regulatory proteins in the gill during seawater acclimation of chum salmon (Oncorhynchus keta) fry

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Salmonid fishes, chum salmon (Oncorhynchus keta) have the developed adaptive strategy to withstand wide salinity changes from the early life stage. This study investigated gene expression patterns of cell membrane proteins in the gill of chum salmon fry on the transcriptome level by tracking the salinity acclimation of the fish in changing environments ranging from freshwater (0 ppt) to brackish water (17.5 ppt) to seawater (35 ppt). Using GO analysis of DEGs, the known osmoregulatory genes and their functional groups such as ion transport, transmembrane transporter activity and metal ion binding were identified. The expression patterns of membrane protein genes, including pump-mediated protein (NKA, CFTR), carrier-mediated protein (NKCC, NHE3) and channel-mediated protein (AQP) were similar to those of other salmonid fishes in the smolt or adult stages. Based on the protein-protein interaction analysis between transmembrane proteins and other related genes, we identified osmotic-related genes expressed with salinity changes and analyzed their expression patterns. The findings of this study may facilitate the disentangling of the genetic basis of chum salmon and better able an understanding of the osmophysiology of the species.

Salinity is one of the critical factors limiting the distribution patterns of all aquatic organisms¹⁻⁴. Salmonid fishes display diverse life-history traits; anadromous individuals that mature in the river from hatching through to juveniles acquire the capacity to tolerate salinity associated with parr-smolt transformation and undergo ocean migrations before returning to rivers for spawning, whereas landlocked types spend their entire life within freshwater^{5,6}. Although migration between habitats is common among salmonid fishes, the seawater acclimation period varies even within anadromous species. Therefore, the timing of river to ocean migration varies from species to species^{5,7,8}.

Chum salmon (Oncorhynchus keta) possess an excellent osmotic plasticity in coping with hyperosmotic or hypoosmotic environments⁹⁻¹¹. During the late embryonic stage, chum salmon have already acquired the hypo-osmoregulatory mechanism by the mitochondria-rich cells (MRCs) in the volk-sac membrane¹². In addition, chum salmon fry whose habitat is freshwater begin to show remarkable seawater adaptability prior to seawater entry, which is not observed in the fry of other salmonids¹³. Chum salmon begin to activate MRCs in the gill at an earlier stage (alevin-fry) and show higher salinity resistance at the fry stage than at the late alevin stages^{11,14,15}.

Most of the salmonid fishes currently in the market are dominated by cultured Atlantic salmon (Salmo salar), whereas the production of chum salmon mostly depends on fishing (FAO, 2019). It is also noteworthy that chum salmon have been studied less when compared to other salmonid fishes. However, chum salmon are a major species of salmonid fishes that return to Korea, and if the feed and the aquaculture system are improved with the help of research on seawater adaptability and growth, they can be developed as a promising aquaculture species in the future.

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	Freshwater (0%)		Brackish water (50%)		Seawater (100%)	
	No. reads	Rate (%)	No. reads	Rate (%)	No. reads	Rate (%)
Mapped reads	91,480,087	94.14	92,338,752	94.45	105,031,902	94.37
Not mapped reads	5,697,147	5.86	5,424,666	5.55	6,268,988	5.63
Reads in pairs	73,700,972	75.84	74,482,876	76.19	84,457,112	75.88
Broken paired reads	17,779,115	18.30	17,855,876	18.26	20,574,790	18.49
Total reads	97,177,234	100.00	97,763,418	100.00	111,300,890	100.00

 Table 1. Mapping statistics of transcriptome reads to the reconstructed transcriptome assembly.

The gill is a primary organ to detect changes in external osmotic pressure and promotes the compensatory active absorption or the excretion of monovalent ions (sodium, potassium, chloride) to maintain the osmolality of body fluids at levels equivalent to approximately one-third of the seawater osmolality¹⁶⁻¹⁸. In seawater, the fish must drink copious amount of seawater and absorb water in the intestines to compensate for the loss of water, while the excess ions are actively excreted from the gills and the kidney¹⁹. In contrast, freshwater teleost cope with the osmotic water load and the ion loss and therefore excrete a large amount of water by producing diluted urine by the kidney and uptake ions through the gills¹⁹. In addition, the gill tissues play crucial roles in physical processes such as gas exchange, nitrogenous waste excretion, and acid-base balance²⁰.

To accomplish these functions implicated in the maintenance of domestic homeostasis, various membrane proteins of ionocytes regulate intracellular ionic concentrations²¹. Ionocytes also regulate acid (H⁺) or base (HCO₃⁻) release to maintain pH homeostasis in the blood^{21,22}. In the euryhaline species, the plasma osmolality was reduced when the seawater adapted fishes (*Acanthopagrus schlegeli* and *Paralichthys orbignyan*) were transferred to freshwater^{23,24}. In contrast, it has been reported that the plasma osmolality increased when freshwater-adapted fishes (*Takifugu obscurus, Synechogobius ommaturus* (R.), and *Chanos chanos*) were moved to brackish-water or seawater^{25–27}. In the diadromous species (*Monopterus albus, S. salar*), the plasma osmolality was gradually decreased during long-term exposure and then was maintained at a certain level^{28,29}. Osmotic control of gill ionocytes is known to involve various membrane proteins such as sodium-potassium ATPase (NKA), sodium-potassium-chloride cotransporter (NKCC), cystic fibrosis transmembrane conductance regulator (CFTR) and aquaporin (AQP)³⁰⁻³². In addition, for research on osmoregulation capabilities of euryhaline fish, it is important to investigate not only the gene expression patterns of transmembrane proteins but also those of interrelated proteins under the influence of salinity stress.

Studies on various aspects of seawater adaptation and osmotic control capacity of salmonid fishes have long been reported. However, a molecular genetic approach to addressing osmotic-related genes involved the osmoregulation and the maintenance of the body homeostasis under salinity changes has rarely been implemented. In addition, studies of the transcriptome level of osmolality-regulating proteins have been rarely reported. Therefore, a deeper understanding of the mechanism underlying the adaptation to salinity stress of the chum salmon may contribute to developing strategies for efficient farming practices for this candidate species. In that regard, this study tried to analyze gene expression patterns of membrane proteins present in ionocytes and a gill tissue with whole transcriptome NGS and qRT-PCR. In addition to that, the gene expression of related proteins was studied as well.

Results

Genome mapping and *de-novo* **assembly of unmapped reads.** The reads obtained from sequencing of each group were trimmed and deposited at Genbank under the Sequence Read Archive (SRA) accessions SRX3932910-SRX3932912. Since chinook salmon are taxonomically close to chum salmon, each group showed a high read-mapping rate of 82 to 83% in Table S1^{6,33}. An additional *de-novo* assembly was conducted on the unmapped reads from the chinook salmon genome assembly, thereby constructing 119,439 counts of N50, 403 bp. Subsequently, a new reference of 197,684 counts (N50 length: 3154 bp, Avr length: 1,428 bp, GC contents: 48%) were constructed by pooling the reads from both the chinook salmon genome assembly and the reads from the *de-novo* assembly. Finally, the reads of each group were mapped to the newly constructed reference (Table 1). The mapping rate of the new reference was 94–95% and the average length was 1428.82 bp.

Functional annotation of reconstructed reference reads. Annotation was conducted on the reads mapped to the newly constructed reference based on a variety of databases. The BLAST annotation based on the NR database identified that a total of 93,542 counts (47.31%) were matched with certain genes. Most of the matched genes originated from chinook salmon (*O. tshawytscha*), which were used as a reference, and the rest of the annotated genes were mostly related to the salmon origin group: rainbow trout (*O. mykiss*, 14.9%), coho salmon (*O. kisutch*, 8.3%), Atlantic salmon (*S. salar*, 6.1%) and Arctic char (*Salvelinus alpinus*, 3.2%) (Fig. S1). GO annotation was carried out on three categories (biological process; BP, molecular function; MF, cellular component; CC) under the condition of level 7, and the results were merged with those of the annotated GO classes were nucleic acid-templated transcription (GO: 0097659) and regulation of nucleic acid-templated transcription (GO: 1903506). Among those, 6,806 reads and 6,469 reads were mapped, respectively (Fig. S2). In the case of the MF category, the genes related to nucleoside-triphosphatase activity (GO: 0017111) and zinc ion binding (GO: 0008270) were annotated frequently, more than any other gene with 2,928 reads and 2,613 reads mapped. As for the CC category, the genes responsible for clathrin-coated vesicle (GO: 0030136) were annotated the most with 342 reads mapped.

Clustering analysis of differential gene expression pattern. DEGs were investigated using transcriptome profiles of gills of chum salmon fry in different salinity environments. Among the pump-mediated transmembrane proteins which are directly involved in osmoregulation, NKA (ATP1a, ATP1b) is known to be associated with metal ion transport (GO: 0030001) and inorganic ion transmembrane transport (GO: 0098660) in BP term, and potassium ion transmembrane transporter activity (GO: 0071805) in MF term (Figs. 1 and 2). Along with ATP1a and ATP1b, the genes involved in metal ion transport, inorganic ion transmembrane transport and potassium ion transmembrane transporter activity showed the pattern of gene expression with chum salmon fry during the environmental alteration: when the chum salmon were transferred from freshwater to brackish water, 1.14 times more genes were expressed than when the fish were transferred from brackish water to seawater. Conversely, when the fish were transferred from brackish water to seawater, there was a 1.16-fold increase in the number of the genes that decreased their expression compared to when the fish were transferred from freshwater to brackish water. NKCC (SLC12a, SLC9a), the carrier-mediated transmembrane protein, is associated with cation transmembrane transport (GO: 0098655), metal ion transport (GO: 0030001), metal ion transport (GO: 0030001), monovalent inorganic cation (GO: 0015672) and inorganic ion transmembrane transport (GO: 0098660) in BP term, and in MF term it is involved in sodium ion transmembrane transporter activity (GO: 0015081) and proton transmembrane transporter (GO: 0015078). The genes involved in cation transmembrane transport, monovalent inorganic cation and proton transmembrane transporter also showed the gene expression pattern: when transferred from freshwater to brackish water, chum salmon fry tended to express more genes than when transferred from brackish water to seawater. The genes were expressed less when the fish were transferred from brackish water to seawater than when transferred from freshwater to brackish water. In addition, there was a tendency of the expression of the genes involved in iron ion binding (GO: 0005506), potassium channel activity (GO: 0008282), and potassium channel complex (GO: 0034705) to increase more remarkably when the chum salmon fry were transferred from freshwater to brackish water than when transferred from brackish water to seawater. In particular, 1.56 times more genes were expressed which were involved in iron ion binding (GO: 0005506) when the salinity was increased from freshwater to brackish water than from brackish water to seawater. The number of genes involved in the potassium channel activity (GO: 0008282), and potassium channel complex (GO: 0034705) was about 1.12 times and 1.15 times, respectively.

The genes showing an increase of expression in both groups, from freshwater to brackish water group and from brackish water to seawater group, were listed in order of fold change. As a result, the types of genes differed between the two groups but showed similar trends in function (Fig. 3). In the salinity change from freshwater to brackish water, an increase in the expression of genes involved in innate immune response and blood coagulation was noticeable. Changes in salinity from brackish water to seawater increased the expression of genes involved in adaptive immunity. In terms of cellular components, the genes involved in binding to the integral component of membrane or cell surface and metal ion binding were expressed in both groups.

