



## Transcriptomic profiles of Japanese medaka (*Oryzias latipes*) in response to alkalinity stress

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**ABSTRACT.** *Oryzias latipes* (Adrianichthyidae), known as Japanese medaka or Japanese killifish, is a small 2-4 cm long fish common in rice paddies in coastal Southeast Asia and is also a popular aquarium fish. It has been widely used as a research model because of its small size and because it is very easy to rear. Alkalinity stress is considered to be one of the major stressors on fish in saline-alkaline water. As very little is known about molecular genetic responses of aquatic organisms to alkalinity stress, we examined genome-wide gene expression profiles of Japanese medaka in response to carbonate alkalinity stress. Adult fish were exposed to freshwater and high carbonate alkaline water in the laboratory. We designed a microarray containing 26,429 genes for measuring gene expression change in the gills of the fish exposed to high carbonate alkalinity stress. Among these genes, 512 were up-regulated and 501 were down-regulated in the gills. These differentially expressed genes can be divided into gene groups using gene ontology, including biological processes, cellular components and molecular function. These gene

groups are related to acid-base and ion regulation, cellular stress response, metabolism, immune response, and reproduction processes. Biological pathways including amino sugar and nucleotide sugar metabolism, porphyrin and chlorophyll metabolism, metabolism of xenobiotics by cytochrome P450, drug metabolism, aminoacyl-tRNA biosynthesis, glycine, serine and threonine metabolism, ascorbate and aldarate metabolism, pentose and glucuronate interconversions, glutathione metabolism, and fructose and mannose metabolism were significantly up-regulated. Alkalinity stress stimulates the energy and ion regulation pathways, and it also slows down the pathways related to the immune system and reproduction.

**Key words:** *Oryzias latipes*; Alkalinity stress; Transcriptomics; Microarray

## INTRODUCTION

Carbonate alkalinity stress is considered to be one of the major stressors on fishes in saline-alkali water. Carbonate alkalinity stress on fishes is related to acid-base regulation (Perry and Gilmour, 2006) and excretion of carbon dioxide (Randall and Tsui, 2006), and is dependent on ionic regulation and osmoregulation in fishes as well (Goss et al., 1994). Major problems for fishes living in alkaline water are that high pH may inhibit ammonia excretion, and increase CO<sub>2</sub> excretion (Parra and Baldisserotto, 2007). A number of studies have reported that high carbonate alkalinity induces mixed respiratory and metabolic alkalosis in freshwater fish (Wilkie and Wood, 1996).

In an extreme natural alkaline environment, some fishes have developed special strategies to adapt to high alkalinity and pH. For example, Lake Magadi tilapia (*Tilapia grahamsi*) excretes all of its waste nitrogen as urea (Wilkie and Wood, 1996); Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) is able to survive in alkaline water because of its ability to reduce ammonia production and possibly stimulate Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchange in the gills by branchial cell chloride proliferation (Wilkie and Wood, 1996), and the scale-less carp (*Gymnocypris przewalskii*) reduces NH<sub>3</sub> production, and accumulates this metabolite in the muscle to attenuate levels in other tissues, and possibly incorporates NH<sub>3</sub> into amino acids (Wang et al., 2003).

In order to acclimate to environments with varied salinities, diverse ion compositions, and varied pH, fishes must have highly efficient ion and osmoregulatory mechanisms (Hwang and Lee, 2007). Ion transportation is an essential function in many animal tissues to modulate ion flux in response to changes of internal and external environments (Scott et al., 2004). The gill is the major site of acid-base regulation in most fish species (Claiborne et al., 2002). Acid-base transfer across the gill is dominated by carbon dioxide (Randall and Tsui, 2006). It is likely that long-term regulation of acid-base balance in fish exposed to alkaline water is dependant upon the independent modulation of Na<sup>+</sup> and Cl<sup>-</sup> movements across the gill epithelium (Wilkie and Wood, 1996).

Fishes need complex changes in physiological and gene regulatory networks, and numerous cellular pathways to acclimate to environmental stressors (Fiol and Kültz, 2007;

Evans, 2010). For osmotic stress, as an example, the osmosensory signal transduction network in fishes includes calcium, mitogen-activated protein kinase, 14-3-3 and macromolecular damage-activated signaling pathways (Fiol and Kültz, 2007). In addition to intracellular signaling mechanisms, the response to osmotic stress in euryhaline fish is harmonized via hormone and paracrine factor-mediated extracellular signaling (Fiol and Kültz, 2007).

Medaka (*Oryzias latipes*) is a model vertebrate commonly used in studies on developmental and evolutionary biology. The genome size of medaka is about one-half that of zebrafish, another common model animal (Takeda and Shimada, 2010). Moreover, considerable sequence information of medaka is available for molecular study (Kasahara et al., 2007). In addition, medaka is adaptable to seawater at most stages examined (Inoue and Takei, 2002), making it a good model for studying mechanisms of acclimation to salt water.

DNA microarray technology has proven to be a powerful tool for the genome-wide assessment of gene transcript levels associated with physiological responses to environmental stressors, such as osmotic stress (Evans, 2010), hypoxia (Zhang et al., 2009) and heat stress (Lockwood et al., 2010). This technology simultaneously detects the expression of many thousands of gene transcripts in response to variations resulting from differences in environmental stressors. Thus, microarrays allow assembly of gene expression in tissues under given conditions (Gibson, 2008). Medaka microarray has been applied to the study of environmental toxicity (Yum et al., 2010) and temperature change (Hirayama et al., 2005). However, this technology has not been utilized in alkalinity stress-related research. The use of microarrays to enhance our understanding of the responses of aquatic animals to alkalinity stress on the molecular level is incipient.

Modulation of expression and activity of some proteins involved in pH or acid-base acclimation of some fish species have been identified, including carbonic anhydrase, Na<sup>+</sup>, K<sup>+</sup>-ATPase, V-H-ATPase. However, molecular genetic response to alkalinity stress is highly complex, and overlaps with responses to other stresses, making it difficult to identify alkalinity-specific responses using traditional single gene or low throughput approaches. Therefore, we determined genome-wide gene expression profiles to better understand how carbonate alkalinity stress influences global gene expression in medaka in the present study.

## MATERIAL AND METHODS

### Experimental fish

Laboratory-raised medaka fish were used for this study. Fish (body weight and total length were  $910 \pm 57$  mg and  $42 \pm 0.7$  mm, respectively,  $N = 30$ ; data reported as means  $\pm$  SEM) were maintained in a 50-L glass tank with filtered dechlorinated tap water for at least two weeks. Temperature, dissolved oxygen, pH, ammonia, and nitrite were maintained at  $26 \pm 1^\circ\text{C}$ ,  $5.8 \pm 0.8$  mg/L,  $7.5 \pm 0.4$ ,  $<0.1$  mg/L, and  $5 \pm 1$  mg/L, respectively.

### Carbonate alkalinity exposure and tissue sampling

Carbonate alkalinity solution was prepared using NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>, and dechlorinated filtered tap water, which was maintained in a plastic tank and aerated for 24 h before use. The carbonate alkalinity was determined every day throughout the exposure

period with acidimetric titrations, and expressed as mM. Exposure tests were conducted in 15-L flow-through tanks (14 cm x 10 cm x 8.5 cm) containing about 10 L of the test solution, each with 15 adult fishes. The 90% solution in the test tanks was changed daily. The fish were exposed to freshwater (FW) (carbonate alkalinity =  $1.5 \pm 0.1$  mM, pH =  $8.4 \pm 0.2$ , pCO<sub>2</sub> =  $4 \pm 1$  torr) and alkaline water (AW) (carbonate alkalinity =  $30.4 \pm 0.4$  mM, pH =  $9.2 \pm 0.0$ , pCO<sub>2</sub> =  $2 \pm 1$  torr) for 96 h, each treatment had three tanks, each with 15 fish. The alkalinity concentration ( $30.4 \pm 0.4$  mM) was tested because our previous study indicates that development of medaka was greatly affected at this alkalinity concentration (Yao et al., 2010). Photoperiod was 16-h light:8-h dark. Water temperature was controlled at  $26^\circ \pm 1^\circ\text{C}$  with a heater. The fish were not fed during the stress experiment. At the end of exposure, the gills of 3 adult fishes in each tank were sampled, and immediately frozen in liquid nitrogen, then stored at  $-80^\circ\text{C}$  for gene expression analysis. Altogether, gills of 9 fish in FW and 9 fish in AW were sampled.

### Oxygen consumption and plasma chemistry analysis

Oxygen consumption of medaka exposed to FW and AW for 96 h was examined. Respirometry trials were conducted in six 0.6-L respirometry chambers (3 for FW, 3 for AW) through which water flowed at a constant rate of 75 mL/s by an external submersible pump. Water was internally recirculated by an internal pump continually at a flow rate of about 75 mL/s. The respirometer was submerged in water to keep temperature constant at  $26 \pm 1^\circ\text{C}$ . The fish for the respirometry trials did not feed 24 h prior to the trials. Fifteen fish were placed in each of the 0.6-L respiration chambers for 96 h. An oxygen probe (YSI 6562, Yellow Springs, OH, USA), calibrated with air-saturated water and zeroed with sodium bisulfate-saturated water, was inserted in the recirculation tube to monitor oxygen concentration of the bypassing water. The external water stopped flowing through the chambers when internal water oxygen concentration reached the target level. Water oxygen concentration in mg/L was recorded every 20 s. Measurements of oxygen consumption lasted 10-30 min, depending on the size of the fish and swimming speed, which was long enough to record a change of 0.5-2.0 mg O<sub>2</sub>/L, but without decreasing the dissolved oxygen concentration below 80% saturation during any measurement. Oxygen consumption was expressed in terms of wet weight of the fish (mg O<sub>2</sub>·kg<sup>-1</sup>·h<sup>-1</sup>). After respirometry trials, the fish were anaesthetized by buffered MS-222 (about 0.5 g/L). Afterwards, 10 fish from each chamber were selected, and their tails were cut off and blood was collected with a glass capillary tube (Hirschmann Laborgeraete, Eberstadt, Germany). The blood collected from 10 fish was pooled and centrifuged at 10,000 g for 30 s. The plasma was collected and used for examining osmolality by a vapor pressure osmometry (Wescor 5520, South Logan, UT, USA). The statistical significance of differences between the two treatments was determined with the Student *t*-test.

### RNA extraction

Total RNA was extracted from 50~100 mg gills from 9 fish in FW and 9 fish in AW using Trizol (Invitrogen, Burlington, ON, Canada) isolation reagent following manufacturer instructions. Total RNA and RNA quality were determined spectrophotometrically (Agilent

Bioanalyzer 2100, Agilent, Wokingham, Berkshire, UK). Total RNA was treated with RQ1 RNase-free DNase (Promega). Then, two separate pools with equal RNA quantities were prepared: one with nine samples in FW treatment and one with nine samples in AW treatment. RNA samples were stored at -80°C following isolation.

### Oligonucleotide microarray fabrication

Sixty-mer oligonucleotide probes for each DFCI (Dana Farber Cancer Institute), OIGI (*O. latipes* Gene Index), TCs (Tentative Consensus sequences) were designed by eArray (Agilent, Santa Clara, CA, USA) based on the data from OIGI, updated July 11, 2008 (release 7.0). The total number of TCs was 26,436 ([http://compbio.dfci.harvard.edu/cgi-bin/tgi/T\\_release.pl?gudb=o\\_latipes](http://compbio.dfci.harvard.edu/cgi-bin/tgi/T_release.pl?gudb=o_latipes); Release 7.0; July 2008). 26,429 60-mer oligonucleotides were successfully designed for 26,429 TCs. Eight microarrays (Agilent 4 x 44k design format, Agilent) were produced by *in situ* oligonucleotide synthesis. The layout of microarray slide consists of 4 duplicated blocks, each block containing a complete set of medaka oligonucleotide probes, positive control, negative control, and empty spot locations.