The enumeration of the genes in fold change order which were highly subject to salinity changes indicated that the types of genes were different between the freshwater to brackish water transfer group and the brackish water to seawater transfer group. However, the functions and the locations of the genes of the two groups showed many similarities (Fig. 3). The following proteins located either in the integral component of the membrane or in the cell surface showed high differential gene expression: single Ig IL-1-related receptor-like isoform X1, 3-oxo-5-beta-steroid 4-dehydrogenase, MHC class I heavy chain, macrophage mannose receptor 1-like and phosphatidylcholine: ceramide cholinephosphotransferase 1-like, serine/threonine-protein phosphatase 6 regulatory subunit 2-like, transmembrane and immunoglobulin domain-containing protein 1-like. Also, the genes involved in metal ion binding, including 4-hydroxyphenylpyruvate dioxygenase-like, activity-dependent neuroprotector homeobox protein-like isoform X1 and histone-lysine N-methyltransferase 2C-like isoform X5 showed high differential gene expression as the salinity increased. However, plasma protease C1 inhibitor-like, fibrinogen beta chain-like isoform X1 and fibrinogen alpha chain-like which were associated with blood coagulation showed unusually high differential gene expression when the fish were transferred from freshwater to brackish water.

PPI networks of osmoregulation related genes. The interaction network of osmoregulatory proteins provides important information about homeostasis responses of fish to salinity changes. PPI network analyses on a total of 59 nodes showed that the genes tended to be grouped according to the functions of the membrane protein genes and each protein was interrelated with each other (Fig. 4). The PPI map consisted of a total of 138 edges and the average local clustering coefficient was 0.638. The average node degree was 4.68 and the PPI enrichment p-value was below 1.0e-16. The functional enrichment analyses of PPI networks indicated that Reactome Pathways was involved in the pathways of transport of small molecules (DRE-382551), ion homeostasis (DRE-5578775), ion transport by P-type ATPases (DRE-936837), aquaporin-mediated transport (DRE-445717) and passive transport by aquaporins (DRE-432047).

The domain and keyword analyses using Uniprot, PFAM, INTERPRO and SMART confirmed the aforementioned finding. As the salinity increased, the membrane protein genes and the interaction proteins of chum salmon fry were variously expressed (Table 2). However, in the gill of chum salmon fry, the genes interacting with membrane proteins were commonly present in the pattern of alternating increases and decreases in expression, rather than a continuous increase or decrease in expression with increasing salinity.

qRT-PCR validation of transmembrane protein genes related to osmoregulation. To confirm the expression patterns of DEGs, representative transmembrane protein genes were selected for qRT-PCR analysis. As shown in Fig. 5, most of the qRT-PCR results of the genes analyzed kept consistent with the high-throughput sequencing data, which confirmed the accuracy and reliability of the sequencing data. Based on qRT-PCR, mRNA expression levels of ATP1a1b, ATP1a1c, ATP1a3, ATP1b1 and CFTR related to pump mediated ion transport in the brackish water vs. freshwater group were almost 1.61-, 1.07-, 1.27-, 1.42 and 2.68 -fold



Figure 1. Gene ontology annotation (by level 7) for functional analysis of differentially expressed genes of *O. keta* fry after transfer from freshwater to brackish water.

of the control respectively, and those in the seawater vs brackish water group were almost 1.63-, 1.31-, 1.70-, 1.01 and 1.17-fold of the control respectively, the expression tended to increase with increasing salinity. On the other hand, in the case of ATP1a1a, as the salinity increased, gene expression tended to decrease by 1.04- and 1.16-fold. As for SLC12a2a and SLC12a2b, a carrier-mediated symporter, gene expression slightly increased in both the brackish water vs. freshwater and the seawater vs. brackish water group. However, SLC12a1 had the fold change value four to five times higher than that of SLC12a2a and SLC12a2b in the two comparative groups. The SLC9a3, a carrier-mediated antiporter, showed a similar pattern to ATP1a1a in which gene expression decreased with the increasing salinity. In the case of AQP, a channel-mediated protein, the fold change value as well as the



Figure 2. Gene ontology annotation (by level 7) for functional analysis of differentially expressed genes of *O. keta* fry after transfer from brackish water to seawater.

expression patterns of the two groups differ from each other according to the isoform type. Based on the mRNA expression level results of qRT-PCR, the following was found: AQP4 showed decreased gene expression in the brackish water vs 0% group by 3.63-fold and increased gene expression in the seawater vs. brackish water group by 3.08-fold. AQP8 and AQP9 showed increased expression in the brackish water vs. freshwater group by 692.98- and 181.44-fold, respectively, and decreased expression in the seawater vs brackish water group by 3.2.75- and 13.42-fold, respectively.

Group	Accession #	Gene description	Log ₂ fold change	Fold change	FDR p-value	Bonferroni
	XP_024294241	I Complement c1q-like protein 2		1443.55	4.27E-12	3.64E-09
	XP_020330357	Alpha-2-HS-glycoprotein-like	10.29	1248.65	1.29E-11	1.18E-08
XP_024263045		4-hydroxyphenylpyruvate dioxygenase-like	9.49	719.48	7.51E-10	8.58E-07
	XP_024235073	Tyrosine aminotransferase-like	9.29	624.38	2.01E-09	2.45E-06
	XP_024298393	Alpha-2-macroglobulin-like	8.94	492.54	0	0
	XP_024274065	Ubiquitin carboxyl-terminal hydrolase 24 isoform X4	8.88	470.90	1.35E-08	1.85E-05
	XP_024258329	Plasma protease C1 inhibitor-like	8.60	386.97	0	0
Brackish	XP_024265252	Complement C3-like	8.54	371.09	6.23E-08	9.56E-05
water (50%)	XP_014071165	Fibrinogen beta chain-like isoform X1	8.44	347.16	0	0
(5070)	XP_024278122	Complement factor H-like	8.29	312.72	1.79E-07	3.00E-04
vs	XP_021477882	Fibrinogen alpha chain-like, partial	8.25	304.69	3.18E-14	2.19E-11
Freshwater	XP_024272679	Single Ig IL-1-related receptor-like isoform X1	8.21	295.77	2.52E-07	4.32E-04
(0%)	XP_020347874	Activity-dependent neuroprotector homeobox protein-like isoform X1	8.19	292.94	2.67E-07	4.59E-04
	XP_020312381	3-oxo-5-beta-steroid 4-dehydrogenase	8.15	283.53	3.26E-07	5.68E-04
	XP_024265107	Serum albumin 2-like	8.10	274.31	0	0
	XP_024241984	AT-rich interactive domain-containing protein 1A-like isoform X4	8.09	272.23	4.16E-07	7.39E-04
	XP_024301333	Nuclear mitotic apparatus protein 1 isoform X1	8.08	271.29	4.26E-07	7.55E-04
	XP_024270126	Complement C3-like	8.01	258.27	0	0
	XP_020324407	Complement c1q-like protein 4	7.94	244.73	0	0
	XP_024298410	Alpha-2-macroglobulin-like isoform X1	7.75	215.04	0	0
	AAB62232	MHC class I heavy chain, partial	14.43	22132.76	1.61.E-02	1.00.E+00
	XP_024286595	Macrophage mannose receptor 1-like	10.51	1455.23	3.65.E-12	3.23.E-09
	XP_024280657	Phosphatidylcholine:ceramide cholinephosphotransferase 1-like	8.00	256.76	5.52.E-07	1.03.E-03
	XP_024268871	Serine/threonine-protein phosphatase 6 regulatory subunit 2-like	7.61	194.89	2.67.E-06	5.74.E-03
	XP_024252207	Meiosis regulator and mrna stability factor 1-like isoform X1	7.59	192.59	2.85.E-06	6.17.E-03
	XP_020364812	Avidin-like	7.51	182.83	0	0
	XP_024271028	Testis-expressed sequence 15 protein isoform X6	7.48	178.85	4.31.E-06	9.64.E-03
Seawater	XP_024273970	Volume-regulated anion channel subunit LRRC8A	7.37	165.10	6.68.E-06	1.55.E-02
(100%)	XP_024258557	Gtpase IMAP family member 7-like isoform X1	7.26	153.64	9.85.E-06	2.37.E-02
vs	XP_024269293	Ras and EF-hand domain-containing protein-like	7.26	153.64	9.85.E-06	2.37.E-02
Brackish	XP_020331541	Transmembrane and immunoglobulin domain-containing protein 1-like	7.26	152.97	7.62.E-11	7.92.E-08
water (50%)	XP_024242164	BRD4-interacting chromatin-remodeling complex-associated protein-like isoform X1	7.01	129.20	2.43.E-05	6.40.E-02
	XP_021420997	Ras-related protein Rab-5C	6.93	122.32	3.55.E-05	9.79.E-02
	XP_024279243	UPF0183 protein c16orf70 homolog isoform X1	6.93	122.31	7.22.E-10	8.44.E-07
	XP_024247258	Histone-lysine N-methyltransferase 2C-like isoform X5	6.83	113.92	5.04.E-05	1.45.E-01
	XP_023999576	Acidic leucine-rich nuclear phosphoprotein 32 family member B-like	6.65	100.17	8.55.E-05	2.61.E-01
	XP_024237673	Protein phosphatase 1 regulatory subunit 26-like isoform X2	6.59	96.35	1.03.E-04	3.21.E-01
	XP_024276407	Transcription initiation factor IIA subunit 1-like isoform X4	6.56	94.06	1.16.E-04	3.65.E-01
	XP_024239944	Tyrosine-protein phosphatase non-receptor type 12-like isoform X2	6.43	86.42	1.73.E-04	5.70.E-01
	XP_024249300	SH3 and PX domain-containing protein 2B-like isoform X1	6.43	86.24	0	0

Figure 3. List of the first 20 genes showing the highest differential expression in salinity changes for the gill of *O. keta* fry.

Discussion

Chum salmon, the excellent osmoregulator, migrate downstream to the sea in their early life stage, acquiring hypo-osmoregulatory capability during the alevin stages. To further examine the potential mechanisms and identify osmotic-regulated genes, we compared the transcriptome of chum salmon gill tissues of two groups: those transferred from freshwater to brackish water and from brackish water to sea water. Among the membrane proteins associated with osmoregulation, NKA subunit isoforms and NHE3 which are pump-mediated proteins, NKCC subunit isoforms which is a carrier-mediated protein and AQP isoforms which is a channel-mediated protein were selected to be investigated in terms of gene expression. Also, gene expression of other proteins was explored which are in the interaction network to which the aforementioned proteins belong.

As the salinity increased, the expression of NKA alpha and beta subunit isoforms slightly increased in the chum salmon fry gill. However, that of ATP1a1a decreased among the subunit isoforms. As mentioned above, the current study observed differences of the gene expression pattern within NKA subunit isoforms with increasing



Figure 4. PPI network map of osmoregulation-related proteins using STRING. The red-colored figure (light to dark) represents the up-regulated protein and the green-colored figure (light to dark) represents the down-regulated protein. The saturation is displayed differently according to fold change (FC). The diamond shape is the main transmembrane protein in this study and the circle shape is a protein interacting with a transmembrane protein. (**A**) illustrates the difference in expression between the brackish water vs freshwater group, and (**B**) illustrates the difference in expression between the seawater vs brackish water group.

salinity. The same tendency was reported in previous studies of such euryhaline fishes as *Dicentrarchus labrax*, *O. mykiss*, *Oreochromis mossambicus*, *Salvelinus alpinus* and *S. salar*³⁴⁻³⁷. This indicates the possibility that either the ion transport activity or the absorption and secretion of ions varies within NKA subunit isoforms³⁶. In this regard, two hypotheses have been reported: one, in the course of sodium and potassium transport, kinetics could vary due to the affinity difference between NKA subunit isoforms and ions, and the other that the concentration of lipid rafts rich in cholesterol and sphingolipids could affect the NKA subunit isoforms activity^{38,39}. Specifically, in the case of the chum salmon fry gill, ATP1a1a was a predominant form in freshwater like other Salmonidae. As the salinity increased, however, ATP1a1b became a predominant form. The other NKA subunits (ATP1a, ATP1b isoforms) were found to have a somewhat lower effect on salinity change than ATP1a1a and ATP1a1b. Like ATP1a1a, gene expression of NHE3, an ion antiport protein, decreased with the increasing salinity. In other words, the sodium ion uptake activity was higher in freshwater than in seawater. This agrees with the research findings of studies of salinity and NHE3 expression in *D. labrax*, *O. mossambicus* and *Gasterosteus aculeatus*⁴⁰⁻⁴³. Another assumption is related to the sodium ion uptake and secretion. NHE3 and NKA present in gill