### Microarray hybridization

Microarray analysis was performed on adult medaka exposed to FW and AW. Sample labeling and hybridization were performed according to the Agilent one-color microarray-based gene expression analysis protocol. Six 44k Agilent test arrays were hybridized with pooled Cyanine-3 (Cy3)-labeled cRNAs gained from the gills of medaka exposed to FW and AW. This microarray design resulted in three arrays for each of the FW and AW treatments. The amplification protocol was started with 2 µg RNA. The pooled RNAs from the gills of all fishes of each replicate of the treatments were labeled using the One-Color low RNA input linear amplification kit (Agilent) according to manufacturer instructions, followed by RNAeasy column purification (QIAGEN, Valencia, CA, USA). Dye incorporation and cRNA yield were evaluated with the NanoDrop ND-1000 Spectrophotometer (Wilmington, DE, USA). Then, the labeled cRNA was prepared for fragmentation by adding 11 µL 10X blocking agent and 2.2 µL 25X fragmentation buffer, heated at 60°C for 30 min, and finally diluted by addition of 55 µL 2X GE hybridization buffer. A volume of 100 µL hybridization solution was then dispensed in the gasket slide and assembled to the microarray slide (each slide containing four arrays). Slides were incubated at 65°C in an Agilent hybridization oven for 17 h, subsequently removed from the hybridization chamber, quickly submerged in GE wash buffer 1 to disassemble the slides and then washed in GE Wash Buffer 1 for approximately 1 min followed by one additional wash in pre-warmed (37°C) GE Wash Buffer 2.

After washing, the arrays were scanned on an Agilent G2565BA DNA microarray scanner (Agilent) at a resolution of 5 µm. The same slide was scanned twice at two different sensitivity levels (XDR Hi 100% and XDR Lo 10%). The two generated images were analyzed together and data were extracted and background subtracted using the standard procedures contained in the Agilent feature extraction software 9.1.

## Array data analysis

Hybridized microarrays were scanned and visualized using an Agilent 2565BA DNA microarray scanner. The scanned images were analyzed with the feature extraction software 9.1 (Agilent). Among these measures the feature extraction software 9.1 flag was used to filter out unreliable probes. Raw data from the Agilent 2565BA were uploaded to GeneSpring GX 10 for quantile normalization. After normalization, differentially expressed genes were identified by unpaired *t*-test. The data addressed here have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO: [www.ncbi.nlm.nih.gov/geo/](http://www.ncbi.nlm.nih.gov/geo/)) and are accessible through the GEO series accession No. GSE27298. Differentially expressed genes identified by the *t*-test were further mined for re-annotation, Gene Ontology (GO) classification and pathway elucidation using the Database for Annotation and Visualization and Integrated Discovery (DAVID <http://david.abcc.ncifcrf.gov/>) (Dennis Jr. et al., 2003), and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000) databases.

Differentially expressed genes were subjected to annotation enrichment analysis using the online functional annotation tool DAVID. *Danio rerio* was used as the background species. Zebrafish gene identifiers were used for this analysis to improve the richness of the output. Significance was calculated by a modified Fisher exact test. From these annotations, GO bar charts were made using 2nd level GO terms based on biological process, molecular function and cellular component. DAVID functional annotation clustering was used to classify the gene functions.

## Gene expression analysis based on real-time RT-PCR

To test whether the changes in gene expression detected with medaka microarray were reproducible by other methods, 10 OIGI sequences were selected for SYBR-based real-time quantitative PCR (qPCR) evaluation. Primers (Table 1) were designed with the Primer 3 program (Rozen and Skaletsky, 2000). Gene expression was assessed using SYBR-based qPCR on LightCycler 1.2 (Roche, Germany). The PCR was carried out with 1  $\mu$ L cDNA, 0.2  $\mu$ L of each primer and 5  $\mu$ L SYBR master mix, in a total volume of 10  $\mu$ L. All qPCRs were run for 1 cycle of 10 s at 95°C, followed by 40 cycles of 5 s at 95°C and 20 s at 60°C. Melting curve analysis was performed following each reaction to confirm that there was only a single product and no primer-dimer artifacts. In addition, representative samples were electrophoresed to verify that only a single product was present. The standard curve of each gene was checked in a linear range with  $\beta$ -actin as an internal control. Negative control reactions were performed for representative samples using RNA that had not been reversely transcribed to control for the possible presence of genomic DNA contamination. Non-template control reactions were also performed to verify that there was no cDNA, PCR product contamination or primer-dimer amplification in the reactions.

A randomly selected sample was used to develop a standard curve for each primer set, and the results were expressed relative to this arbitrary standard. All samples were run in triplicate. Expression of the different samples was normalized to  $\beta$ -actin. Relative changes in the expression of candidate genes were calculated by the comparative  $C_T$  method (Schmittgen and Livak, 2008).



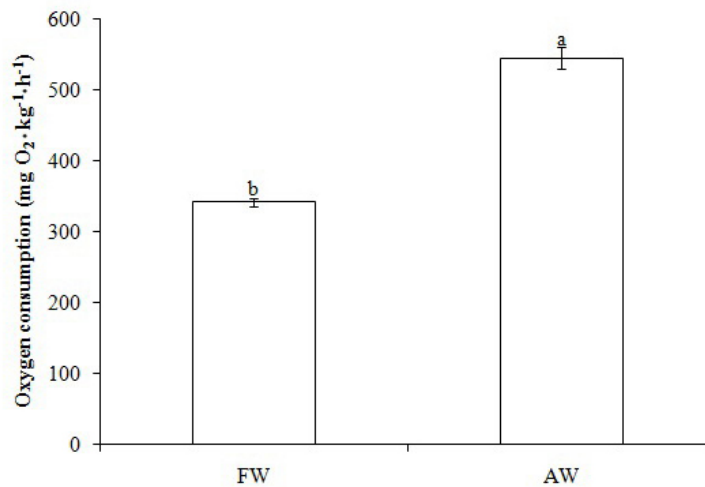
**Table 1.** Primers designed for qPCR of 10 *Oryzias latipes* Gene Indexes.

Sequence source	Tentative annotation, hit coverage	Sequence (5'-3') (forward, reverse)	Cycles
TC84688	Cathepsin L, partial (28%)	CGTGTGGTGGGGTTAC AGTGGTTGTTCGGTCTTTGG	30
TC80053	Calcyclin, partial (34%)	CACGAACCAGAACAG TTTACTAATCTTCCCTCCACAG	30
TC72720	Solute carrier family 2, partial (97%)	TGGCATCATATACATCAACC CGTCAACATCCTCTTCAC	30
TC81188	Carbonic anhydrase, partial (91%)	TGAACGGAACCAATACG CAGGAAAACCTCCACGAC	30
TC80284	Vertebrate ATP-binding cassette sub-family F, partial (33%)	CAAGCAAGTGAAGAGGAAAGATG GCATCAGAGTCCACCTCATC	30
TC62791	HoxB5a, complete	AAGAGTCCACTTCAACC CTTCCACTTCATCCTACG	30
TC74266	50-kD glycoprotein, complete	GCGATGCTGTGATGACTAC CATGTGATTGTGTACTCCAAGAC	30
TC79955	Hemoglobin embryonic subunit alpha, complete	CCTGCCAACTTCAAAGATTCTC CCAGAGCCAGAGCCAAAAG	30
TC64825	Orphan nuclear receptor SHP, partial (59%)	TGATGAACAAGCCACCATGATTG AGAGACAAGTCTGCCACTGTG	30
TC78371	Outer membrane autotransporter barrel protein, partial (1%)	GCATCCTCTGGAGTCAACAG AAACCCTCAAAGCACACCG	30
S74868	$\beta$ -actin	CTGGACTTCGAGCAGGAGAT AGGAAAGGAGGCTGGAAAGAG	30

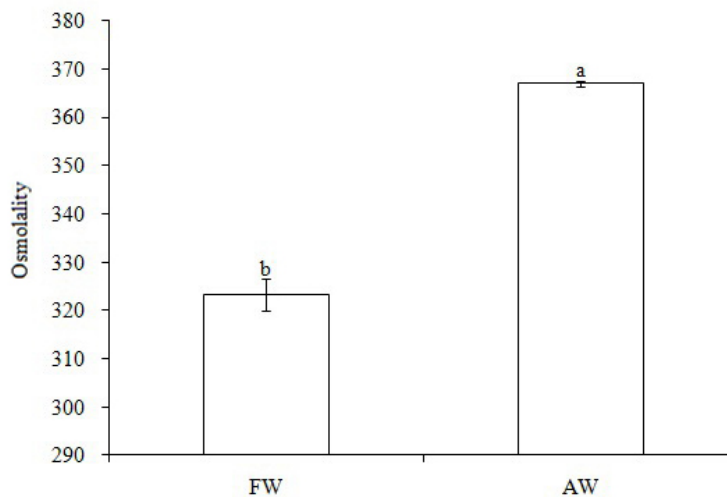
## RESULTS AND DISCUSSION

### Oxygen consumption and plasma chemistry

Exposure to high carbonate alkalinity (30.4 mM) water for 96 h resulted in a high oxygen consumption of medaka (Figure 1). Marked increase in plasma osmolality (Figure 2) was observed at 96 h post-exposure.



**Figure 1.** Oxygen consumption (means  $\pm$  SEM, N = 3) of *Oryzias latipes* exposed to freshwater (FW) and 30.4 mM carbonate alkalinity water (AW) for 96 h. Different letters above the bars indicate statistically significant differences between means ( $P < 0.05$ ).



**Figure 2.** Plasma concentration of osmolality (means  $\pm$  SEM, N = 3) in the blood of *Oryzias latipes* exposed to freshwater (FW) and 30.4 mM carbonate alkalinity water (AW) for 96 h. Different letters above the bars indicate statistically significant differences between means ( $P < 0.05$ ).



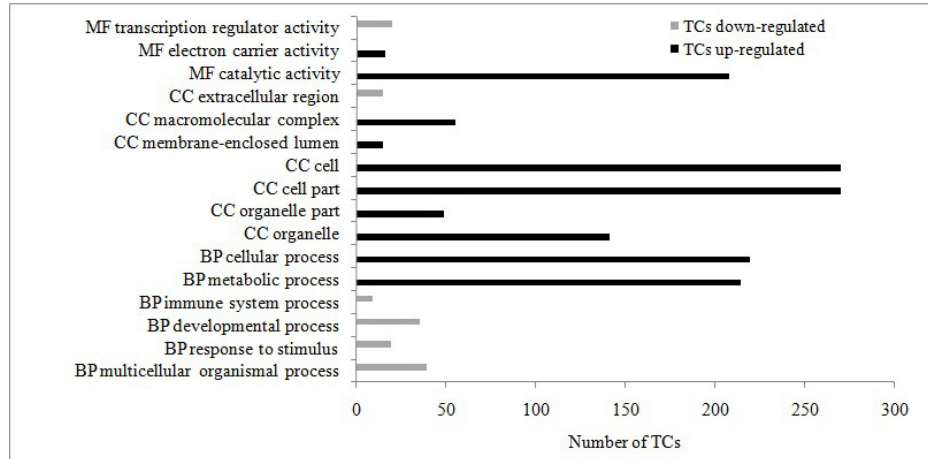
## Alkalinity stress-responsive genes in medaka

Gene expression profiles may reflect the interaction of environmental stresses and the host transcript (Gibson, 2008). Salinization of land and water owing to climate change has become a major problem in land and aquatic ecosystems worldwide. Alkalinity stress is considered to be one of the major stressors on fish in saline-alkali water. The detected signal intensities in the present study suggest that medaka gills had statistically significant gene expression under alkalinity stress compared to the control ( $P < 0.05$ ). Tests show that 3.8% (1013 gene targets) of the features on the arrays were significantly different ( $P < 0.05$ , fold change  $>1.5$ ) between the FW and AW treatment, of which 512 genes were up-regulated and 501 genes were down-regulated (see Supplementary Tables 1 and 2).