Gene (Davisoration)NomeNecession (20, disapports)100% - stopAdde phosphates (2) synomalACP2XM, Q449038711.6.91.21Aquaporin 4AQP4XM, Q419135141.6.91.21Aquaporin 7AQP4XM, Q441015211.8.8-1.67Aquaporin 8.1AQP4XM, Q44401581100.44-1.02Aquaporin 8.1AQP4XM, Q444015811.01.4-1.01Aquaporin 10AQP1XM, Q44403581-1.01-1.01Aquaporin 11AQP1XM, Q44403581-1.01-1.01Aquaporin 12AQP1XM, Q44403581-1.01-1.01Aquaporin 12AQP1XM, Q4440351-1.01-1.01Agmanor nanopersisinAVP1XM, Q4440341-1.01-1.01Areine mchytinandraseASMTXM, Q44403411.08-1.01Arbine N+K + transporting abunit alpha 10ATP1XM, Q44403411.08-1.01ATPae N+K + transporting abunit alpha 10ATP1XM, Q44403411.18-1.01ATPae N+K + transporting abunit alpha 10ATP1XM, Q44403411.10-1.01ATPae N+K + transporting abunit alpha 10ATP1XM, Q44403411.10-1.02ATPae N+K + transporting abunit alpha 10				Fold change	
Acid proprints 2, yunsca 2, unface 3 AVI2 XML 22439357.1 -1.63 1.73 Adpuncyme 1, 2, unface 3 AVI2 XML 22439357.1 1.83 -1.67 Aquiportin 3.1 AQPS XML 02443757.1 1.83 -1.62 Aquiportin 3.1 AQPS XML 02443757.1 1.83 -1.62 Aquiportin 1.1 AQPS XML 02443757.1 1.86 -1.01 Aguiportin 1.1 AQP12 XML 02443757.1 -1.02 -2.64 Aguinor rin 1. AQP12 XML 02443757.1 -1.03 -3.35 Arginine terms etch colled-coll 1 RSRC1 XKL 0244301.1 -1.06 -1.34 Arginine terms etch colled-coll 1 ATP114 XKL 0244302.1 -1.06 1.11 Arginine terms etch colled-coll 1 ATP114 XKL 0244301.1 -1.06 1.11 Arginine terms etch colled-coll 1 ATP114 XKL 0244302.1 -1.06 1.11 Arginine terms etch colled-coll 1 ATP114 XKL 0244302.1 -1.06 1.12 Argine terms etch colled-coll 1 ATP114 XKL 02444305.1	Gene (Danio rerio)	Symbol	Accession # (O. tshawytscha)	50% vs 0%	100% vs 50%
Aderacopro heta 2. nuface aNBR2aKN. 024791011.601.21Aquporin 7AQPNK. 02479101.1.85-1.67Aquporin 8.1.AQPS.1.NK. 024491052.1.1.85-1.67Aquporin 8.1.AQPS.1.NK.024491052.1.1.64-21.22Aquporin 10AQPS.1.XK.02444058.11.62-21.22Aquporin 11AQP11XK.02444058.11.62-1.61Aquporin 12AQP12XK.02444578.11.70-1.01Arginins/entri-fox-holle-offBSRC1SK.0295773.1.1.14-1.25Arginins/entri-fox-holle-offBSRC1SK.0295773.1.1.14-1.25Arginins/entri-fox-holle-offASMTSK.0295773.1.1.14-1.25Arginins/entri-fox-holle-offASMTSK.0295773.1.1.14-1.25Arginins/entri-fox-holle-offASMTSK.0295773.1.1.16-1.25Arginins/entri-fox-holle-offASMTSK.0295773.1.1.16-1.25Arginins/entri-fox-holle-offASMTSK.0295773.1.1.16-1.25Arginins/entri-fox-holle-offASMTSK.0295761.1.1.10-1.25Arginins/entri-fox-holle-offASMTSK.0295761.1.1.10-1.26Arginins/entri-fox-holle-offASMTSK.0244571.1.1.10-1.02Arginins/entri-fox-holle-offASMTSK.02445761.1.1.10-1.02Arginins/entri-fox-holle-offASMTSK.02445761.1.1.10-1.02Arginins/entri-fox-holle-offASMT	Acid phosphatase 2, lysosomal	ACP2	XM_024439857.1	-4.64	5.84
Apageorin 4 NOP4 XM. 202401623.1 1.45 -1.67 Aquiportin 7 AQP5 XM. 202401623.1 7.45 -1.62 Aquiportin 8.1 AQP5 XM. 202401623.1 7.45 -1.62 Aquiportin 9.6 XM. 202442058.1 7.64 -1.12 -1.01 Aquiportin 12 AQP12 XM. 202442155.1 -1.01 -1.01 -2.04 Arginine vargoresin AVP XM. 0244255.1 -1.02 -2.04 Arginine vargoresin AVP XM. 02442030.1 -1.04 -2.35 Arginine vargoresin AVP XM. 0244203.1 -2.06 -2.66 Argine vargoresin gubanti alpla 1 ATP113 XM. 0244372.1 -1.06 -1.11 Argine vargoresin gubanti alpla 1 ATP113 XM. 0244372.1 -1.06 1.11 -1.07 Argine vargoresin gubanti alpla 1 ATP113 XM. 0244372.1 -1.06 1.11 -1.07 Argine vargoresin gubanti alpla 1 ATP113 XM. 0244372.1 -1.06 1.11 -1.07 Argine vargoresin gubanti alpla 1 </td <td>Adrenoceptor beta 2, surface a</td> <td>ADRB2a</td> <td>XM_024393505.1</td> <td>-1.50</td> <td>1.21</td>	Adrenoceptor beta 2, surface a	ADRB2a	XM_024393505.1	-1.50	1.21
Apagemin 7 KU 02401652.1 185 -1-67 Aqueporin 7 KM 024413576.1 743.3 11.62 Aqueporin 96 AQ96.1 KM 02444584.1 10.64 -11.22 Aqueporin 11 AQP11 KM 02444584.1 10.64 -11.22 Aqueporin 12 AQP12 KM 02444584.1 1.68 -10.1 Arginine vicino-th colled-cell RSRC KM 024440301.1 3.13 -3.85 Arenit methyltransferae ASMT KM 02441034.1 -2.47 -2.69 ATPase Ka-//K-transporting submit alpla 1 ATP1a1 KM 02441034.1 -1.40 1.11 ATPase Ka-//K-transporting submit alpla 1 ATP1a1 KM 02444058.1 1.13 1.07 ATPase Ka-//K-transporting submit alpla 1 ATP1a1 KM 02444085.1 1.14 1.18 ATPase Ka //K-transporting submit alpla 1 ATP1a1 KM 0244085.61 1.14 1.18 ATPase Ka //K-transporting submit alpla 1 ATP1b2 KM 0244085.61 1.61 1.14 ATPase Ka //K-transporting submit alpla 1 ATP1b2 KM 02443972.1 -	Aquaporin 4	AQP4	XM_024375186.1	-1.63	1.73
Apageorin 9.1 XM.02843376.1 ?43.33 -1.62 Aqueporin 9.b AQP9b XM.02444028.1 100.44 -21.22 Aqueporin 11 AQP12 XM.02444028.1 -7.61 Aquiporin 12 AQP12 XM.02442155.1 -7.10 - Arginine expension AVP XM.02449755.1 -7.10 - Arginine recorder citch cells cells 1 RBRC1 XM.02449755.1 -7.69 -2.64 Arginine recorder citch cells cells 1 RBRC1 XM.0244970.1 1.14 -1.25 Arginine recorder citch cells cells 1 RBRC1 XM.0244971.1 -1.60 1.11 ATPace N+/K-transporting subunit alpha 1 ATP1al XM.0244971.1 -1.60 1.11 ATPace N+/K-transporting subunit leta 2 ATP1ab XM.02449766.1 1.14 -1.81 ATPace N+/K-transporting subunit leta 3 ATP1ab XM.02449766.1 1.11 -1.23 Argeoring subunit leta 3 ATP1ab XM.02449766.1 1.11 -1.24 ATPace N+/K-transporting subunit leta 3 ATP1ab XM.02449766.1 1.10	Aquaporin 7	AQP7	XM_024401632.1	1.85	-1.67
Aquiporin 7b AQP9b XM_02440281 100.44 2122 Aquiporin 11 AQP11 XM_024412851 -1.01 - Aggintor/stric-fric-foolded-coll RS XM_0244128551 -1.01 - Aggintor/stric-fric-foolded-coll RS XM_0244128551 -1.01 - - Argintor/stric-fric-foolded-coll RS XM_024412851 -1.01 -	Aquaporin 8a.1	AQP8a.1	XM_024433576.1	743.33	-13.62
Aquaporin 11 AQP11 XM_02441584.1 -8.88 -1.01 Aquiporin 12 AQP12 XM_02441333.1 -1.17 -1.01 Arginine vaporesin AVP XM_0243735.1 -7.39 -26.48 Arginine vaporesin XM_024407353.1 -1.17 -1.01 Arginine vaporesin XM_024407953.1 1.73 -2.69 ArThas N+/K + transporting submit alpha 1a ATP1a1a XM_024410741.1 -1.00 1.11 ArThas N+/K + transporting submit alpha 1 ATP1a1a XM_02441055.1 1.14 1.14 ATPase N+/K + transporting submit alpha 1 ATP1a1a XM_02441055.1 1.14 1.18 ATPase N+/K + transporting submit beta 3 ATP1b2a XM_02439116.1 1.61 -1.04 ATPase N+/K + transporting submit beta 3 ATP1b2a XM_02439055.1 1.14 1.18 Capor-dependent protein Kinase catalytic submit alpha PRKACAa XM_02439184.1 -1.63 1.21 Cascin Kinase Lepislon CSNKK XM_02439184.1 -1.63 1.21 Cascin Kinase Lepislon 1.11 -1.28	Aquaporin 9b	AQP9b	XM_024440258.1	100.44	-21.22
Aquiportin 12 AQP12 KM_024421335.1 -1.17 -1.01 Arginine/strine-trich colled-coll 1 KSR_021375.1 27.50 -2.648 Arginine/strine-trich colled-coll 1 KSR_01 KR_00235720.1 1.14 -1.23 Arsentie methyftransferase ASSMT KR_002437520.1 1.14 -1.23 ArtPase Na //K + transporting subunit alpha 16 ATP1a16 XM_024445742.1 1.08 1.34 ATPase Na //K + transporting subunit alpha 16 ATP1a16 XM_02444658.1 1.13 1.07 ATPase Na //K + transporting subunit beta 2 ATP1b2a XM_02444658.1 1.161 -1.04 ATPase Na //K + transporting subunit beta 2 ATP1b2b XM_024394116.1 1.61 -1.04 ATPase Na //K + transporting subunit beta 3 ATP1b2b XM_024399314.1 -1.63 1.21 Case Na //K + transporting subunit beta 3 ATP1b2b XM_02439814.1 -1.63 1.22 Case Na //K + transporting subunit beta 3 ATP1b2b XM_024381561.1 -1.63 1.23 Case Na //K + transporting subunit beta 3 ATP1abb XM_02444281.1 1.	Aquaporin 11	AQP11	XM 024445684.1	-8.88	-1.01
Arginine vasopressin AVP XM.0243973351 27.80 -26.48 Arginine vasopressin KRC1 XR.0024307351 1.14 -1.13 Arsenite methyransferase ASSMT XR.002440091 3.13 -3.85 ATPase Na //K + transporting subunit alpha 1a ATP1 tala XR.002440091 -247 -2.49 ATPase Na //K + transporting subunit alpha 1c ATP1 tala XR.0024405742.1 -1.08 1.34 ATPase Na //K + transporting subunit alpha 3 ATP1 tal XR.002440585.1 1.14 -1.18 ATPase Na //K + transporting subunit beta 3 ATP1 taba XR.002440585.1 -1.14 -1.27 ATPase Na //K + transporting subunit beta 3b ATP1 taba XR.002439058.1 -1.63 -1.27 ATPase Na //K + transporting subunit beta 3b ATP1 taba XR.002439058.1 -1.64 -1.27 ATPase Na //K + transporting subunit beta 3b ATP1 taba XR.002439058.1 -1.63 -1.21 Casen Inase L episIon COIKCI XR.002439064.1 -1.64 -1.22 Carber also 1 DERL XR.00243896.1 -1.65	Aquaporin 12	AQP12	 XM 024421535.1	-1.17	-1.01
Arginino/scine Instruction RSRC1 RR.002955720.1 1.14 -1.23 Arenite methyltransferase AS3MT XM.024405019.1 3.13 -3.35 ArPase Nar /K + transporting submit alpha 16 ATP Ial XM.024443742.1 1.08 1.34 ATPase Nar /K + transporting submit alpha 16 ATP Ial XM.02444374.1 -1.00 1.11 ATPase Nar /K + transporting submit beta 1 ATP Ial XM.024441658.1 1.13 1.07 ATPase Nar /K + transporting submit beta 2 ATP Iab XM.024441658.1 1.14 -1.24 ATPase Nar /K + transporting submit beta 2 ATP Iab XM.024349116.1 1.61 -1.04 ATPase Nar /K + transporting submit beta 3 ATP Iab XM.024390584.1 -1.28 -1.28 Camp-dependent protein kinasc calify its submit alpha PEKACAa XM.024390584.1 -1.48 1.21 Casei Kinase L epsilon COLECI XM.024390584.1 -1.48 1.29 -1.66 Carcati 3 CTXN3 XM.02439057.1 1.34 -1.05 Disheredled segment polarity protein DEKLI XM.024430575.1 <t< td=""><td>Arginine vasopressin</td><td>AVP</td><td>XM 024397535.1</td><td>27.90</td><td>-26.48</td></t<>	Arginine vasopressin	AVP	XM 024397535.1	27.90	-26.48
Assnite methyltransferase ASSNT XM_024403019.1 3.13 -3.85 ATDse Na+/K+ transporting subunit alpha 1a ATP 1a1a XM_02441203.4.1 -2.47 -2.89 ATDse Na+/K+ transporting subunit alpha 1c ATP 1a1b XM_02444732.1 1.08 1.34 ATDse Na+/K+ transporting subunit bla 1 ATP 1b1b XM_024444732.1 -1.00 1.11 ATDse Na+/K+ transporting subunit bla 1 ATP 1b1b XM_024441658.1 1.14 1.18 ATDse Na+/K+ transporting subunit bla 2 ATP 1b2b XM_024394116.1 1.04 -1.24 ATDse Na+/K+ transporting subunit bla 2b ATP 1b2b XM_02439688.1 -1.63 1.21 Casen hinase (rapioln CSNK 12 XM_02439058.1 -1.16 1.22 Casen hinase (rapioln CSNK 12 XM_02439057.1 358.79 -1.56 Cortexin 3 Crixin 3 M_02439156.1 -1.44 -1.10 Derlin 1 DERL1 XM_024407576.1 1.04 -1.10 Daskevelice segment polarity protein 2 DV12 XM_02439085.1 -1.14 -1.26	Arginine/serine-rich coiled-coil 1	RSRC1	 XR 002955720.1	1.14	-1.23
ATPase Na+/K+ transporting subunit alpha 1a ATP tala XM_024412034.1 -2.47 -2.69 ATPase Na+/K+ transporting subunit alpha 1b ATP tala XM_02444374.1 1.00 1.11 ATPase Na+/K+ transporting subunit alpha 3 ATP tala XM_02444374.1 1.00 1.11 ATPase Na+/K+ transporting subunit beta 2 ATP tala XM_024443563.1 1.13 1.07 ATPase Na+/K+ transporting subunit beta 20 ATP taba XM_024394118.1 -1.24 -1.27 Camp-dependent protein kinase catalytic subunit beta 20 ATP tabb XM_02437662.1 1.11 -1.28 Caselt kinase 1, epsilon COIKCI2 XM_024390584.1 -1.63 1.21 Caselt kinase 1, epsilon COIKCI2 XM_02439027.1 38.79 -1.56 Cortexin 3 CTXN3 XM_02430854.1 -1.44 -1.10 Defin 1 DERL1 XM_0244084.1 1.16 1.29 Defin 2 DVL2 XM_02430754.1 1.14 -1.26 Defin 3 DEX XM_024408756.1 1.04 -1.10 Defin 4 <t< td=""><td>Arsenite methyltransferase</td><td>AS3MT</td><td>XM 024403019.1</td><td>3.13</td><td>-3.85</td></t<>	Arsenite methyltransferase	AS3MT	XM 024403019.1	3.13	-3.85