## Functional classification of the differentially expressed genes

Most of differentially expressed genes identified in this study are listed as unknown in the annotation section of the GenBank database. Thus, we performed comparative genome analyses by retrieving these DNA sequences and comparing these genes against the KEGG database using *D. rerio* as the background species. In order to get more annotation information, a loose threshold ( $P < 0.05$ , fold change  $>1.1$ ) was used. This process yielded zebrafish orthologs ( $E < 0.005$ ) for 70% (1939) of differentially expressed medaka genes. These medaka genes were treated as known genes and their zebrafish counterparts' Entrez Gene IDs were used for GO grouping, and biological pathway analysis using *D. rerio* as the background species. One thousand and five differentially expressed genes match with annotated DAVID gene objects. Zebrafish IDs corresponding to differentially expressed medaka transcripts and to all genes represented on the array were then used to define a "gene list" and a "background" in DAVID. As the cut-off P value was set to count = 2 and ease = 0.1, the differently expressed genes fell into a number of GO groups on the second level.

As shown in Figure 3, there are 10 GO groups for up- and 6 for down-regulated genes. The number of up-regulated genes was higher than that of down-regulated genes. Most of the up-regulated genes were grouped into catalytic activity, metabolic process, cellular process, cell and cell part with the highest oxygen consumption rate (Figure 1), indicating that the alkalinity stress speeds up general metabolic and catalytic processing. The physiological responses of organisms to changes in environmental conditions, such as high CO<sub>2</sub> (Todgham and Hofmann, 2009), osmotic stress (Evans, 2010; Lockwood et al., 2010), heat stress (Podrabsky and Somero, 2004), are energetic cost processes. In many fish species it is known that acclimation to hyper-saline water leads to an over-expression of the genes participating in osmoregulation as compared to the acclimation to iso-osmotic conditions (Evans, 2010). Indeed, fish acclimated to high carbonate alkalinity water face several pressures, including acid-base (Perry and Gilmour, 2006), osmolality disturbances (Figure 2) and ion imbalance (Goss et al., 1994). In this case, fish must maintain their homeostasis by activating energetically expensive ionic and osmotic regulation mechanisms. This is supported by the presence of several transcripts of genes encoding for ion binding proteins, such as carbonic anhydrase, glutathione-S-transferase and beta-type globin (Table 2). These proteins play a pivotal role in ionic regulation. On the other hand, identification of genes encoding for oxidation reduction such as cytochrome P450 suggests a high energetic demand for medaka acclimation to high alkalinity stress.



**Figure 3.** Number of gene ontology groups of medaka differentially expressed genes exposed to alkalinity stress. MF = Molecular function; BP = biological process; CC = cellular component; TCs = Tentative Consensus sequences.

**Table 2.** Up-regulated genes in the gills of medaka exposed to carbonate alkalinity stress.

TCs	Zebrafish Entrez Gene ID	Fold change	Gene name (Zebrafish description)
Acid-base and ion regulation			
TC64429	30331	1.57	Carbonic anhydrase
TC81188	791844	8.10	Carbonic anhydrase XV b
TC76034	564924	1.94	ATPase, Cu <sup>2+</sup> transporting, alpha polypeptide
TC80873	448856	9.80	S100 calcium binding protein A1
TC86511	100150781	1.20	Solute carrier family 30 (zinc transporter), member 5
TC74393	321324	1.45	Solute carrier family 39 (zinc transporter), member 1
TC64151	368907	1.39	ATPase, H <sup>+</sup> transporting, lysosomal, V1 subunit C, isoform 1
TC80039	324140	4.20	Serum/glucocorticoid regulated kinase 1
TC82123	777614	1.26	Zgc:152898; similar to two pore calcium channel protein
TC66488	555922	1.59	Similar to potassium voltage-gated channel, Shaker-related subfamily, beta member 1
TC67007	335821	1.46	Zgc:8589; similar to aquaporin 1
Apoptosis			
TC71948	559355	1.34	BCL2-like 13 (apoptosis facilitator)
TC67450	114401	1.18	Bcl2-like
TC77936	559018	1.46	Clusterin-like
TC85108	559007	1.12	Death-associated protein 3
Cell cycle			
TC72575	793158	1.54	Cell division cycle 42, like
TC75544	445392	1.59	F-box protein 5
TC71874	192295	1.56	Cyclin A2
TC87801	368316	1.43	Cyclin B2
TC63530	80973	1.40	Cell division cycle 2
Cellular stress response			
Protein homeostasis			
TC66063	406294	1.14	Glutaredoxin 5 homolog ( <i>Saccharomyces cerevisiae</i> )
TC77350	326857	1.63	Glutamate-cysteine ligase, catalytic subunit
TC87281	494477	2.39	Transferrin receptor 1a
TC74299	406289	1.33	Thioredoxin domain containing 5
TC77854	565155	1.60	Heat shock protein 90-alpha 2
TC71195	321945	1.26	AHA1, activator of heat shock protein atpase homolog 1, like
Antioxidant defense			
TC77135	564619	1.23	Transferrin-a; Rho-class glutathione S-transferase

Continued on next page

**Table 2.** Continued.

TCs	Zebrafish Entrez Gene ID	Fold change	Gene name (Zebrafish description)
TC67219	406736	1.32	Zgc:56518; similar to microsomal glutathione-S-transferase
TC80729	325449	2.07	Metallothionein 2; metallothionein; kinesin family member 20A
TC66068	553575	1.72	Zgc:110010; similar to glutathione reductase
Toxicant, metal and xenobiotic defense			mitochondrial precursor
TC88886	406731	2.32	Zgc:123097; UDP glucuronosyltransferase 1 family a, b; UDP glucuronosyltransferase 1 family
TC63674	282559	1.69	Aldehyde dehydrogenase 3 family, member D1
TC79029	553969	1.17	Cytochrome P450, family 3, subfamily A, polypeptide 65
TC76397	399485	12.67	Cytochrome P450, family 2, subfamily J, polypeptide 21
TC66471	792041	1.15	Thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A)
TC65409	406467	1.55	ATP-binding cassette, subfamily F (GCN20), member 1
TC71048	100149614	1.44	Similar to ATP-binding cassette, subfamily F (GCN20), member 3
Metabolism			
Energy metabolism			
TC86848	321664	2.04	Aldolase b, fructose-bisphosphate
TC79787	100003895	1.33	ADP-ribosylation factor interacting protein 1 (arfaptin 1)
Electron transport chain			
TC87387	325922	1.15	Retinol saturase (all-trans-retinol 13,14-reductase)
TC86740	664750	1.43	Protoporphyrinogen oxidase
TC70864	140540	1.13	Cytochrome c oxidase subunit 2
TC72168	555604	1.50	NADPH oxidase 1
TC86017	393829	1.12	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8
TC62782	140531	1.84	NADH-ubiquinone oxidoreductase chain 1
Mitochondrial membrane transporters			
TC79031	100005717	1.29	Carnitine palmitoyltransferase II
TC73439	393638	1.29	Translocase of inner mitochondrial membrane 50 homolog (yeast)
TC62966	64607	1.97	Aminolevulinic acid, delta-, synthetase 2
TC69117	394133	1.39	Aldehyde dehydrogenase 4 family, member A1
TC76181	558883	1.16	Translocated promoter region (to activated MET oncogene)
TC79274	335236	1.36	Transmembrane protein 48
TC67553	406852	1.31	Nucleoporin 133
TC76902	337424	1.28	ADP-ribosylation factor-like 8Ba
Carbohydrate metabolism			
TC66110	116991	2.24	UDP-glucose dehydrogenase
TC75325	678540	2.29	UDP-galactose-4-epimerase
TC73743	393461	2.23	GDP-mannose 4,6-dehydratase
TC64070	378727	1.98	Phosphoenolpyruvate carboxykinase 1 (soluble)
TC79120	791728	1.22	Renin binding protein
TC69528	445487	1.53	Fucosidase, alpha-L-2, plasma
TC65066	386663	1.85	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4, like
TC84984	570579	1.48	Wu:fj78b06; similar to glucose-6-phosphate 1-dehydrogenase
Amino acid metabolism			
TC88744	393423	1.21	Argininosuccinate lyase
TC65754	447864	2.12	Argininosuccinate synthetase 1; similar to argininosuccinate synthetase
TC87003	378847	1.30	5-methyltetrahydrofolate-homocysteine methyltransferase
TC88179	550524	1.29	Cystathionine-beta-synthase a
TC65385	317738	3.06	ELOVL family member 6, elongation of long chain fatty acids (yeast)
TC65615	266799	1.47	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)
TC65820	566537	1.76	Slc22a11-132p20.4; similar to amino acid system A transporter-like
TC75021	100007704	1.83	Similar to solute carrier family 7 (cationic amino acid transporter, y <sup>+</sup> system), member 8
TC67983	550373	1.21	Peptidylprolyl isomerase E (cyclophilin E)
Lipid metabolism			
TC75954	445025	1.30	Zgc:92251; similar to sodium/bile acid co-transporter 7

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**Table 2.** Continued.

TCs	Zebrafish Entrez Gene ID	Fold change	Gene name (Zebrafish description)
Vitamin metabolism			
TC72880	30295	24.82	Rhodopsin
TC70178	777635	1.59	Procollagen-lysine 1,2-oxoglutarate 5-dioxygenase 1a
TC64004	403338	1.45	Glycine N-methyltransferase
TC63892	322055	2.80	Cystathionase (cystathionine gamma-lyase); cystathionase (cystathionine gamma-lyase), like
Nucleotide metabolism			
TC63071	30733	1.53	Ribonucleotide reductase M2 polypeptide
TC70658	406383	1.89	Cytidylate kinase
TC76038	550560	1.82	Ectonucleoside triphosphate diphosphohydrolase 6 (putative function)
TC62666	550420	1.98	Guanylate cyclase 1, soluble, alpha 3
TC65833	393603	1.18	cAMP-regulated phosphoprotein 19a

TCs = Tentative Consensus sequences.

Most of the down-regulated genes were grouped into multicellular organismal process, developmental process, transcription regulator activity, response to stimulus, extracellular region, and immune system process (Figure 3), indicating a decrease in disease resistance of medaka when exposed to carbonate alkalinity stress. Environmental stressors, such as salinity and heat stress, may cause immune suppression in aquatic animals (Prophete et al., 2006; Green and Barnes, 2010), and lead to maladaptation. The presence of several transcripts of genes encoding for immune response proteins, such as major histocompatibility complex (MHC), suggests that alkalinity stress has elicited an immune response in medaka fish.

In the section below, we describe the changes in mRNA expression in five main processes, including: acid-base and ion regulation, cellular stress response, metabolism, immune response, and reproduction (Tables 2 and 3). This custom category was based on the functional annotation clustering analysis by DAVID (classification stringency set as medium).

### Acid-base and ion regulation

Regulation of ion transport proteins such as carbonic anhydrase, Na<sup>+</sup>, K<sup>+</sup>-ATPase and ATPase, H<sup>+</sup> transportation is essential to acid-base and osmoregulatory processes in fish (Kang et al., 2008; Gilmour and Perry, 2009). In this study, an 8.1- and 1.6-fold increase in mRNA of the CAXV and CA (carbonic anhydrase) was found during 96 h of high alkalinity stress. Carbonic anhydrase, a zinc enzyme that catalyzes reversible reactions of CO<sub>2</sub> with water (Tufts et al., 2003), is responsible for converting CO<sub>2</sub> into HCO<sub>3</sub><sup>-</sup> and H<sup>+</sup> ions. Carbonic anhydrase plays a key role in both acid-base and ionic regulation in fish by providing the acid-base equivalents that function as counter-ions for NaCl movement and that are excreted to maintain or restore acid-base balance at both the gills and kidney (Gilmour and Perry, 2009). Nitrogen end-products have diverse physiological functions in different animal groups, including acid-base regulation, osmoregulation and buoyancy. Animals excrete a variety of nitrogen waste products, such as ammonia, urea and uric acid. Aquatic animals excrete mostly ammonia. The major problems for fishes in alkaline water are the inhibition of ammonia excretion and increase in CO<sub>2</sub> excretion (Parra and Baldisserotto, 2007). In alkali water, there is less H<sup>+</sup> available for transforming NH<sub>3</sub> into NH<sub>4</sub><sup>+</sup>, so that the NH<sub>3</sub> gradient from blood to water decreases. When medaka was placed in alkali water, a transitory metabolic alkalosis may occur, which resulted in the genes in nitrogen metabolism such as highly expressed CA2 mRNA to interconvert carbon dioxide and bicarbonate to maintain acid-base balance, and to facilitate carbon dioxide transport out of the gills.

**Table 3.** Down-regulated genes in the gills of medaka exposed to carbonate alkalinity stress.

TCs	Zebrafish Entrez Gene ID	Fold change	Gene name (Zebrafish description)
Acid-base and ion regulation			
TC65263	504083	1.38	Zgc:110418; similar to potassium channel subfamily K member 6
TC77323	368255	1.48	Chloride intracellular channel 4
Cellular stress response			
Protein homeostasis			
TC82521	799177	1.41	Heat shock factor binding protein 1-like
Toxicant, metal and xenobiotic defense			
TC74995	557525	1.58	Si:dkey-239i20.2; similar to flavin-containing monooxygenase FMO1
TC87468	557008	1.22	Novel protein similar to vertebrate aldehyde dehydrogenase 3 family
TC68274	116713	1.37	Aldehyde dehydrogenase 1 family, member A2
TC73159	447849	1.12	Zgc:92194; similar to nuclear receptor coactivator 5 (ncoa-5)
TC66847	565865	1.24	Si:dkey-245f7.1; similar to nuclear receptor-interacting protein 2
TC64825	403010	1.71	Nuclear receptor subfamily 0, group B, member 2a
Development			
TC79119	30037	1.66	Similar to tenascin C; tenascin C
TC83533	570960	1.69	Zgc:158824; pre-B-cell leukemia transcription factor 1a
TC71844	100005469	1.59	Fibronectin 1
TC75996	30264	1.67	T-box gene 16
TC74173	566796	1.23	Laminin, alpha 4
TC65915	554050	1.33	Integrin, beta-like 1
TC64936	58150	1.46	Heart and neural crest derivatives expressed transcript 2
TC68595	497649	1.56	Transcription factor 7 (T-cell specific, HMG-box)
TC78017	83496	1.62	BMP and activin membrane-bound inhibitor ( <i>Xenopus laevis</i> ) homolog
TC72996	799300	1.82	Zgc:66156; similar to myosin heavy chain
TC68178	335098	1.53	Sprouty homolog 2
TC86481	386723	1.54	Friend leukemia integration 1b
TC85163	569047	1.19	Forkhead box p1b
TC69056	405843	1.36	Forkhead box Q1
TC65686	393959	1.43	Forkhead box L1
TC65627	30180	2.08	Growth differentiation factor 6b
Kinome			
TC69853	447930	1.80	Zgc:103639; similar to myosin light chain 2
TC67029	406728	1.32	Mitogen-activated protein kinase kinase 1
TC63675	100002318	1.34	Im:7136778; similar to mitogen-activated protein kinase 13
TC69379	790934	2.15	Mitogen-activated protein kinase-activated protein kinase 3
TC66976	553696	1.12	Zgc:112257; similar to LIM and senescent cell antigen-like domains
TC86513	393813	1.14	PDZ and LIM domain 7
Metabolism			
Energy metabolism			
TC66930	553755	1.14	ATP synthase, H <sup>+</sup> transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
Carbohydrate metabolism			
TC64384	30497	1.32	Lactate dehydrogenase B4
Amino acid metabolism			
TC81763	335573	1.90	Protein tyrosine phosphatase, non-receptor type 6
TC81376	568277	8.89	SET binding factor 1
TC63031	334304	2.59	Elastase 2-like
TC70135	556307	2.05	Zgc:158605; serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
TC62634	140425	1.54	Guanylyl cyclase 2
TC69379	790934	2.15	Mitogen-activated protein kinase-activated protein kinase 3
TC78001	100006858	1.57	Casein kinase 1, epsilon
TC62864	335339	1.57	Male germ cell-associated kinase
TC71719	100001160	1.58	Fibroblast growth factor receptor 4
TC85640	325084	1.68	V-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)

Continued on next page

**Table 3.** Continued.

TCs	Zebrafish Entrez Gene ID	Fold change	Gene name (Zebrafish description)
TC88729	558900	1.63	RAB6B, member RAS oncogene family
TC82622	541515	1.74	Myosin 1b-like 2
TC69367	327396	1.77	Ras-related associated with diabetes
TC83650	100001337	1.67	Slow myosin heavy chain 3; similar to slow myosin heavy chain 3
TC65046	335396	1.24	Splicing factor, arginine/serine-rich 5a
TC76014	794311	1.61	Flavin containing monooxygenase 5
TC71098	322614	2.18	Arginase, type II
Lipid metabolism			
TC67400	442923	1.45	3-oxoacid coa transferase 1a
TC66334	402958	1.28	Zgc:77336; im:7142624; similar to probable lipid phosphate
Phosphatase PPAPDC3			
TC84023	324345	1.56	Wu:fc26c03; similar to choline transporter-like protein 2
Vitamin metabolism			
TC65526	558106	1.34	Hypothetical LOC558106; similar to vitamin K-dependent protein Z precursor
Nucleotide metabolism			
TC86873	492467	1.62	Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type
TC79351	406251	1.63	RNA binding motif protein 39a
Protein synthesis - translational control			
TC64820	322237	1.12	Eukaryotic translation initiation factor 1B
TC69371	562897	1.65	Similar to RIKEN cdna 2810012L14
TC85263	436759	1.59	Ribosomal protein L38
Immune response			
TC65777	368614	1.43	Si:busm1-194e12.11; similar to MHC class II alpha subunit
TC62736	550387	2.51	Zgc:111997
TC80282	571282	1.74	Similar to H-2 class II histocompatibility antigen, A-K alpha chain; novel protein with a class II histocompatibility antigen, alpha domain and an immunoglobulin C1-set domain
TC83664	100003911	2.09	Chemokine CXCL-c13d
TC73740	553699	2.38	Vitronectin a
TC81021	541408	1.73	Similar to marapsin; zgc:101788
TC67829	58113	1.70	Invariant chain-like protein 1
TC69939	30553	1.38	Superoxide dismutase 1, soluble
Reproduction			
TC75004	445065	5.28	Hypothetical protein LOC100151043; corticotropin-releasing hormone binding protein
TC66699	572500	1.29	Similar to RAS-like, estrogen-regulated, growth inhibitor

TCs = Tentative Consensus sequences.

The serum and glucocorticoid regulated kinase 1 (SGK-1) gene that plays an important role in ion homeostasis (Lang et al., 2006) was significantly up-regulated by high alkalinity stress. SGK-1 plays an important role in the regulation of epithelial ion transport, such as Na<sup>+</sup> handling in renal tubular of mice (Loffing et al., 2006). This kinase is rapidly regulated at the transcriptional level. In addition, SGK-1 may influence the activities of other ion-transporters such as the Na<sup>+</sup>-K<sup>+</sup>-ATPase, Na<sup>+</sup>/H<sup>+</sup> exchanger and Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> co-transporters (Lang et al., 2006). These properties allow SGK-1 to integrate numerous signaling inputs into varied cellular responses.

The mitogen-activated protein kinase (MAPK) is a family of enzymes that are involved in osmosensory signal transduction in yeast, vertebrates and other eukaryotes (Kültz, 2001). They are key elements of protein phosphorylation cascades that integrate and amplify signals from osmosensors to activate appropriate downstream targets mediating physiological acclimation (Kültz, 2001). It plays an important role in osmosensory signal transduction in fish (Fiol and Kültz, 2007). Under high alkalinity stress, MAPK1 and MAPK3 MAPK13 mRNA expression in medaka gill tissue were down-regulated 1.3-2.15-fold (Table 3). The decrease



in MAPK expression was backed by a coincident decrease of other MAPK relevant genes-phosphatidic acid phosphatase 2, together with the increase of the plasma osmolality (Figure 2), indicating a stimulation of acid-base and ion regulation of medaka under alkalinity stress.

### Cellular stress response

Several cellular stress response-related genes of medaka were differentially expressed during alkalinity stress, such as, protein homeostasis, antioxidant defense and toxicant, metal and xenobiotic defense (Table 2). The cellular stress response is a universal mechanism of extraordinary physiological/pathophysiological significance. It represents a defense reaction of cells to environmental forces inflicted on macromolecules (Kültz, 2005). Medaka may turn on its defense system in response to relative high alkalinity stress, but mortality increases greatly when the alkalinity exceeds its tolerance (Yao et al., 2010). At a cellular level, cells can quantify stress and activate a death program (apoptosis) when tolerance limits are exceeded.

### Metabolism

As mentioned above, the alkalinity stress speeded up general metabolic and catalytic processes of medaka. Fish gills are highly oxidative tissues, and their oxygen requirement increases when fishes are exposed to environmental stresses, such as salinity (Vijayan et al., 1996). The energy requirement for the gills is through oxidation of glucose and lactate obtained from the circulation (Tseng and Hwang, 2008). When fishes are exposed to alkalinity stress, the glycolysis process is up-regulated by carbohydrate metabolism (Table 2), so medaka may spend more energy under alkalinity stress.

Lipid phosphate phosphatase (LPPs) regulates cell signaling by modifying the concentrations of lipid phosphates versus the dephosphorylated products (Brindley and Pilquill, 2009). LPP mRNA was down-regulated under high alkalinity stress in medaka gill tissue (Table 3), which is similar to *Gillichthys mirabilis* exposed to hyper- and hypo-osmotic stress (Evans, 2010).

### Immune response

MHC belongs to the immunoglobulin superfamily of proteins, and interacts with the T-cell subsets through a specific T-cell receptor (TCR), and initiates the adaptive immune response (Kurosawa and Hashimoto, 1997). MHC has been studied intensively, and is consequently one of the best characterized genetic complexes in vertebrates because of its key function in the immune response. MHC gene expression is always down-regulated when medaka fish are exposed to environmental stresses, such as low temperature (Rodrigues et al., 1998). Because MHC class II antigens play an important role in generating an immune response to bacterial and fungal pathogens, down-regulation of these genes during alkalinity stress probably accounts for the susceptibility of fish to alkalinity-related diseases such as *Prymnesium parvum*. How salinity or alkalinity up- or down-regulates the immune responses remains unknown. In this study, MHC class II and invariant chain-like protein I (Table 3) were down-regulated when medaka was exposed to high alkalinity, indicating that the immune system response of medaka was slowed down under this kind of stress.



## Reproduction

Some genes taking part in the regulation of gonadotropin gene expression were down-regulated in medaka exposed to alkalinity stress. The genes involved in GnRH (gonadotropin-releasing hormone) signaling pathway including mitogen-activated protein kinase, v-src sarcoma viral oncogene homolog, and matrix metalloproteinase 14 beta were down-regulated (Table 3). And other genes playing important roles in fish reproduction, such as corticotropin-releasing hormone binding protein and estrogen-regulated gene were down-regulated (Table 3). Dysregulation of these genes agrees with our previous results that reproduction of medaka was inhibited under high carbonate alkalinity stress (Yao et al., 2010).

As the cut-off P value was set to  $<0.05$ , count = 2 and ease = 0.1, we found that 10 pathways were up-regulated significantly in dealing with alkalinity stress, including amino sugar and nucleotide sugar metabolism, porphyrin and chlorophyll metabolism, metabolism of xenobiotics by cytochrome P450, drug metabolism, aminoacyl-tRNA biosynthesis, glycine, serine and threonine metabolism, ascorbate and aldarate metabolism, pentose and glucuronate interconversions, glutathione metabolism, and fructose and mannose metabolism (Table 4).