ATPase Na+/K+ transporting subunit alpha 1b ATP latb XM_02444374.1. 1.08 1.34 ATPase Na+/K+ transporting subunit alpha 1c ATP latc XM_02444374.1. -1.00 1.11 ATPase Na+/K+ transporting subunit beta 1 ATP lat XM_02444058.1. 1.14 1.18 ATPase Na+/K+ transporting subunit beta 2 ATP lib1 XM_02434011. -1.04 -1.04 ATPase Na+/K+ transporting subunit beta 2 ATP lib2 XM_02434011. -1.12 -1.27 ATPase Na+/K+ transporting subunit beta 3 ATP lib2 XM_02439084.1. -1.04 -1.28 Camp-dependent protein kinase catalytic subunit alpha CRKAC XM_02439082.1. -1.03 1.21 Cascin kinast _ repaidin CSKIce XM_02439082.1. -3.09 1.53 Cotexin 3 CTXN3 XM_02439302.1. 388.79 -1.39 1.33 Cystic fibrosis transmembrane conductance regulator CFTR XM_02439385.1. 1.16 1.29 -1.40 Derlin 1 Derlin 2 DVL2 XM_02439385.1. -1.30 1.35 Claif fordilary acide protein	ATPase Na+/K+ transporting subunit alpha 1a	ATP1a1a	XM 024412034.1	-2.47	-2.69
ATPase Na+/K+ transporting subunit alpha 1 ATP 1a1c XM_024443741.1 -1.00 1.11 ATPase Na+/K+ transporting subunit beta 1 ATP 1a1 XM_024441651.1 1.13 1.07 ATPase Na+/K+ transporting subunit beta 2 ATP 1b2 XM_02444556.1 1.14 1.18 ATPase Na+/K+ transporting subunit beta 20 ATP 1b2 XM_024394118.1 -1.24 -1.27 ATPase Na+/K+ transporting subunit beta 20 ATP 1b2 XM_024394118.1 -1.23 -1.23 Camp-dependent protein kinase catalytic subunit alpha PRKACAa XM_02437052.1 -2.00 1.83 Callectin sub-family member 12 COLECI XM_02439027.1 -3.69 1.63 -1.27 Cartexin 3 CTXN3 XM_02424084.1 1.16 1.29 -1.00 Derlin 1 DERL XM_02442084.1 1.16 -1.02 -1.00 Derlin 2 DV12 XM_02439055.1 1.04 -1.10 Derlin 3 DV12 XM_02438905.1 -1.34 -1.26 SYD domain containing in trasport regulator 6 EYD 50 XM_02438905.1 -1.35	ATPase $Na+/K+$ transporting subunit alpha 1b	ATP1a1b	XM 024443742.1	1.08	1.34
ATPase Na+/K+ transporting subunit bapha 3 ATP1a3 XM_024441658.1 1.13 1.07 ATPase Na+/K+ transporting subunit beta 1 ATP1b1 XM_024441658.1 1.14 1.18 ATPase Na+/K+ transporting subunit beta 2 ATP1b2a XM_024394116.1 1.61 -1.04 ATPase Na+/K+ transporting subunit beta 30 ATP1b2b XM_024396561.1 -1.11 -1.28 Camp-dependent protein kinase calaptic subunit beta 30 ATP1b2b XM_024390581.1 -1.61 -1.27 Casin funge protein kinase calaptic subunit alpha PKRACAa XM_024390581.1 -1.61 -1.28 Collectin sub-family member 12 COLEC12 XM_024393027.1 -356.7 -1.56 Cortexin 3 CTNN3 XM_024393087.1 -1.44 -1.10 Derlin 1 Derlin 2 DV12 XM_024393085.1 1.11 -1.26 Pipdermal growth factor EGR XM_024393085.1 -1.14 -1.26 FyD domain containing ion transport regulator 6 FYD5 XM_024393085.1 -1.34 -1.26 FYD domain containing ion transport regulator 6 FYD5 XM	ATPase $Na+/K+$ transporting subunit alpha 1c	ATP1a1c	XM_024443741_1	-1.00	1.11
ATPase Na+/K+ transporting submit beta 1 ATP ibl XM_024408556.1 1.14 1.18 ATPase Na+/K+ transporting submit beta 2 ATP ib2a XM_024391118.1 -1.24 -1.27 ATPase Na+/K+ transporting submit beta 3b ATP ib2b XM_0243976682.1 -1.11 -1.28 Camp-dependent protein kinase catalytic submit alpha PRKACAA XM_024397621.1 -2.00 1.83 Collectin sub-family member 12 COLEC12 XM_024397621.1 -2.00 1.83 Cortexin 3 CYTXN3 XM_024397621.1 -2.00 1.83 Cortexin 3 CYTXN3 XM_024397621.1 -2.00 1.64 Derlin 1 DERL1 XM_024497561.1 1.04 -1.10 Diskevelled segment polarity protein 2 DVL2 XM_024376942.1 1.25 -1.70 Epidermal growth factor EGF XM_024376942.1 1.28 -1.30 -1.34 -1.26 FXVD domain containing ion transport regulator 6 FXVD 6 XM_024387040.1 1.52 -1.63 Gilal finfulnary acide protein GFAP XM_024437800.1 1.52	ATPase Na+/K+ transporting subunit alpha 3	ATP1a3	XM 024441658.1	1.13	1.07
ATPase Na+/K + transporting subunit beta 2 ATP b2a XM_024394116.1 1.61 -1.04 ATPase Na+/K + transporting subunit beta 2b ATP b2b XM_024394118.1 -1.24 -1.27 ATPase Na+/K + transporting subunit beta 3b ATP b2b XM_024390584.1 -1.68 1.21 Casen it kinase 1, epsilon CSNK1e XM_024390572.1 -2.00 1.83 Collectin sub-family member 12 COLEC12 XM_02439057.1 -3.69 -1.56 Cortexin 3 CTXN3 XM_02439057.1 -1.64 1.11 -1.57 Derlin 1 DERL1 XM_024439062.1 1.14 -1.10 Derlin 1 DERL2 XM_02439062.1 2.55 -1.70 Eis variant 5b EFV 5b XM_02439062.1 2.55 -1.70 Eis variant 5b EFV 5b XM_02439062.1 2.55 -1.70 Eis variant 5b EFV 5b XM_02438905.1 1.85 -1.70 Eis variant 5b EFV 5b XM_02438905.1 1.52 -1.70 Eis variant 5b EFV 5b XM_02438905.1	ATPase $Na+/K+$ transporting subunit beta 1	ATP1b1	XM_024408556.1	1 14	1.18
ATPase Na+/K+ transporting subunit beta 2b ATP b2b XM_024394118.1 -1.24 -1.27 ATPase Na+/K+ transporting subunit beta 3b ATP b2b XM_024390584.1 -1.63 1.21 Camp-dependent protein kinase catalytic subunit alpha PRKACAa XM_024390584.1 -1.63 1.21 Casen kinast , repaion COIECI2 XM_024390584.1 -1.63 1.21 Casen kinast , repaion COIECI2 XM_024390584.1 -1.63 1.29 Cortexin 3 CrtxN3 XM_024391564.1 1.04 -1.10 Derlin 1 DERL1 XM_024397524.1 1.29 -1.90 Epidermal growth factor EGF XM_024390462.1 2.55 -1.70 Es variant 5b EYVD6 XM_024390462.1 2.55 -1.70 Es variant 5b EYVD6 XM_024390462.1 2.55 -1.70 Es variant 5b EYVD6 XM_024390451.1 -1.36 1.35 Gial fibrillary acidic protein GEAP XM_02439300.1 1.52 -1.63 Gowth hormone releasing hormone receptor, like HHNRI XM_024392700.1 1.52	ATPase $Na+/K+$ transporting subunit beta 2a	ATP1b2a	XM_024394116.1	1.61	-1.04
ATPase Na+/K+ transporting subunit beta 3b ATP1b3b XM_024376682.1 1.11 -1.28 Camp-dependent protein kinase catalytic subunit alpha PKKACAA XM_0243990584.1 -1.63 1.21 Casein kinase 1, epsilon COECI2 XM_0243990584.1 -1.63 1.21 Casein kinase 1, epsilon COECI2 XM_024399057.1 358.79 -1.56 Cortexin 3 CTXN3 XM_0243938564.1 -3.49 1.63 Cysic fibrosis transmembrane conductance regulator CFR XM_024405756.1 1.04 -1.10 Derlin 1 DERL1 XM_02449393885.1 1.11 -1.05 Dishevelled segment polarity protein 2 DVL2 XM_024393985.1 -1.34 -1.26 EXYD domain containing ion transport regulator 6 FXYD6 XM_024389695.1 -1.30 -1.35 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_02438700.1 1.52 -1.63 Growth hormone releasing hormone recepton, like GHRHRI XM_0243877.1 -1.23 1.31 Mabaguini, ring finger 1a MGKN1a XM_02443870.1 1.71	ATPase $Na+/K+$ transporting subunit beta 2b	ATP1b2b	XM 0243941181	-1.24	-1.27
International and the second	ATPase $Na+/K+$ transporting subunit beta 3b	ATP1b3b	XM_024376682.1	1.11	-1.28
Camp Appricture Product Nature Callay is a North April FIND FIND FIND Casein Kinas I, epsion CNK1e XM_02433927.11 -2.00 1.83 Collectin sub-family member 12 COLEC12 XM_0243393027.11 358.79 -1.56 Cortexin 3 CTXN3 XM_0243393027.11 358.79 -1.56 Cortexin 3 CTXN3 XM_0243393027.11 1.64 -1.10 Derlin 1 DERL1 XM_0244393858.1 1.11 -1.05 Disherelled segment polarity protein 2 DVL2 XM_0243393858.1 1.13 -1.30 Eis variant 5b ETV5b XM_024339805.1 -1.34 -1.26 EXYD domain containing ion transport regulator 6 FXYD6 XM_024337800.1 1.52 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_0243350.1 -1.30 -1.35 Glaid fordingray acidic protein GEAP XM_0243350.1 -1.22 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_02443350.1 -1.02 -1.02 Heart and neural coretair derivatrivatres	Camp_dependent protein kinase catalytic subunit alpha	PRKACAa	XM_024390584.1	-1.63	1.20
Caskin Anase Frequencin CONKT Xm_024397721.1 =2.000 1.037 Collectin aub framing member 12 COLEC12 XM_024381564.1 =3.49 1.63 Cystic fibrosis transembrane conductance regulator CFTR XM_0244381564.1 =3.49 1.63 Cystic fibrosis transembrane conductance regulator CFTR XM_0244037564.1 1.04 =1.10 Derlin 1 DERL1 XM_02440375764.1 1.29 -1.90 Epidermal growth factor EGF XM_0243976794.1 1.29 -1.90 Epidermal growth factor EGF XM_02449257.1 -1.34 -1.26 EX variant 5b ETV5b XM_024442135.1 -1.30 -1.35 Gial aborital protein GEF XM_02443850.1 1.152 -1.63 Golgi-associtad PDZ and colled-coll motif containing GOPC XM_02443575.1 -1.24 -1.30 Leurine carboy methyltransferase 1 LCMT1 XM_0244357.1 -1.23 1.31 Maboguni, ring finger 1a MGRN1a XM_0244357.1 -1.42 1.30 Numethyltransferase 1	Casein kinase 1 ansilon	CSNK10	XM_024370721.1	-1.05	1.21
Contextin 3 CLUCUL1 XM_02433502.1 -3.49 1.63 Cystic fibrosis transmembrane conductance regulator CTXN3 XM_02431554.1 -3.49 1.63 Cystic fibrosis transmembrane conductance regulator CFTR XM_024405756.1 1.04 -1.10 Derlin 1 DERLI XM_024393851.1 1.11 -1.05 Dishevelled segment polarity protein 2 DVL2 XM_0243976794.1 1.29 -1.90 Epidermal growth factor EGF XM_024389695.1 -1.34 -1.26 SYD domain containing ion transport regulator 6 FYYD 6 XM_02438502.1 18.95 -17.99 Golgi-associated PDZ and coled-coil motif containing GOPC XM_024385676.1 1.37 -1.36 Growth hormone releasing hormone receptor, like GHRHRI XM_02438576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_02438576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_02438576.1 1.20 -1.02 NPA like domain containing 2 NIPAL2 RR_0025212.1 -1.02	Callectin sub family member 12	COLECIA	XM_024373721.1	259 70	1.65
Christi And Christi Arabition -1.49 1.03 Cysic fibrosis transmembrane conductance regulator CFTR XM_0244408756.1 1.04 -1.10 Derlin 1 DERL1 XM_0244085756.1 1.04 -1.10 Derlin 2 DERL2 XM_024309885.1 1.11 -1.05 Eibkevelled segment polarity protein 2 DVL2 XM_02430962.1 2.55 -1.70 Eis variant 5b ETV5b XM_024380695.1 -1.34 -1.26 FXYD domain containing ion transport regulator 6 FXYD6 XM_024438300.1 1.85 -1.79 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_024425320.1 1.895 -1.79 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_024425576.1 .7.13 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_02443576.1 .1.04 1.44 NIPA like domain containing 2 NIPA L2 XR_002438797.1 -1.23 1.31 Maboguin, ring finger 1a MGRN1a XM_02443523.1 1.15 -1.47 PDZ domain containing 1 <td>Contextin 3</td> <td>CTVN2</td> <td>XM_024393027.1</td> <td>330.79</td> <td>-1.50</td>	Contextin 3	CTVN2	XM_024393027.1	330.79	-1.50
Cysic transmensatic conductance regulator CF1 R XM_024424084.1 1.16 1.29 Derlin 1 DERLI XM_0244376756.1 1.04 -1.10 Derlin 2 DERL XM_024376756.1 1.29 -1.90 Epidermal growth factor EG XM_02438965.1 -1.34 -1.26 Ext variant 5b CM_02438965.1 -1.30 -1.35 Glial fibrillary acidic protein GFA XM_024385300.1 18.95 -17.99 Golgi-associated PDZ and colled-coll motif containing GOPC XM_024438576.1 1.37 -1.36 Eavariant formone receptor, like GHRHRI XM_024438576.1 1.37 -1.36 Corowth hormone releasing hormone receptor, like GHRHRI XM_02443576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024438570.1 -1.20 -1.02 Maboguin, ring finger 1a MGRN1a XM_024438570.1 -1.22 -1.02 -1.02 N=nethylpurine DNA glycosylase MPG XM_024403931.1 3.73 5.43 Pivi-like RNA-mediated gene silencing 2 <	Cortexin 5	CIANS	XM_024381564.1	-5.49	1.03