**Table 4.** Up-regulated pathways in the gills of medaka exposed to carbonate alkalinity stress.

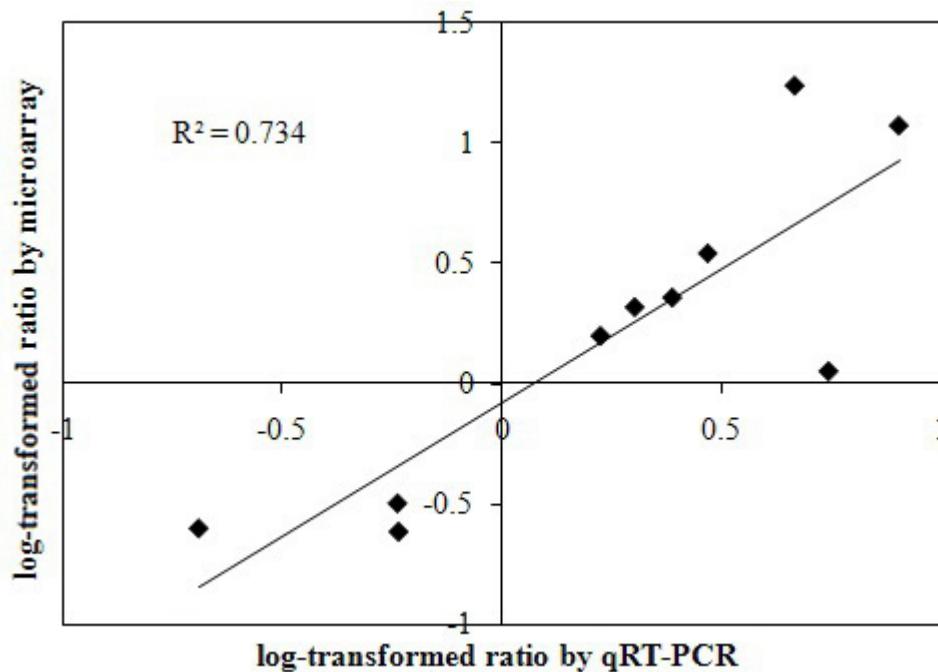
Target	Genes found (N)	P value
Amino sugar and nucleotide sugar metabolism	11	4.65E-05
Porphyrin and chlorophyll metabolism	8	3.35E-04
Metabolism of xenobiotics by cytochrome P450	6	0.0033
Drug metabolism	6	0.0033
Aminoacyl-tRNA biosynthesis	7	0.0076
Glycine, serine and threonine metabolism	6	0.0130
Ascorbate and aldarate metabolism	4	0.0174
Pentose and glucuronate interconversions	4	0.0219
Glutathione metabolism	6	0.0303
Fructose and mannose metabolism	6	0.0408

## Real-time PCR validation

The validation involved qPCR analysis of mRNA abundance for 10 genes. The directionality of responses to alkalinity stress obtained through qPCR is in full agreement with microarrays. As shown in Figure 4, inductions and suppressions of the gene expression were in line with the microarray data.

## CONCLUSION

To our knowledge, this is the first study employing a medaka high-density oligonucleotide microarray to detect global gene expression alterations in response to alkalinity stress. Alkalinity stress stimulated the energy and ion regulated processes, and slowed down the gene expression related to the immune system and reproduction in medaka. The alkalinity-regulated genes characterized in the present study may be convenient entry points to study the molecular basis of alkalinity stress. The physiological role of these genes in alkalinity stress response remains to be explored. In the future, how alkalinity triggers regulation of expression of these genes needs to be addressed.



**Figure 4.** Scatter plot comparing the log-transformed ratio measured by qRT-PCR analysis (x-axis) and by microarray (y-axis).  $R^2 = 0.734$ .

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## REFERENCES

- Brindley DN and Pilquill C (2009). Lipid phosphate phosphatases and signaling. *J. Lipid. Res.* 50: S225-S230.
- Claiborne JB, Edwards SL and Morrison-Shetlar AI (2002). Acid-base regulation in fishes: cellular and molecular mechanisms. *J. Exp. Zool.* 293: 302-319.
- Dennis G Jr, Sherman BT, Hosack DA, Yang J, et al. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.* 4: 3.
- Evans TG (2010). Co-ordination of osmotic stress responses through osmosensing and signal transduction events in fishes. *J. Fish Biol.* 76: 1903-1925.
- Fiol DF and Kültz D (2007). Osmotic stress sensing and signaling in fishes. *FEBS J.* 274: 5790-5798.
- Gibson G (2008). The environmental contribution to gene expression profiles. *Nat. Rev. Genet.* 9: 575-581.

- Gilmour KM and Perry SF (2009). Carbonic anhydrase and acid-base regulation in fish. *J. Exp. Biol.* 212: 1647-1661.
- Goss GG, Wood CM, Laurent P and Perry SF (1994). Morphological responses of the rainbow trout (*Oncorhynchus mykiss*) gill to hyperoxia, base (NaHCO<sub>3</sub>) and acid (HCl) infusions. *Fish Physiol. Biochem.* 12: 465-477.
- Green TJ and Barnes AC (2010). Reduced salinity, but not estuarine acidification, is a cause of immune-suppression in the Sydney rock oyster *Saccostrea glomerata*. *Mar. Ecol. Prog. Ser.* 402: 161-170.
- Hirayama M, Nakaniwa M, Mitani H and Watabe S (2005). Gene expression profiles for medaka *Oryzias latipes* associated with cold and warm temperatures in cDNA microarray. *Comp. Biochem. Phys.* 141: S354-S355.
- Hwang PP and Lee TH (2007). New insights into fish ion regulation and mitochondrion-rich cells. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 148: 479-497.
- Inoue K and Takei Y (2002). Diverse adaptability in *oryzias* species to high environmental salinity. *Zoolog. Sci.* 19: 727-734.
- Kanehisa M and Goto S (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 28: 27-30.
- Kang CK, Tsai SC, Lee TH and Hwang PP (2008). Differential expression of branchial Na<sup>+</sup>/K<sup>+</sup>-ATPase of two medaka species, *Oryzias latipes* and *Oryzias dancena*, with different salinity tolerances acclimated to fresh water, brackish water and seawater. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 151: 566-575.
- Kasahara M, Naruse K, Sasaki S, Nakatani Y, et al. (2007). The medaka draft genome and insights into vertebrate genome evolution. *Nature* 447: 714-719.
- Kültz D (2001). Evolution of osmosensory MAP kinase signaling pathways. *Am. Zool.* 41: 743-757.
- Kültz D (2005). Molecular and evolutionary basis of the cellular stress response. *Annu. Rev. Physiol.* 67: 225-257.
- Kurosawa Y and Hashimoto K (1997). How did the primordial T cell receptor and MHC molecules function initially? *Immunol. Cell Biol.* 75: 193-196.
- Lang F, Bohmer C, Palmada M, Seebohm G, et al. (2006). (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. *Physiol. Rev.* 86: 1151-1178.
- Lockwood BL, Sanders JG and Somero GN (2010). Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. *J. Exp. Biol.* 213: 3548-3558.
- Loffing J, Flores SY and Staub O (2006). Sgk kinases and their role in epithelial transport. *Annu. Rev. Physiol.* 68: 461-490.
- Parra JEG and Baldisserotto B (2007). Effect of Water pH and Hardness on Survival and Growth of Freshwater Teleosts. In: *Fish Osmoregulation* (Baldisserotto B, Mancera JM and Kapoor BG, eds.). Science Publishers, Enfield, 139.
- Perry SF and Gilmour KM (2006). Acid-base balance and CO<sub>2</sub> excretion in fish: unanswered questions and emerging models. *Respir. Physiol. Neurobiol.* 154: 199-215.
- Podrabsky JE and Somero GN (2004). Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* 207: 2237-2254.
- Prophete C, Carlson EA, Li Y, Duffy J, et al. (2006). Effects of elevated temperature and nickel pollution on the immune status of Japanese medaka. *Fish Shellfish Immunol.* 21: 325-334.
- Randall DJ and Tsui TK (2006). Tribute to R. G. Boutilier: acid-base transfer across fish gills. *J. Exp. Biol.* 209: 1179-1184.
- Rodrigues PN, Hermesen TT, van Maanen A, Taverne-Thiele AJ, et al. (1998). Expression of MhcCyca class I and class II molecules in the early life history of the common carp (*Cyprinus carpio* L.). *Dev. Comp. Immunol.* 22: 493-506.
- Rozen S and Skaletsky HJ (2000). Primer 3 on the WWW for General Users and for Biologist Programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (Krawetz S and Misener S, eds.). Humana Press, Totowa, 365-386.
- Schmittgen TD and Livak KJ (2008). Analyzing real-time PCR data by the comparative C<sub>T</sub> method. *Nat. Protoc.* 3: 1101-1108.
- Scott GR, Richards JG, Forbush B, Isenring P, et al. (2004). Changes in gene expression in gills of the euryhaline killifish *Fundulus heteroclitus* after abrupt salinity transfer. *Am. J. Physiol. Cell Physiol.* 287: C300-C309.
- Takeda H and Shimada A (2010). The art of medaka genetics and genomics: what makes them so unique? *Annu. Rev. Genet.* 44: 217-241.
- Todgham AE and Hofmann GE (2009). Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO<sub>2</sub>-driven seawater acidification. *J. Exp. Biol.* 212: 2579-2594.
- Tseng YC and Hwang PP (2008). Some insights into energy metabolism for osmoregulation in fish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 148: 419-429.
- Tufts BL, Esbaugh A and Lund SG (2003). Comparative physiology and molecular evolution of carbonic anhydrase in the erythrocytes of early vertebrates. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 136: 259-269.
- Vijayan M, Morgan J, Sakamoto T, Grau E, et al. (1996). Food-deprivation affects seawater acclimation in tilapia: hormonal and metabolic changes. *J. Exp. Biol.* 199: 2467-2475.

- Wang YS, Gonzalez RJ, Patrick ML, Grosell M, et al. (2003). Unusual physiology of scale-less carp, *Gymnocypris przewalskii*, in Lake Qinghai: a high altitude alkaline saline lake. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 134: 409-421.
- Wilkie MP and Wood CM (1996). The adaptations of fish to extremely alkaline environments. *Comp. Biochem. Phys. B* 113: 665-673.
- Yao ZL, Lai QF, Zhou K, Rizalita RE, et al. (2010). Developmental biology of medaka fish (*Oryzias latipes*) exposed to alkalinity stress. *J. Appl. Ichthyol.* 26: 397-402.
- Yum S, Woo S, Kagami Y, Park HS, et al. (2010). Changes in gene expression profile of medaka with acute toxicity of Arochlor 1260, a polychlorinated biphenyl mixture. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 151: 51-56.
- Zhang Z, Ju Z, Wells MC and Walter RB (2009). Genomic approaches in the identification of hypoxia biomarkers in model fish species. *J. Exp. Mar. Biol. Ecol.* 381: S180-S187.

**SUPPLEMENTARY MATERIAL**

**Supplementary Table 1.** List of up-regulated genes in the gills of medaka exposed to carbonate alkalinity stress ( $P < 0.05$ , fold change  $> 1.5$ ).