Derkin 1 DERL 1 XM_02440578.1 1.04 -1.10 Dishevelled segment polarity protein 2 DFL2 XM_02439388.1 1.11 -1.05 Dishevelled segment polarity protein 2 DVL2 XM_02439695.1 2.55 -1.70 Explarmal growth factor EGF XM_02439695.1 -1.34 -1.26 EXYD domain containing ion transport regulator 6 FYYD6 XM_02442135.1 -1.30 -1.35 Gial fibrillary acidic protein GEAP XM_02443570.1 1.895 -17.99 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_02443676.1 1.37 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_02443676.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_02443250.1 -1.04 1.44 NIPAL2 XR_002952192.1 -1.02 -1.02 -1.02 N=methylpurine DNA glycosylase MPG XM_02443234.11 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_02443234.11 1.16 -1.44 NPM-tibke	Cystic fibrosis transmembrane conductance regulator	CFIK DEDL1	XM_024424084.1	1.16	1.29
Dernin 2 DERL2 XM_02435885.1 1.11 -1.05 Dishevelide segment polarity protein 2 DVL2 XM_02437674.1 1.29 -1.90 Epidermal growth factor EGF XM_024380695.1 -1.34 -1.26 Ex vrainat 5b ETV 5b XM_02438695.1 -1.30 -1.35 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_02438520.1 18.95 -1.79 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_02438570.1 1.37 -1.36 Growth hormone releasing hormone receptor, like GHRHRI XM_024438773.1 -1.23 1.31 Mahogunin, ring finger 1a MGRN1a XM_024438250.1 -1.04 1.44 NPAL2 RX_002952192.1 -1.02 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_024439251.1 -1.37 -3.54 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024439251.1 -1.02 -1.02 N=nethylpurine DNA glycosylase MPG XM_02443931.1 .1.5 -1.47 PDZ domain containin	Derlin 1	DERLI	XM_024405756.1	1.04	-1.10
Dishevalida segment potarity protein 2 DVL2 NM_0243/6794.1 1.29 -1.90 Explacemal growth factor EGF XM_024390462.1 2.55 -1.70 Ets variant 5b ETV5b XM_024390462.1 2.55 -1.70 Ets variant 5b ETV5b XM_024389205.1 -1.34 -1.26 FXYD domain containing in transport regulator 6 FXYD6 XM_02438520.1 18.95 -17.99 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_02438520.1 -1.63 -1.63 Growth hormone recessing hormone receptor, like GHRHRI XM_024438793.1 -1.23 1.31 Heart and neural crest derivatives expressed 2 HAND2 XM_02438795.1 -1.02 1.34 Mahogunin, ring finger 1 MCN11 XM_02433250.1 -1.04 1.44 NIPA like domain containing 1 PDZKI XM_02443243.1 1.15 -1.47 PDZ domain containing 1 PDZKI XM_02443243.1 1.15 -1.42 PD2 domain containing 1 PDZKI XM_02447505.1 -1.22 1.35 Ret	Derlin 2	DERL2	XM_024393885.1	1.11	-1.05
Epicermai growth factor EAP XM_024390462.1 2.53 -1.70 Ets variant 5b ETV5b XM_024390695.1 -1.34 -1.26 FXYD domain containing ion transport regulator 6 FYYD6 XM_024389695.1 -1.30 -1.35 Glial fibrillary acidic protein GFAP XM_024385320.1 18.95 -17.99 Golgi-associated PDZ and colled-coil motif containing GOPC XM_024378000.1 1.52 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_02438576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_02433570.1 -1.02 1.31 Mabogunin, ring finger 1a MGRN1a XM_02433243.1 1.15 -1.02 N-methylpurine DNA glycosylase MPG XM_024433243.1 1.15 -1.47 DPZ domain containing 1 PDVXL XM_024439391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_02443751.1 -1.42 1.30 Retinoschisin 1a RS1a XM_02442537.1 -4.34 4.69	Dishevelled segment polarity protein 2	DVL2	XM_024376794.1	1.29	-1.90
Hs variant 5b EV 5b XM_02438955.1 -1.34 -1.26 FXYD domain containing ion transport regulator 6 FXYD 6 XM_024482135.1 -1.30 -1.35 Gilal fibrillary acidic protein GFAP XM_024385320.1 18.95 -17.99 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_024428575.1 -2.19 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_024438576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024433250.1 -1.04 1.44 NIPAL XR_024332320.1 -1.02 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_024438243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_02443520.1 -1.42 1.30 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_02443750.1 -1.26 2.46 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_0244387965.1 -1.42 1.30 Retinoschisin 1a RS1a XM_0244387965.1 -1.22 1.35 Rh 1.55 1.15 -1.16	Epidermal growth factor	EGF	XM_024390462.1	2.55	-1.70
FXTD domain containing ion transport regulator 6 FXTD6 XM_02442155.1 -1.30 -1.35 Glial fibrillary acidic protein GFAP XM_024385320.1 18.95 -17.99 Glogia-associated PDZ and coiled-coil motif containing GOPC XM_024378000.1 1.52 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_024436576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024435270.1 -1.04 1.44 Mahogunin, ring finger 1a MGRN1a XM_024433230.1 -1.04 1.44 NIPA like domain containing 2 NIPAL2 XR_0024323243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_02443231.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024420391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_02442781.1 -1.42 1.30 Retinoschisin 1a R51a XM_0244257.1 -4.34 4.69 Rh associated glycoprotein (gene/pseudogene) RHAG XM_024437707.1 1.23 -1.15 <td>Ets variant 5b</td> <td>ETV5b</td> <td>XM_024389695.1</td> <td>-1.34</td> <td>-1.26</td>	Ets variant 5b	ETV5b	XM_024389695.1	-1.34	-1.26
Glain Ibrillary acidic protein GRAP XM_02438530.1 18.95 -1/.99 Golgi-associated PDZ and coiled-coil motif containing GOPC XM_02437800.1 1.52 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_024435576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024435250.1 -1.23 1.31 Mahogunin, ring finger 1a MGRN1a XM_024433250.1 -1.04 1.44 NIPAL XR_00295192.1 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_02443323.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_02449391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_02449301.1 -1.42 1.30 Retinoschisin 1a RS1a XM_02443257.1 -4.34 4.69 Rh associated glycoprotein (gene/pseudogene) RHBG XM_024397707.1 1.23 -1.15 Rh associated glycoprotein (gene/pseudogene) RHBG XM_02439755.1 -1.09 1.01 Ring finger protein	FXYD domain containing ion transport regulator 6	FXYD6	XM_024442135.1	-1.30	-1.35
Golgi-associated PD2 and colled-coil motif containing GOPC XM_024432800.1 1.52 -1.63 Growth hormone releasing hormone receptor, like GHRHRI XM_024432521.1 -2.19 -1.39 Heart and neural crest derivatives expressed 2 HAND2 XM_024436576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024433250.1 -1.04 1.44 NIPALike domain containing 2 NIPAL2 XR_002433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024439243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_02443931.1 -1.26 2.46 Potasium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_02443518.1 -1.42 1.30 Retinoschisin 1a RS1a XM_024435965.1 -1.42 1.30 Retinoschisin 1a RS1a XM_024435965.1 -1.22 1.35 Rh associated glycoprotein (gene/pseudogene) RHBG XM_024435965.1 -1.22 1.35 Rhomboid 5 homolog 1a RHBG XM_024435965.1 -1.19 1.06	Glial fibrillary acidic protein	GFAP	XM_024385320.1	18.95	-17.99
Growth hormone receptor, like GHRHRI XM_02442521.1 -2.19 -1.39 Heart and neural crest derivatives expressed 2 HAND2 XM_024436576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024387973.1 -1.23 1.31 Mahogunin, ring finger 1a MGRN1a XM_024433250.1 -1.04 1.44 NIPA like domain containing 2 NIPAL2 XR_002952192.1 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_024433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024409391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024445818.1 -1.42 1.30 Retinoschisin 1a RS1a XM_02442637.1 -4.34 4.69 Rh associated glycoprotein RHAG XM_02442637.1 -1.22 1.35 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024437707.1 1.23 -1.15 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024387965.1 -1.09 1.01 <t< td=""><td>Golgi-associated PDZ and coiled-coil motif containing</td><td>GOPC</td><td>XM_024378000.1</td><td>1.52</td><td>-1.63</td></t<>	Golgi-associated PDZ and coiled-coil motif containing	GOPC	XM_024378000.1	1.52	-1.63
Heart and neural crest derivatives expressed 2 HAND2 XM_024436576.1 1.37 -1.36 Leucine carboxyl methyltransferase 1 LCMT1 XM_024436576.1 -1.23 1.31 Mahogunin, ring finger 1a MGRN1a XM_024433250.1 -1.04 1.44 NIPA like domain containing 2 NIPAL2 XR_002952192.1 -1.02 -1.02 -1.07 PDZ domain containing 1 PDZK1 XM_024433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024445818.1 -1.42 1.30 Retinoschisin 1a RS1a XM_024445818.1 -1.42 1.30 Retinoschisin 1a RS1a XM_02442537.1 -4.34 4.69 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_02443515.1 -1.22 1.35 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_02438765.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024388934.1 1.52 1.52 Solute carrier family 1 member 14 SLC6a14 XM_02443892.1 -2.06 2.03 Solute carrier family 10 member 13 SLC12a2 XM_024388934.1 1.52 <t< td=""><td>Growth hormone releasing hormone receptor, like</td><td>GHRHRI</td><td>XM_024422521.1</td><td>-2.19</td><td>-1.39</td></t<>	Growth hormone releasing hormone receptor, like	GHRHRI	XM_024422521.1	-2.19	-1.39
Leucine carboxyl methyltransferase 1 LCMT1 XM_024387973.1 -1.23 1.31 Mahogunin, ring finger 1a MGRN1a XM_024433250.1 -1.04 1.44 NIPA like domain containing 2 NIPAL2 XR_002952192.1 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_024433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024403931.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024445818.1 -1.42 1.30 Retinoschisin 1a RS1a XM_024422637.1 -4.34 4.69 Rh associated glycoprotein RHAG XM_024437515.1 -1.22 1.35 Rhomboid 5 homolog 1a RHBG XM_02437707.1 1.23 -1.16 Ring finger protein 5 RNF5 XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_02438941.1 1.52 1.52 Solute carrier family 1 member 2b SIC1a2b XM_024489894.1 1.52 1.52 Solute carrier family 1 member 14 SIC6a14	Heart and neural crest derivatives expressed 2	HAND2	XM_024436576.1	1.37	-1.36
Mahogunin, ring finger 1a MGRN1a KM_202433250.1 -1.04 1.44 NIPA like domain containing 2 NIPAL2 XR_002952192.1 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_024433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024409391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024417801.1 -1.26 2.46 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_024422637.1 -4.34 4.69 Rt associated glycoprotein RHAG XM_024437515.1 -1.22 1.35 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024437515.1 -1.15 -1.14 Scinte carrier family 1 member 16 SCARF1 XM_024488934.1 1.52 1.52 Solute carrier family 0 member 14 SLC6a14 XM_0244388934.1 1.52 1.52 <td>Leucine carboxyl methyltransferase 1</td> <td>LCMT1</td> <td>XM_024387973.1</td> <td>-1.23</td> <td>1.31</td>	Leucine carboxyl methyltransferase 1	LCMT1	XM_024387973.1	-1.23	1.31
NIPA like domain containing 2 NIPAL2 XR_002952192.1 -1.02 -1.02 N-methylpurine DNA glycosylase MPG XM_024433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_02440391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024417801.1 -1.26 2.46 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_0244257.1 -4.34 4.69 Rt hassociated glycoprotein RHAG XM_02437707.1 1.23 -1.15 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_02437707.1 1.23 -1.16 Ring finger protein 5 RNF5 XM_02437707.1 1.15 -1.06 Scavenger receptor class F, member 1 SCARF1 XM_0243796104.1 1.15 -1.06 Scavenger receptor class F, member 14 SCARF1 XM_02443921.1 -2.06 2.03 Solute carrier family 1 member 2b SLC1a2b XM_02443921.1 -2.06 2.03 Solute carrier family 9 member 3 SLC9a3 XM_0244396240.1 -2.00 -2.22	Mahogunin, ring finger 1a	MGRN1a	XM_024433250.1	-1.04	1.44
N-methylpurine DNA glycosylase MPG XM_024433243.1 1.15 -1.47 PDZ domain containing 1 PDZK1 XM_024409391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024417801.1 -1.26 2.46 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_024445818.1 -1.42 1.30 Retinoschisin 1a RS1a XM_024422637.1 -4.34 4.69 Rh associated glycoprotein (gene/pseudogene) RHAG XM_024377707.1 1.23 -1.15 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024396104.1 1.15 -1.16 Scavenger receptor class F, member 1 SCARF1 XM_02444509.1 -1.15 -1.14 Serine threonine kinase 39 STK39 XM_024448931.1 1.52 1.52 Solute carrier family 1 member 2 SLC9a3 XM_024449802.1 -2.06 2.03 Solute carrier family 12 member 3 SLC12a2 XM_02448080.1 -2.20 -2.22 <td>NIPA like domain containing 2</td> <td>NIPAL2</td> <td>XR_002952192.1</td> <td>-1.02</td> <td>-1.02</td>	NIPA like domain containing 2	NIPAL2	XR_002952192.1	-1.02	-1.02
PDZ domain containing 1 PDZK1 XM_024409391.1 3.73 5.43 Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024417801.1 -1.26 2.46 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_024422637.1 -4.34 4.69 Retinoschisin 1a RS1a XM_02432637.1 -4.34 4.69 Rh associated glycoprotein (gene/pseudogene) RHAG XM_02437707.1 1.23 -1.15 Rh mily, B glycoprotein (gene/pseudogene) RHBG XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024387965.1 -1.09 1.01 Scavenger receptor class F, member 1 SCARF1 XM_02438934.1 1.52 1.52 Solute carrier family 1 member 2b SLC1a2b XM_02443892.1 -2.06 2.03 Solute carrier family 9 member 3 SLC1a2b XM_02443862.1 -2.00 -2.22 Solute carrier family 12 member 2a SLC12a2a XM_02443862.1 -2.00 -2.22 Solute carrier family 12 member 2a SLC12a2b XM_02444805.1 1.28 1.56 Solute carrier family 12 member 2a SLC12a2b XM	N-methylpurine DNA glycosylase	MPG	XM_024433243.1	1.15	-1.47
Piwi-like RNA-mediated gene silencing 2 PIWIL2 XM_024417801.1 -1.26 2.46 Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_024422637.1 -4.34 4.69 Retinoschisin 1a RS1a XM_024422637.1 -4.34 4.69 Rh associated glycoprotein (gene/pseudogene) RHAG XM_024377707.1 1.23 -1.15 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_02443091.4 1.15 -1.06 Scavenger receptor class F, member 1 SCARF1 XM_024445009.1 -1.15 -1.14 Serine threonine kinase 39 STK39 XM_024413392.1 -2.06 2.03 Solute carrier family 1 member 2b SLC1a2b XM_024449802.1 -2.00 -2.22 Solute carrier family 9 member 3 SLC29a3 XM_02444802.1 -0.02 -2.22 Solute carrier family 12 member 2a SLC12a2b XM_02444802.1 7.02 4.87 Solute carrier family 12 member 2a SLC12a1 XM_024440802.1 1.28 1.56 Solute carrier family 12 member 2a SLC12a2b	PDZ domain containing 1	PDZK1	XM_024409391.1	3.73	5.43
Potassium inwardly-rectifying channel, subfamily J, member 1b KCNJ1b XM_024445818.1 -1.42 1.30 Retinoschisin 1a RS1a XM_024422637.1 -4.34 4.69 Rh associated glycoprotein RHAG XM_024377707.1 1.23 -1.15 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_02437515.1 -1.22 1.35 Rhomboid 5 homolog 1a RHBG XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_02445009.1 -1.15 -1.14 Scavenger receptor class F, member 1 SCARF1 XM_024485009.1 -1.15 -1.14 Serine threonine kinase 39 STK39 XM_0244338934.1 1.52 1.52 Solute carrier family 1 member 2b SLC1a2b XM_024427745.1 2.53 -2.25 Solute carrier family 9 member 3 SLC9a3 XM_02440802.1 -0.02 -2.22 Solute carrier family 12 member 1a SLC12a2b XM_02440802.1 7.02 4.87 Solute carrier family 12 member 2b SLC12a2b XM_02441805.1 1.28 1.56	Piwi-like RNA-mediated gene silencing 2	PIWIL2	XM_024417801.1	-1.26	2.46
Retinoschisin 1a RS1a XM_024422637.1 -4.34 4.69 Rh associated glycoprotein RHAG XM_024377707.1 1.23 -1.15 Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024377515.1 -1.22 1.35 Rhomboid 5 homolog 1a RHBG XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024386936.1 1.15 -1.16 Scavenger receptor class F, member 1 SCARF1 XM_024388934.1 1.52 1.52 Solute carrier family 1 member 2b SLC1a2b XM_024413392.1 -2.06 2.03 Solute carrier family 9 member 3 SLC6a14 XM_024427745.1 2.53 -2.25 Solute carrier family 1 member 1 SLC12a1 XM_02441392.1 -2.00 -2.22 Solute carrier family 1 member 3 SLC12a1 XM_02441805.1 1.28 1.56 Solute carrier family 12 member 1 SLC12a2b XM_02441805.1 1.28 1.56 Solute carrier family 12 member 2a SLC12a2b XM_024417016.1 1.27 -1.34 Transient recep	Potassium inwardly-rectifying channel, subfamily J, member 1b	KCNJ1b	XM_024445818.1	-1.42	1.30
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Rh family, B glycoprotein (gene/pseudogene) RHBG XM_024437515.1 -1.22 1.35 Rhomboid 5 homolog 1a RHBDF1 XM_024387965.1 -1.09 1.01 Ring finger protein 5 RNF5 XM_024387965.1 -1.09 1.01 Scavenger receptor class F, member 1 SCARF1 XM_02445009.1 -1.15 -1.14 Serine threonine kinase 39 STK39 XM_024438934.1 1.52 1.52 Solute carrier family 1 member 2b SLC1a2b XM_024413392.1 -2.06 2.03 Solute carrier family 6 member 14 SLC6a14 XM_024396240.1 -2.00 -2.22 Solute carrier family 12 member 3 SLC12a1 XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2a SLC12a2b XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC14a2 XM_024417109.1 1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_024417109.1 -1.09 1.07	Rh associated glycoprotein	RHAG	XM_024377707.1	1.23	-1.15
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Scavenger receptor class F, member 1 SCARF1 XM_024445009.1 -1.15 -1.14 Serine threonine kinase 39 STK39 XM_024388934.1 1.52 1.52 Solute carrier family 1 member 2b SLC1a2b XM_024413392.1 -2.06 2.03 Solute carrier family 6 member 14 SLC6a14 XM_024427745.1 2.53 -2.25 Solute carrier family 9 member 3 SLC9a3 XM_024396240.1 -2.00 -2.22 Solute carrier family 12 member 1 SLC12a1 XM_02440802.1 7.02 4.87 Solute carrier family 12 member 2a SLC12a2a XM_024418405.1 1.28 1.56 Solute carrier family 12 member 2b SLC14a2 XM_024417109.1 1.09 1.07 Solute carrier family 14 member 2 SLC14a2 XM_024417109.1 -1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_024381824.1 1.08 -1.25 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Ring finger protein 5	RNF5	XM_024396104.1	1.15	-1.06
Serine threonine kinase 39 STK39 XM_024388934.1 1.52 1.52 Solute carrier family 1 member 2b SLC1a2b XM_024413392.1 -2.06 2.03 Solute carrier family 6 member 14 SLC6a14 XM_024427745.1 2.53 -2.25 Solute carrier family 9 member 3 SLC9a3 XM_024396240.1 -2.00 -2.22 Solute carrier family 12 member 1 SLC12a1 XM_02440802.1 7.02 4.87 Solute carrier family 12 member 2a SLC12a2a XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC14a2 XM_024417109.1 -1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_02440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Scavenger receptor class F, member 1	SCARF1	XM_024445009.1	-1.15	-1.14
Solute carrier family 1 member 2b SLC1a2b XM_024413392.1 -2.06 2.03 Solute carrier family 6 member 14 SLC6a14 XM_024427745.1 2.53 -2.25 Solute carrier family 9 member 3 SLC9a3 XM_024396240.1 -2.00 -2.22 Solute carrier family 12 member 1 SLC1a1 XM_02440802.1 7.02 4.87 Solute carrier family 12 member 2a SLC12a2a XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC14a2 XM_024417109.1 1.09 1.07 Transient receptor potential cation channel, subfamily V, member 4 TRPV4 XM_02440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Serine threonine kinase 39	STK39	XM_024388934.1	1.52	1.52
Solute carrier family 6 member 14 SLC6a14 XM_024427745.1 2.53 -2.25 Solute carrier family 9 member 3 SLC9a3 XM_024396240.1 -2.00 -2.22 Solute carrier family 12 member 1 SLC12a1 XM_02440802.1 7.02 4.87 Solute carrier family 12 member 2a SLC12a2 XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC14a2 XM_024417109.1 1.09 1.07 Transient receptor potential cation channel, subfamily V, member 4 TRPV4 XM_024410227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Solute carrier family 1 member 2b	SLC1a2b	XM_024413392.1	-2.06	2.03
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Solute carrier family 12 member 1 SLC12a1 XM_024440802.1 7.02 4.87 Solute carrier family 12 member 2a SLC12a2a XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC12a2b XM_024417716.1 1.27 -1.34 Transient receptor potential cation channel, subfamily V, member 4 TRPV4 XM_024417109.1 -1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_02440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Solute carrier family 9 member 3	SLC9a3	XM_024396240.1	-2.00	-2.22
Solute carrier family 12 member 2a SLC12a2a XM_024381566.1 1.48 1.26 Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC14a2 XM_024417716.1 1.27 -1.34 Transient receptor potential cation channel, subfamily V, member 4 TRPV4 XM_024417109.1 -1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_02440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Solute carrier family 12 member 1	SLC12a1	XM_024440802.1	7.02	4.87
Solute carrier family 12 member 2b SLC12a2b XM_024418405.1 1.28 1.56 Solute carrier family 14 member 2 SLC14a2 XM_024417716.1 1.27 -1.34 Transient receptor potential cation channel, subfamily V, member 4 TRPV4 XM_024417109.1 -1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_024440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25	Solute carrier family 12 member 2a	SLC12a2a	XM_024381566.1	1.48	1.26
Solute carrier family 14 member 2 SLC14a2 XM_024417716.1 1.27 -1.34 Transient receptor potential cation channel, subfamily V, member 4 TRPV4 XM_024417109.1 -1.09 1.07 Ubiquitin specific peptidase 10 USP10 XM_024440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25 Continued V V V V V V	Solute carrier family 12 member 2b	SLC12a2b	XM_024418405.1	1.28	1.56
Transient receptor potential cation channel, subfamily V, member 4TRPV4XM_024417109.1-1.091.07Ubiquitin specific peptidase 10USP10XM_024440227.1-1.561.82Valosin containing proteinVCPXM_024381824.11.08-1.25Continued	Solute carrier family 14 member 2	SLC14a2	XM_024417716.1	1.27	-1.34
Ubiquitin specific peptidase 10 USP10 XM_024440227.1 -1.56 1.82 Valosin containing protein VCP XM_024381824.1 1.08 -1.25 Continued VCP	Transient receptor potential cation channel, subfamily V, member 4	TRPV4	XM_024417109.1	-1.09	1.07
Valosin containing protein VCP XM_024381824.1 1.08 -1.25 Continued	Ubiquitin specific peptidase 10	USP10	XM_024440227.1	-1.56	1.82
Continued	Valosin containing protein	VCP	XM_024381824.1	1.08	-1.25
	Continued				