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_1050_P1414333748	TC63668	799977	1.501355	up	1E-61
CUST_22262_P1414333748	TC84882	449656	1.501972	up	2E-52
CUST_16526_P1414333748	TC79146	394039	1.503811	up	1E-38
CUST_5169_P1414333748	TC67787	560091	1.504662	up	8E-20
CUST_25657_P1414333748	TC88280	678628	1.507466	up	1E-152
CUST_20169_P1414333748	TC82789	30709	1.508469	up	1E-69
CUST_19925_P1414333748	TC82545	436906	1.508974	up	1E-103
CUST_18493_P1414333748	TC81113	100007272	1.510119	up	0.035
CUST_6724_P1414333748	TC69342	563726	1.510697	up	0.0001
CUST_24139_P1414333748	TC86761	559733	1.511316	up	0.018
CUST_20096_P1414333748	TC82716	560091	1.51569	up	6E-11
CUST_6877_P1414333748	TC69495	406605	1.515769	up	2E-86
CUST_178_P1414333748	TC62796	563937	1.51619	up	2E-33
CUST_4502_P1414333748	TC67120	564619	1.516589	up	9E-71
CUST_6782_P1414333748	TC69400	406625	1.51821	up	9E-31
CUST_22963_P1414333748	TC85583	559931	1.527209	up	3E-61
CUST_3992_P1414333748	TC66610	565728	1.527532	up	9E-79
CUST_18931_P1414333748	TC81551	100005564	1.527723	up	4E-14
CUST_453_P1414333748	TC63071	30733	1.527737	up	0
CUST_7499_P1414333748	TC70117	494155	1.528129	up	1E-122
CUST_6611_P1414333748	TC69229	791145	1.528442	up	1E-82
CUST_14717_P1414333748	TC77336	30709	1.528717	up	7E-42
CUST_10961_P1414333748	TC73579	100150573	1.529973	up	0.85
CUST_66148	TC66148	406813	1.530297	up	5E-79
CUST_3530_P1414333748	TC69845	393203	1.531143	up	2E-20
CUST_7227_P1414333748	TC75282	445139	1.531953	up	1.2
CUST_12663_P1414333748	TC82641	566767	1.532666	up	1.4
CUST_20021_P1414333748	TC79893	378480	1.532318	up	1E-11
CUST_17273_P1414333748	TC63095	393569	1.532621	up	8E-78
CUST_477_P1414333748	TC68841	567949	1.532609	up	0.26
CUST_6223_P1414333748	TC69528	445487	1.534896	up	2E-41
CUST_6910_P1414333748	TC70630	561921	1.536371	up	7E-30
CUST_8012_P1414333748	TC75127	406663	1.536462	up	2E-87
CUST_12508_P1414333748	TC71773	565691	1.537198	up	0.54
CUST_9155_P1414333748	TC87422	327263	1.538585	up	5E-35
CUST_24799_P1414333748	TC80552	406312	1.540318	up	0.91
CUST_17932_P1414333748	TC71958	323956	1.540885	up	2E-60

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_12380_P1414333748	TC74999	100148429	1.543063	up	4.7
CUST_24928_P1414333748	TC87551	100150174	1.543565	up	0.0005
CUST_9957_P1414333748	TC72575	793158	1.543902	up	1E-104
CUST_12811_P1414333748	TC75430	569243	1.544164	up	4.7
CUST_3414_P1414333748	TC66032	564886	1.54459	up	1E-79
CUST_9983_P1414333748	TC72601	336650	1.54467	up	9E-32
CUST_3967_P1414333748	TC66585	81584	1.545289	up	2E-66
CUST_2791_P1414333748	TC65409	406467	1.545337	up	3E-13
CUST_17961_P1414333748	TC80581	561018	1.546234	up	3E-80
CUST_23691_P1414333748	TC86312	324023	1.549046	up	0
CUST_5020_P1414333748	TC67638	553723	1.551293	up	2E-54
CUST_16202_P1414333748	TC78821	571333	1.551635	up	5E-61
CUST_13265_P1414333748	TC75884	321383	1.552054	up	1E-54
CUST_14946_P1414333748	TC77565	554780	1.553388	up	1.2
CUST_10540_P1414333748	TC73158	100003148	1.553721	up	3E-48
CUST_16034_P1414333748	TC78653	562550	1.554234	up	5.9
CUST_15976_P1414333748	TC78595	564651	1.555121	up	0.99
CUST_25201_P1414333748	TC87824	569039	1.555512	up	1.8
CUST_16131_P1414333748	TC78750	100001684	1.555858	up	9.6
CUST_10568_P1414333748	TC73186	100000278	1.556595	up	1E-07
CUST_9256_P1414333748	TC71874	192295	1.557095	up	1E-21
CUST_3749_P1414333748	TC66367	492707	1.557748	up	3E-89
CUST_4636_P1414333748	TC67254	793123	1.557874	up	2.7
CUST_19354_P1414333748	TC81974	571327	1.557927	up	0.39
CUST_5821_P1414333748	TC68439	559170	1.558009	up	3
CUST_6671_P1414333748	TC69289	321383	1.560745	up	1E-54
CUST_13565_P1414333748	TC76184	393648	1.562743	up	9E-96
CUST_7271_P1414333748	TC69889	100148877	1.563766	up	0.000004
CUST_11671_P1414333748	TC74290	436748	1.563792	up	9E-98
CUST_16225_P1414333748	TC78844	100002754	1.564425	up	9E-37
CUST_23070_P1414333748	TC85690	406264	1.564778	up	2
CUST_2941_P1414333748	TC65559	445107	1.564969	up	3E-79
CUST_1811_P1414333748	TC64429	30331	1.566152	up	1E-126
CUST_10787_P1414333748	TC73405	798311	1.566651	up	5E-14
CUST_246_P1414333748	TC62864	335339	1.569679	up	1E-116
CUST_14880_P1414333748	TC77499	560703	1.570598	up	2E-53
CUST_3929_P1414333748	TC66547	324023	1.574817	up	0.054

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_13553_P1414333748	TC76172	795668	1.618165	up	2E-07
CUST_12766_P1414333748	TC75385	560911	1.618485	up	1E-36
CUST_633_P1414333748	TC63251	792285	1.620886	up	0.0001
CUST_19699_P1414333748	TC82319	393239	1.621462	up	9E-35
CUST_8156_P1414333748	TC70774	569875	1.621952	up	6E-39
CUST_22557_P1414333748	TC85177	474329	1.624152	up	3E-44
CUST_12944_P1414333748	TC75563	569051	1.62669	up	4E-16
CUST_9285_P1414333748	TC71903	100006838	1.627024	up	4E-79
CUST_2470_P1414333748	TC65088	553969	1.627574	up	0
CUST_1336_P1414333748	TC63954	266962	1.628082	up	1E-118
CUST_20867_P1414333748	TC83487	560127	1.628432	up	1.6
CUST_10559_P1414333748	TC73177	558878	1.629185	up	0.99
CUST_8985_P1414333748	TC71603	563855	1.630549	up	3E-78
CUST_20794_P1414333748	TC83414	393796	1.630686	up	6E-82
CUST_19635_P1414333748	TC82255	797134	1.631266	up	0.82
CUST_11925_P1414333748	TC74544	569548	1.631596	up	0.000007
CUST_11696_P1414333748	TC74315	322527	1.632984	up	6E-54
CUST_14731_P1414333748	TC77350	326857	1.634389	up	2E-11
CUST_20341_P1414333748	TC82961	403049	1.635539	up	1.3
CUST_16255_P1414333748	TC78874	572267	1.636922	up	1.9
CUST_2532_P1414333748	TC65150	553969	1.638375	up	1E-25
CUST_7757_P1414333748	TC70375	541486	1.639349	up	7E-20
CUST_11742_P1414333748	TC74361	570652	1.639588	up	1.8
CUST_24538_P1414333748	TC87161	436842	1.643553	up	9E-53
CUST_16143_P1414333748	TC78762	791152	1.645912	up	2E-28
CUST_15230_P1414333748	TC77849	100151617	1.646075	up	1E-27
CUST_390_P1414333748	TC63008	30481	1.646317	up	1E-126
CUST_25794_P1414333748	TC88417	567938	1.647547	up	0.67
CUST_9560_P1414333748	TC72178	751728	1.650841	up	2E-33
CUST_10202_P1414333748	TC72820	171481	1.651806	up	2E-33
CUST_26384_P1414333748	TC89008	558940	1.653636	up	2E-30
CUST_6022_P1414333748	TC68640	559931	1.654952	up	9E-53
CUST_4298_P1414333748	TC66916	559775	1.65518	up	6E-46
CUST_15119_P1414333748	TC77738	100004674	1.657601	up	2E-74
CUST_1379_P1414333748	TC63997	550522	1.658005	up	1E-129
CUST_13024_P1414333748	TC75643	572121	1.661495	up	0.4
CUST_5074_P1414333748	TC67692	436842	1.666742	up	2E-88
CUST_8483_P1414333748	TC71101	791152	1.668904	up	2E-48

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_22804_P1414333748	TC85424	327626	1.57593	up	0.002
CUST_3941_P1414333748	TC66559	394111	1.57659	up	2E-70
CUST_13892_P1414333748	TC76511	550476	1.57671	up	0.00005
CUST_23051_P1414333748	TC85671	100151272	1.578759	up	0.13
CUST_21263_P1414333748	TC83883	797628	1.580393	up	4E-93
CUST_21347_P1414333748	TC83967	192297	1.581871	up	1E-104
CUST_2514_P1414333748	TC65132	100126022	1.582082	up	2E-41
CUST_12311_P1414333748	TC74930	402900	1.582478	up	4E-42
CUST_4364_P1414333748	TC66982	100000633	1.583248	up	1.1
CUST_23531_P1414333748	TC86152	100003686	1.584243	up	0.003
CUST_2859_P1414333748	TC65477	555211	1.585734	up	5E-55
CUST_7560_P1414333748	TC70178	777635	1.586303	up	7E-25
CUST_24068_P1414333748	TC86690	553389	1.587731	up	4E-81
CUST_3870_P1414333748	TC66488	555922	1.58804	up	1E-104
CUST_12783_P1414333748	TC75402	768158	1.588529	up	9E-74
CUST_3412_P1414333748	TC66030	567403	1.58899	up	1E-50
CUST_23171_P1414333748	TC85791	100034459	1.589096	up	6.5
CUST_12925_P1414333748	TC75544	445392	1.589803	up	0.00003
CUST_23233_P1414333748	TC85853	558079	1.590995	up	0.55
CUST_16422_P1414333748	TC79042	550522	1.591318	up	1E-22
CUST_22207_P1414333748	TC84827	567075	1.592365	up	5.2
CUST_8014_P1414333748	TC70632	386663	1.592547	up	0
CUST_12932_P1414333748	TC75551	393856	1.592634	up	2E-40
CUST_7460_P1414333748	TC70078	564342	1.593895	up	1E-42
CUST_9607_P1414333748	TC72225	100148002	1.595565	up	1.1
CUST_15235_P1414333748	TC77854	565155	1.596345	up	0
CUST_12423_P1414333748	TC75042	324023	1.598084	up	7E-62
CUST_25041_P1414333748	TC87664	100126029	1.602739	up	7E-15
CUST_17958_P1414333748	TC80578	550522	1.603111	up	5E-47
CUST_22445_P1414333748	TC85065	550522	1.603556	up	1E-123
CUST_8010_P1414333748	TC70628	751635	1.60354	up	2E-52
CUST_4094_P1414333748	TC66712	555795	1.60819	up	1E-26
CUST_8611_P1414333748	TC71229	550522	1.612537	up	1E-129
CUST_8112_P1414333748	TC70730	794613	1.613044	up	2E-19
CUST_5329_P1414333748	TC67947	321283	1.615288	up	8E-49
CUST_10282_P1414333748	TC72900	393796	1.616104	up	1E-18
CUST_5743_P1414333748	TC68361	393452	1.61661	up	0.074

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**Supplementary Table 1. Continued.**

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs COG] fold change	[C32G vs COG] regulation	dre e value
CUST_20865_P1414333748	TC83485	568029	1.669407	up	0
CUST_20909_P1414333748	TC83529	100149983	1.673113	up	2.9
CUST_23552_P1414333748	TC86173	450032	1.675415	up	2E-35
CUST_23909_P1414333748	TC86531	572335	1.676346	up	0.021
CUST_10356_P1414333748	TC72974	321245	1.679301	up	1E-164
CUST_5972_P1414333748	TC68590	415176	1.68076	up	8E-85
CUST_21893_P1414333748	TC84513	100148015	1.681148	up	0.31
CUST_15396_P1414333748	TC78015	565172	1.681374	up	3E-49
CUST_7297_P1414333748	TC69915	799265	1.68222	up	3E-10
CUST_18980_P1414333748	TC81600	556118	1.683656	up	0.013
CUST_7305_P1414333748	TC69923	799116	1.68466	up	3E-27
CUST_24865_P1414333748	TC87488	567769	1.685012	up	1E-84
CUST_13397_P1414333748	TC76016	393209	1.685659	up	3.5
CUST_9842_P1414333748	TC72460	555550	1.686601	up	1.7
CUST_10102_P1414333748	TC72720	373140	1.687896	up	0
CUST_13042_P1414333748	TC75661	571333	1.687982	up	5E-08
CUST_17857_P1414333748	TC80477	100151233	1.688257	up	7.9
CUST_5890_P1414333748	TC68508	569399	1.68845	up	7E-51
CUST_1056_P1414333748	TC63674	282559	1.688628	up	0
CUST_18884_P1414333748	TC81504	556118	1.689734	up	0.015
CUST_24628_P1414333748	TC87251	557305	1.69012	up	1.6
CUST_545_P1414333748	TC63163	767691	1.690823	up	1E-67
CUST_21216_P1414333748	TC83836	550522	1.692946	up	8E-52
CUST_11948_P1414333748	TC74567	325885	1.69457	up	1E-116
CUST_22900_P1414333748	TC85520	563121	1.696785	up	0.72
CUST_5014_P1414333748	TC67632	286831	1.697467	up	2.1
CUST_6681_P1414333748	TC69299	563121	1.697479	up	0.68
CUST_14953_P1414333748	TC77572	793736	1.698215	up	3
CUST_23640_P1414333748	TC86261	393648	1.700551	up	2E-72
CUST_8653_P1414333748	TC71271	100150315	1.701191	up	5E-18
CUST_6224_P1414333748	TC68842	795335	1.7015	up	0.7
CUST_11755_P1414333748	TC74374	797351	1.702053	up	1E-69
CUST_3480_P1414333748	TC66098	100329124	1.703984	up	0.62
CUST_10149_P1414333748	TC72767	57969	1.706339	up	2E-89
CUST_1316_P1414333748	TC63934	613246	1.706344	up	0
CUST_1224_P1414333748	TC63842	100004001	1.706519	up	3E-62
CUST_2301_P1414333748	TC64919	100004977	1.707123	up	1E-113
CUST_13621_P1414333748	TC76240	100149456	1.710569	up	9E-11

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_5899_P1414333748	TC68517	100004977	1.711911	up	1E-128
CUST_17087_P1414333748	TC79707	100002856	1.712597	up	1
CUST_3450_P1414333748	TC66068	553575	1.719085	up	7E-98
CUST_10323_P1414333748	TC72941	60637	1.719383	up	9E-97
CUST_10507_P1414333748	TC73125	404617	1.721287	up	1E-31
CUST_4497_P1414333748	TC67115	100006563	1.731099	up	4
CUST_21507_P1414333748	TC84127	368352	1.732247	up	3E-79
CUST_24256_P1414333748	TC86878	30766	1.73342	up	1E-59
CUST_20939_P1414333748	TC83559	406625	1.734808	up	1E-167
CUST_15085_P1414333748	TC77704	406625	1.734962	up	1E-144
CUST_356_P1414333748	TC62974	100004977	1.739322	up	3.3
CUST_1970_P1414333748	TC64588	100147853	1.74495	up	0.095
CUST_22739_P1414333748	TC85359	393460	1.745041	up	9E-14
CUST_9737_P1414333748	TC72355	599333	1.746946	up	2E-96
CUST_23345_P1414333748	TC63347	550352	1.74896	up	0.061
CUST_729_P1414333748	TC63737	799570	1.750868	up	2.5
CUST_1119_P1414333748	TC85965	100009623	1.75297	up	1E-67
CUST_3202_P1414333748	TC65820	566537	1.75581	up	0
CUST_197_P1414333748	TC62815	64607	1.76006	up	2E-37
CUST_24953_P1414333748	TC87576	100005752	1.762561	up	3.6
CUST_3654_P1414333748	TC66272	100150699	1.767008	up	1E-64
CUST_7957_P1414333748	TC70575	406467	1.767092	up	3E-91
CUST_16741_P1414333748	TC79361	447810	1.769459	up	4.2
CUST_18443_P1414333748	TC81063	100151058	1.770508	up	0.65
CUST_18296_P1414333748	TC80916	100141333	1.773357	up	8E-38
CUST_4499_P1414333748	TC65985	567972	1.776865	up	2E-38
CUST_23143_P1414333748	TC67117	30507	1.778153	up	9E-64
CUST_19558_P1414333748	TC85763	436911	1.779138	up	1E-101
CUST_786_P1414333748	TC82178	572182	1.781437	up	1E-46
CUST_8265_P1414333748	TC63404	492485	1.78163	up	8E-18
CUST_15812_P1414333748	TC70883	386589	1.787875	up	1.6
CUST_21922_P1414333748	TC78431	563802	1.791204	up	1E-175
CUST_4570_P1414333748	TC84542	568244	1.791299	up	1E-19
CUST_16152_P1414333748	TC67188	59931	1.791522	up	2E-70
CUST_3678_P1414333748	TC78771	793139	1.795483	up	4E-34
CUST_18474_P1414333748	TC66296	100151052	1.796292	up	
CUST_18474_P1414333748	TC81094			up	

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**Supplementary Table I. Continued.**

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_1128_P1414333748	TC63746	447810	1.797715	up	0
CUST_5571_P1414333748	TC68189	550560	1.797773	up	8E-61
CUST_3036_P1414333748	TC65654	325922	1.804101	up	0
CUST_13574_P1414333748	TC76193	81538	1.80512	up	2E-57
CUST_2827_P1414333748	TC65445	541508	1.807054	up	3E-13
CUST_13537_P1414333748	TC76156	445225	1.807997	up	6E-70
CUST_17936_P1414333748	TC80556	556118	1.8081	up	0.73
CUST_2304_P1414333748	TC64922	394109	1.811866	up	5E-74
CUST_18833_P1414333748	TC81453	559931	1.8121	up	3E-69
CUST_4516_P1414333748	TC67134	553952	1.814262	up	2E-48
CUST_19402_P1414333748	TC82022	796019	1.816967	up	2E-16
CUST_8911_P1414333748	TC71529	393837	1.817378	up	8E-29
CUST_6092_P1414333748	TC68710	100150701	1.817743	up	0.31
CUST_21371_P1414333748	TC83991	559431	1.822617	up	0.38
CUST_13419_P1414333748	TC76038	550560	1.82292	up	7E-78
CUST_22906_P1414333748	TC85526	445107	1.823138	up	6E-96
CUST_16476_P1414333748	TC79096	570542	1.825179	up	2E-08
CUST_12402_P1414333748	TC75021	100007704	1.828798	up	0.00003
CUST_25490_P1414333748	TC88113	572007	1.830556	up	0.36
CUST_7873_P1414333748	TC70491	569169	1.830941	up	9E-73
CUST_9546_P1414333748	TC72164	140820	1.831138	up	6E-26
CUST_21188_P1414333748	TC83808	324345	1.832939	up	2.2
CUST_8166_P1414333748	TC70784	795748	1.835911	up	4E-73
CUST_164_P1414333748	TC62782	140531	1.836669	up	1E-110
CUST_6728_P1414333748	TC69346	100148638	1.839198	up	2E-21
CUST_16577_P1414333748	TC79197	572267	1.839359	up	2.6
CUST_26080_P1414333748	TC88703	100005128	1.840171	up	6.1
CUST_20671_P1414333748	TC83291	57969	1.842869	up	5E-76
CUST_15997_P1414333748	TC78616	406349	1.842913	up	2E-58
CUST_17025_P1414333748	TC79645	393433	1.843469	up	3E-28
CUST_9653_P1414333748	TC72271	567416	1.845435	up	3E-11
CUST_13449_P1414333748	TC76068	100002992	1.845673	up	8E-64
CUST_2448_P1414333748	TC65066	386663	1.85107	up	2E-85
CUST_10508_P1414333748	TC73126	100006354	1.852291	up	0.68
CUST_21600_P1414333748	TC84220	100000517	1.854172	up	1E-66
CUST_18388_P1414333748	TC81008	563278	1.854378	up	0.12
CUST_6235_P1414333748	TC68853	321283	1.855133	up	2E-34
CUST_2251_P1414333748	TC64869	393394	1.860125	up	1E-79

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_789_P1414333748	TC63407	797351	1.860682	up	7E-49
CUST_21561_P1414333748	TC84181	553723	1.866708	up	6E-16
CUST_22790_P1414333748	TC85410	100147895	1.868127	up	2E-58
CUST_16850_P1414333748	TC79470	557843	1.869738	up	1.2
CUST_16704_P1414333748	TC79324	57969	1.875475	up	1E-79
CUST_11713_P1414333748	TC74332	373140	1.879978	up	1E-38
CUST_12872_P1414333748	TC75491	796968	1.885674	up	0.12
CUST_8040_P1414333748	TC70658	406383	1.890108	up	4E-99
CUST_5992_P1414333748	TC68610	566840	1.899453	up	2E-43
CUST_18960_P1414333748	TC81580	402914	1.906282	up	3E-29
CUST_1973_P1414333748	TC64591	352913	1.906666	up	4E-50
CUST_13650_P1414333748	TC76269	30516	1.907231	up	1E-113
CUST_6058_P1414333748	TC68676	494077	1.90838	up	1E-147
CUST_21991_P1414333748	TC84611	550517	1.910667	up	4E-49
CUST_24810_P1414333748	TC87433	406387	1.918014	up	1E-59
CUST_7096_P1414333748	TC69714	560778	1.930118	up	1E-124
CUST_6980_P1414333748	TC69598	794173	1.932368	up	6E-56
CUST_13415_P1414333748	TC76034	564924	1.941378	up	6E-38
CUST_7096_P1414333748	TC79475	794519	1.942085	up	4.1
CUST_14897_P1414333748	TC82519	565503	1.946869	up	5E-48
CUST_12335_P1414333748	TC77516	100006838	1.947819	up	2E-13
CUST_14925_P1414333748	TC74954	569185	1.947918	up	2.8
CUST_19794_P1414333748	TC82414	562396	1.955282	up	0.61
CUST_1143_P1414333748	TC63761	563660	1.956006	up	2.4
CUST_1452_P1414333748	TC62966	436832	1.957353	up	0.001
CUST_2051_P1414333748	TC64070	64607	1.973992	up	8E-83
CUST_48_P1414333748	TC84671	378727	1.975913	up	1E-114
CUST_7664_P1414333748	TC62666	550420	1.976301	up	1E-141
CUST_26207_P1414333748	TC70282	30268	1.981252	up	0.018
CUST_10743_P1414333748	TC88830	568268	1.981252	up	0.018
CUST_6778_P1414333748	TC73361	334173	1.98394	up	2.2
CUST_7950_P1414333748	TC69396	555765	1.986095	up	0.0003
CUST_23398_P1414333748	TC70568	100002992	1.989128	up	1E-64
CUST_10503_P1414333748	TC86018	562906	1.989524	up	4E-72
CUST_16689_P1414333748	TC73121	100150846	1.992906	up	8
CUST_3926_P1414333748	TC79309	777627	1.997562	up	0.000001
	TC66544	550268	1.998183	up	0
			2.004729	up	5E-90

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_22020_P1414333748	TC84640	793139	2.01543	up	1E-24
CUST_8696_P1414333748	TC71314	393909	2.019158	up	1E-113
CUST_17664_P1414333748	TC80284	406467	2.020376	up	7E-09
CUST_538_P1414333748	TC63156	386701	2.025635	up	0
CUST_19766_P1414333748	TC82386	393909	2.030202	up	3E-35
CUST_13824_P1414333748	TC76443	100150625	2.03203	up	1.7
CUST_21388_P1414333748	TC84008	407620	2.034202	up	0
CUST_24226_P1414333748	TC86848	321664	2.035019	up	4E-20
CUST_11246_P1414333748	TC73864	100005964	2.036426	up	2E-08
CUST_10492_P1414333748	TC73110	100002565	2.037712	up	2.7
CUST_6476_P1414333748	TC69094	334240	2.046788	up	1.3
CUST_13609_P1414333748	TC76228	559789	2.052683	up	1E-72
CUST_20483_P1414333748	TC83103	793139	2.053421	up	7
CUST_14824_P1414333748	TC77443	567631	2.054309	up	1E-83
CUST_15783_P1414333748	TC78402	558261	2.056387	up	1E-79
CUST_11753_P1414333748	TC74372	100149782	2.05661	up	4E-38
CUST_17429_P1414333748	TC80049	100149782	2.063774	up	3E-07
CUST_9454_P1414333748	TC72072	100004978	2.069198	up	3.3
CUST_18109_P1414333748	TC80729	325449	2.071873	up	5E-48
CUST_10803_P1414333748	TC73421	321453	2.077669	up	1E-170
CUST_20556_P1414333748	TC83176	403137	2.080235	up	6.8
CUST_10314_P1414333748	TC72932	368684	2.080998	up	3E-25
CUST_24021_P1414333748	TC86643	403138	2.082388	up	5.8
CUST_3696_P1414333748	TC66314	777736	2.08557	up	0.71
CUST_11425_P1414333748	TC74044	100151170	2.08946	up	0.002
CUST_15078_P1414333748	TC77697	30643	2.094785	up	4E-15
CUST_26418_P1414333748	TC89042	798998	2.10453	up	8E-42
CUST_14352_P1414333748	TC76971	406558	2.115048	up	1E-175
CUST_3136_P1414333748	TC65754	447864	2.122042	up	5E-76
CUST_18152_P1414333748	TC80772	792970	2.123961	up	1.2
CUST_20006_P1414333748	TC82626	563855	2.131727	up	3E-78
CUST_9883_P1414333748	TC72501	436894	2.133305	up	6E-99
CUST_14979_P1414333748	TC77598	572862	2.136404	up	4E-30
CUST_16532_P1414333748	TC79152	768288	2.144167	up	0
CUST_2798_P1414333748	TC65416	550268	2.152133	up	1E-50
CUST_11741_P1414333748	TC74360	562105	2.154536	up	5E-69
CUST_18849_P1414333748	TC81469	562906	2.162296	up	2.5
CUST_13649_P1414333748	TC76268	445405	2.162795	up	1E-29