			Fold change	
Gene (Danio rerio)	Symbol	Accession # (O. tshawytscha)	50% vs 0%	100% vs 50%
WNK lysine deficient protein kinase 1b	WNK1b	XM_024425441.1	-1.09	1.10
WNK lysine deficient protein kinase 3	WNK3	XM_024384949.1	1.02	1.25
WNK lysine deficient protein kinase 4	WNK4	XM_024388112.1	-1.28	1.55
linked Kx blood group (mcleod syndrome)	XK	XM_024409767.1	-1.84	1.79

Table 2. List of proteins interacting with transmembrane proteins present in the STRING database and differences in expression between each group.

mitochondria-rich cells (MRC) either uptake or secrete sodium ions. Hence, if the amount of sodium ion uptake decreases as the result of salinity increase, the amount of secretion will decrease as well. As a result, it is assumed that gene expression of ATP1a1a, a predominant form in freshwater fish, decreased with salinity increase.

In addition, it has been reported that rather than proton-pumping ATPase, a pump-mediated protein, NHE3 was activated in a yolk-sac membrane to balance hydrogen in the freshwater environment⁴². When they were transferred from freshwater to brackish water, the gill of chum salmon fry expressed more CFTR than when transferred from brackish water to seawater. That is, CFTR was greatly expressed when chum salmon fry were first exposed to salt stress. A similar pattern was observed in the gill of *F. heteroclitus*. It is known that Cl-secretion of CFTR in epithelial cells is controlled by adjusting the number of CFTR channels. This is known to be the result of the activation of the protein kinase A (PKA) caused by cAMP. Aquaporin, a channel-mediated protein, which is characterized by passive diffusion, was involved in the central nervous system (CNS) in cases of AQP4, AQP8 and AQP9 used in this study⁴⁴. On the basis of functional features, AQP4 and AQP8 are classified as water-permeable aquaporin and AQP9 as aquaglyceroporin or permeable to water, glycerol and urea and AQP8 as permeable to water and urea⁴⁵. Additionally, AQP8 and AQP9 are known to have ammonia transport capabilities⁴⁶.

In this study, in the gill of chum salmon fry, AQP8 and AQP9 were expressed to a great extent when the fish were transferred from freshwater to brackish water. According to the findings of the studies of AQPs in *F. heteroclitus, Lateolabrax maculatus, O. nerka* and *S. salar*, AQP8 and AQP9 are rarely expressed in gills under the normal condition. The performance of qRT-PCR confirmed that the same was true for chum salmon fry in which AQP8 and AQP9 were hardly expressed in the gill of chum salmon fry in freshwater^{47–50}. However, gene expression of AQP8 and AQP9 in the gill of chum salmon fry sharply increased with salinity increase. Likewise, gene expression of the AQP8 and AQP9 increased in the intestine of *A. japonica* and *O. nerka* in seawater in previous studies^{47,51}. However, what underlies the expression pattern has not been clearly found so far. There are two possibilities: one that AQP8 and AQP9 would be expressed to secret ammonium and the other that the sudden movement of water molecules would cause the expression increase for the ion balance in and out of the body. As the salinity increases, the concentration of ammonium increases simultaneously, causing the secretion of ammonium in the body of the fish. In the process, the gill is reported to be involved in the secretion^{52,53}.

Gene expression of AQP4 decreased with salinity increase and then increased again to control the cell-volume. This was observed in the case of transient receptor potential cation channel subfamily V member (TRPV4) which was present in the same interaction network as AQP4. However, the fold change value of TRPV4 was much lower than that of AQP4⁵⁴. In previous research, AQP4 was expressed the most in the gill tissue of *Eptatretus burgeri* in the process of seawater adaptation, confirming the understanding that water transport is facilitated by an osmotic gradient in the gill⁵⁵. That was in line with the findings of the current study. Referring to the PPI network analysis, transmembrane proteins were divided into five groups: NKA subunit isoforms group, AQP group, NKCC, CFTR and NHE3 group. The interaction between the groups can be seen on the map. NKCC1 and NKCC2 expressed in the gill of chum salmon fry were in the interaction network in which lysine deficient protein kinase (WNK), a chloride ion protein, was present. Also, both WNK3 which was 'with no lysine' family of serine-threonine protein kinase and NKCC1 which was in the same interaction network as STK39 (=SPAK) had the same expression pattern in chum salmon fry in the case of salinity increase. This was a similar tendency as seen in the WNK signaling pathway. Among NKA subunit isoforms, ATP1a1a had a similar expression pattern with FXYD6 which was a small membrane protein affecting gene expression of NKA alpha and beta complex. This agrees with the findings of a study on rats that discovered that FXYD6 was co-localized with NKA and FXYD6 bordered epithelial cells⁵⁶. In addition to this, further research on the interaction network of various membrane proteins and analyses of gene expression patterns are expected to provide valuable information to research on functions of osmoregulatory proteins of fish.

Of special note is that while most of the fishes studied in other relevant research were in the smolt or the adult stage, the chum salmon used in this study were in the fry stage. Interestingly, the chum salmon fry showed a similar expression tendency to other fishes in the smolt or the adult stage although the expression of some essential membrane protein genes NKA, NKCC, NHE3, CFTR, and AQP which are involved in osmotic pressure control showed different patterns. The findings of this study confirm that chum salmon have excellent seawater adaptability early on, even in their fry stage. In addition, the comparative analysis of the gene expression patterns of the freshwater to brackish water group and the brackish water to seawater group indicates that 66% of the genes analyzed showed different expression patterns in both groups. In this respect, the types and the expression trends of various genes involved in balancing the body with a rapid increase in various ions were revealed.

This study was conducted on chum salmon, the species with the best osmotic control among salmonid fishes. It is expected that investigating the membrane proteins expressed in the gill of *O. keta*, which has a seawater





acclimation ability even in the fry stage and studying the interaction network of membrane proteins and other various genes will contribute to future research on the osmoregulation pathway.

Materials and Methods

Ethics statement. All the experimental procedures with all the fish were performed and approved according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Gangneung-Wonju National University (GWNU-2019-21). Furthermore, all the authors of this study have completed Animal Welfare & Ethics Course certification under the CITI program, research ethics and compliance training program.

Salinity challenge and sample collection. The chum salmon at the alevin stage, one month after hatching, were transferred from the Korea Fisheries Resources Agency (FIRA) to the laboratory and reared in tanks with re-circulating freshwater for two months. Water temperature was maintained at 12 ± 1 °C. Aeration was provided continuously to maintain dissolved oxygen levels at 9.0 ± 0.5 mg/L. Fish were fed daily with commercial pellets and blood worms and fasted for 24 hours prior to the experiment.

In order to establish a stable seawater acclimation method, preliminary experiments were conducted by varying the salt concentration and the acclimation period. When the chum salmon fry were transferred from freshwater to seawater, most of the fry died within 20 days after the transfer. However, most of the chum salmon fry adapting to brackish water prior to moving to seawater survived approximately 20 days after the transfer. Based on these experiments, a methodology was established in which the individuals showed high survival rates within a short seawater acclimation period. No mortality was observed in the experimental group during salinity exposure. For the hyperosmotic challenge, fish (fry, average body weight and length = 0.6 ± 0.12 g and 4.87 ± 0.23 cm) were directly transferred to brackish water (50% seawater;17.5 ppt) for one day and acclimated in 100% seawater (35 ppt) for one day. Sampling (N = 5 in each group) was conducted at the same time points for the challenge. The 300 ppm of 2-phenoxyethanol (Sigma Aldrich Co, St. Louis, USA) was used as an anesthetic for sampling and gill tissues were extracted and stored at -80 °C.

Library construction and illumina sequencing. Total RNA was extracted from the gill using RNAiso Reagent (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. Further purification was preceded by RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) and RNase-free DNase set (Qiagen). The RNA-seq libraries were constructed using the Truseq stranded mRNA prep kit (Illumina, San Diego, Calif., USA). They were sequenced with a 2×101 bp (paired-end) read module using the Illumina Hiseq. 2500 platform. The raw sequencing files were generated using the Illumina base-calling software (CASAVA v1.8.2 with ASCII Q-score offset 33). Read-through adapter sequences, low-quality sequences (limit = 0.05), ambiguous nucleotides (maximal 2 nucleotides) were removed using CLC Genomics Workbench 11.0 (CLC Bio, QIAGEN) (Table S2).

Genome mapping and *de-novo* **assembly of unmapped reads.** A schematic representation of the RNA-seq reference reconstitution and analysis pipeline is shown in Fig. 6. The chum salmon used in this study had no genome assembly information available as a reference. However, there was genome assembly information for chinook salmon (*O. tshawytscha*, GenBank assembly accession: GCF_002872995.1), which is taxonomically close to chum salmon among *Oncorhynchus* spp⁵⁷. Trimmed sequences of each group were mapped to the chinook salmon reference. In addition, unmapped reads in the genome sequence of chinook salmon were *de-novo* assembled using CLC Genomics Workbench 11.0 under conditions of Kmer size 45, bubble size 50 and minimum contig length of 150 bp. Therefore, a new reference was constructed by combining both *de-novo* assembly data and the reads mapped to the reference genome. Finally, the annotation process was performed by mapping the trimmed readings to the newly constructed reference. The mapping was set under the following conditions: mismatch cost = 2, insertion cost = 3, length fraction = 0.5, similarity fraction = 0.8, Auto-detect paired distances.

Annotation of reconstructed reference sequences and differential expressed genes analysis. The mapping of the reads was performed with BLASTx-based annotation using BLAST2GO PRO v 5.2.5. BLASTx homology searches were carried after dynamic translation against NCBI non-redundant protein sequences (NR) database using the default cut-off parameters of E-value, 1.0e-3 and the word size of BLAST parameters of 3. In addition to the functional annotation, InterProScan v 5.34–73.0⁵⁸, Gene ontology (GO; http:// geneontology.org), GO-Slim, EggNOG v 4.5.1⁵⁹ were used and the results were merged with the BLASTx annotation. The read counts that were mapped to the reconstructed reference were normalized to reads per kilobase of transcript per million mapped reads (RPKM) as the expression values. The false discovery rate (FDR) p-value less than 0.05 was used as a statistical value for differential expressed genes (DEG) screening and classified it into four groups of up-regulation (FC \geq 1.5), moderately up-regulation (1 < FC < 1.5), down-regulation (FC \leq -1.5) and moderately down-regulation (-1.5 < FC < -1) based on fold change (FC), respectively.

Investigation and analysis of protein-protein interaction (PPI) networks. To investigate the expression of the membrane protein genes, the types of the proteins related to the network of the interaction among the membrane protein genes, analyses were conducted based on zebra fish (*Danio rerio*) database in STRING v11.0 (https://string-db.org/) on the condition of minimum required interaction score >0.5 and active interaction sources were as follows: Textmining, Experiments, Databases, Co-expression, Neighborhood, Gene Fusion, and Co-occurrence. Of the representative osmoregulatory membrane proteins, ATPase transporters (ATP1a1a, ATP1a1b, ATP1a3, ATP1b1, CFTR), symporters (SLC12a1, SLC12a2), antiporter (SLC9a3) and passive transporters (AQP4, AQP8, AQP9) were selected to be analyzed, and the expression of the various protein genes known to be related to the interaction network was investigated. Finally, an interaction networks map was completed on the multiple proteins used in the analyses and visualized with Cytoscape v3.7.1 software⁶⁰.

RNA-seq data validation by quantitative real-time RT-PCR (qRT-PCR). The qRT-PCR was performed to verify expression patterns of differential expression genes in representative membrane protein genes of ionocytes as a result of RNA-Seq data. For qRT-PCR validation, normalization of the total RNA concentration between groups was performed, and cDNA was synthesized using PrimeScript RT reagent kit (Takara) with random primer and oligo-dTs. Primer design for qRT-PCR validation was based on the trimmed reads of chum salmon transcriptome sequencing. Specific primer pairs for membrane protein genes, including elongation factor 1 alpha as a qRT-PCR reference gene⁶¹ were constructed based on RNA-seq results (Table S3). qRT-PCR



Figure 6. Schematic diagram of the transcriptome construction and the pipeline for annotation.



was conducted using a Thermal Cycler Dice TM real-time PCR system (Takara) and SYBR premix Ex TaqII Kit (Takara). The qRT-PCR was carried out in triplicate on each sample. The thermal cycling was performed as follows: denaturation at 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, and annealing at 60 °C for 30 s. qRT-PCR results were expressed as mean \pm standard error (SEM) and performed with one-way ANOVA with significant level p < 0.05 using SPSS 25.0 software.

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Author contributions

The preparation of fish sample (including critical care of fishes), gene-expression analyses, and data analysis and manuscript draft were conducted by S.Y.L.; H.J.L. helped the quantitative real-time PCR experiment and improved the manuscript; Y.K.K. developed and supervised the experiment, and modified the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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