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**Supplementary Table 1. Continued.**

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_5422_P1414333748	TC68040	794337	2.163419	up	0.71
CUST_20079_P1414333748	TC82699	100001258	2.166049	up	0.74
CUST_8842_P1414333748	TC71460	336471	2.166803	up	0.002
CUST_4213_P1414333748	TC66831	100002245	2.202849	up	0.000001
CUST_7699_P1414333748	TC70317	321496	2.223404	up	2E-70
CUST_1125_P1414333748	TC73743	393461	2.227318	up	3E-28
CUST_2288_P1414333748	TC64906	321496	2.230035	up	2E-70
CUST_23421_P1414333748	TC86041	100150668	2.237217	up	0.67
CUST_3492_P1414333748	TC66110	116991	2.240732	up	0
CUST_5658_P1414333748	TC68276	561933	2.245024	up	1E-86
CUST_4949_P1414333748	TC67567	100005197	2.252708	up	1E-60
CUST_15197_P1414333748	TC77816	555962	2.256443	up	2E-29
CUST_20723_P1414333748	TC83343	566517	2.259451	up	3E-78
CUST_26242_P1414333748	TC88865	58938	2.276655	up	2E-36
CUST_22795_P1414333748	TC85415	678540	2.278473	up	1E-169
CUST_12706_P1414333748	TC75325	566212	2.285543	up	7E-43
CUST_5962_P1414333748	TC68580	386701	2.291285	up	0
CUST_9205_P1414333748	TC71823	100007474	2.301009	up	3E-68
CUST_20523_P1414333748	TC83143	100005596	2.305075	up	2.9
CUST_21261_P1414333748	TC83881	100001026	2.311522	up	0.18
CUST_4590_P1414333748	TC67208	393335	2.311664	up	6E-65
CUST_19543_P1414333748	TC82163	406731	2.314158	up	2E-17
CUST_26263_P1414333748	TC88886	56822	2.321463	up	3E-29
CUST_20695_P1414333748	TC83315	114367	2.322158	up	1E-142
CUST_7016_P1414333748	TC65260	447864	2.334991	up	1E-117
CUST_2642_P1414333748	TC65260	568997	2.336488	up	5E-32
CUST_18170_P1414333748	TC80790	100147886	2.340991	up	8E-31
CUST_14163_P1414333748	TC76782	559931	2.342146	up	5E-83
CUST_17016_P1414333748	TC79636	567023	2.346145	up	1.2
CUST_25318_P1414333748	TC87941	494477	2.349019	up	1E-119
CUST_14099_P1414333748	TC76718	406828	2.38611	up	1E-136
CUST_24658_P1414333748	TC87281	378477	2.38739	up	0.49
CUST_10611_P1414333748	TC73229	406828	2.40445	up	6E-45
CUST_22026_P1414333748	TC84646	406828	2.4371	up	2.438885
CUST_7648_P1414333748	TC70266	791147	2.452802	up	2E-67
CUST_17101_P1414333748	TC79721	321453	2.462583	up	7E-18
CUST_22068_P1414333748	TC84688	100001568	2.462583	up	0.0001
CUST_24229_P1414333748	TC86851		2.47172	up	

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_17174_P1414333748	TC79794	10002245	2.499939	up	0.00002
CUST_21010_P1414333748	TC83630	100148413	2.506785	up	3.7
CUST_9773_P1414333748	TC72391	565972	2.510358	up	1E-107
CUST_16053_P1414333748	TC78672	561639	2.511761	up	0.86
CUST_11461_P1414333748	TC74080	570437	2.516529	up	4.3
CUST_22047_P1414333748	TC84667	566390	2.518271	up	4.8
CUST_7526_P1414333748	TC70144	767638	2.533341	up	6E-59
CUST_632_P1414333748	TC63250	751646	2.545378	up	0.87
CUST_12909_P1414333748	TC75528	393676	2.547501	up	5E-90
CUST_22907_P1414333748	TC85527	436740	2.550007	up	1.2
CUST_2510_P1414333748	TC65128	796521	2.576378	up	4E-29
CUST_12168_P1414333748	TC74787	797503	2.580027	up	6E-35
CUST_426_P1414333748	TC63044	30341	2.588263	up	1E-58
CUST_10344_P1414333748	TC72962	566212	2.594836	up	9E-32
CUST_17539_P1414333748	TC80159	30645	2.61809	up	3E-19
CUST_19256_P1414333748	TC81876	403019	2.630709	up	1E-55
CUST_10048_P1414333748	TC72666	563265	2.643338	up	5E-25
CUST_4043_P1414333748	TC66661	566581	2.656291	up	0
CUST_7110_P1414333748	TC69728	445116	2.658501	up	1E-105
CUST_18230_P1414333748	TC80850	406731	2.667862	up	1E-137
CUST_4879_P1414333748	TC67497	325557	2.68097	up	1E-149
CUST_25305_P1414333748	TC87928	100151170	2.689204	up	3E-10
CUST_6152_P1414333748	TC68770	560341	2.713023	up	8E-22
CUST_6425_P1414333748	TC69043	322055	2.719115	up	1E-26
CUST_9993_P1414333748	TC72611	393246	2.763995	up	0.35
CUST_1274_P1414333748	TC63892	322055	2.797835	up	0
CUST_10987_P1414333748	TC73605	569258	2.798142	up	6.5
CUST_20074_P1414333748	TC82694	100002245	2.799943	up	0.077
CUST_19660_P1414333748	TC82280	559516	2.817421	up	0.032
CUST_5110_P1414333748	TC67728	493627	2.864817	up	7E-34
CUST_6592_P1414333748	TC69210	795236	2.881843	up	3.7
CUST_17129_P1414333748	TC79749	30123	2.888996	up	2.3
CUST_173_P1414333748	TC62791	30317	2.966959	up	2E-72
CUST_4650_P1414333748	TC67268	100149969	2.98463	up	4E-81
CUST_4515_P1414333748	TC67133	449552	2.994059	up	3E-07
CUST_8348_P1414333748	TC70966	436732	2.997656	up	1E-33
CUST_17665_P1414333748	TC80285	567846	3.037911	up	0.043
CUST_2767_P1414333748	TC65385	317738	3.057566	up	1E-114

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_17330_P1414333748	TC79950	567846	3.068644	up	0.042
CUST_9375_P1414333748	TC71993	323269	3.082744	up	2E-62
CUST_14151_P1414333748	TC76770	30616	3.134665	up	0.39
CUST_7510_P1414333748	TC70128	571850	3.151876	up	1E-139
CUST_15344_P1414333748	TC77963	100151170	3.152278	up	7E-34
CUST_2972_P1414333748	TC65590	100144567	3.217869	up	4E-32
CUST_11979_P1414333748	TC74598	30245	3.22156	up	1.6
CUST_8682_P1414333748	TC71300	791216	3.260074	up	1E-104
CUST_7947_P1414333748	TC70565	325599	3.322079	up	7E-58
CUST_6784_P1414333748	TC69402	553744	3.335331	up	2E-21
CUST_4661_P1414333748	TC67279	335651	3.341081	up	1E-99
CUST_2349_P1414333748	TC64967	245699	3.353819	up	1.1
CUST_14873_P1414333748	TC77492	447878	3.379912	up	5E-47
CUST_24023_P1414333748	TC86645	550411	3.396086	up	1E-53
CUST_24916_P1414333748	TC87539	327566	3.478166	up	8E-93
CUST_12265_P1414333748	TC74884	100036547	3.480915	up	0.048
CUST_8322_P1414333748	TC70940	100003066	3.512906	up	0.00002
CUST_24112_P1414333748	TC86734	557459	3.533389	up	2.6
CUST_5543_P1414333748	TC68161	393483	3.571868	up	3E-23
CUST_8489_P1414333748	TC71107	30128	3.602231	up	0
CUST_14457_P1414333748	TC77076	432374	3.678984	up	7E-49
CUST_22655_P1414333748	TC85275	393483	3.701699	up	3E-31
CUST_12746_P1414333748	TC76324	571214	3.72584	up	0.58
CUST_10985_P1414333748	TC75365	550322	3.771113	up	1E-37
CUST_17419_P1414333748	TC80039	324140	3.846087	up	2E-58
CUST_9122_P1414333748	TC71740	492647	4.198755	up	0
CUST_21142_P1414333748	TC83762	447878	4.211862	up	2E-38
CUST_17486_P1414333748	TC80106	544656	4.294793	up	5E-74
CUST_6060_P1414333748	TC68678	566212	4.315548	up	2E-33
CUST_17599_P1414333748	TC80219	359841	4.363059	up	3E-41
CUST_6961_P1414333748	TC69579	406513	4.438208	up	3E-97
CUST_18904_P1414333748	TC81524	563388	4.455945	up	2.5
CUST_9148_P1414333748	TC71766	100001788	4.463423	up	0.009
CUST_4005_P1414333748	TC66623	571762	4.603817	up	0.64
CUST_11647_P1414333748	TC74266	100038797	4.648498	up	0.097
CUST_2069_P1414333748	TC64687	449866	4.687213	up	0
CUST_18317_P1414333748	TC80937	359841	4.692396	up	2E-37
			4.782817	up	2E-43

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_22548_P1414333748	TC85168	767700	4.79233	up	4E-40
CUST_10361_P1414333748	TC72979	568975	4.836072	up	1E-23
CUST_16882_P1414333748	TC79502	327284	4.838827	up	9E-34
CUST_9173_P1414333748	TC71791	100001788	4.876383	up	0.2
CUST_2843_P1414333748	TC65461	100151287	5.013922	up	1E-94
CUST_16448_P1414333748	TC79068	100150209	5.238195	up	1.2
CUST_5320_P1414333748	TC67938	192294	5.260218	up	0.79
CUST_6321_P1414333748	TC68939	565984	5.272771	up	4E-36
CUST_12385_P1414333748	TC75004	445065	5.277498	up	1E-109
CUST_10875_P1414333748	TC73493	378866	5.556612	up	8E-40
CUST_17335_P1414333748	TC79955	30597	5.598797	up	4E-60
CUST_23586_P1414333748	TC86207	84041	5.689823	up	7E-96
CUST_5299_P1414333748	TC67917	432374	5.74124	up	4E-72
CUST_23479_P1414333748	TC86100	100148380	5.80966	up	0.64
CUST_10533_P1414333748	TC73151	567411	6.191104	up	2.4
CUST_5873_P1414333748	TC68491	799308	6.408137	up	1E-31
CUST_14334_P1414333748	TC76953	368352	6.579829	up	7E-08
CUST_5487_P1414333748	TC68105	492816	6.651357	up	4E-45
CUST_7633_P1414333748	TC70251	415138	6.84565	up	1E-129
CUST_21660_P1414333748	TC84280	563244	6.880057	up	2.2
CUST_25773_P1414333748	TC88396	561967	6.926927	up	1E-151
CUST_24668_P1414333748	TC87291	30343	7.001814	up	8E-22
CUST_7652_P1414333748	TC70270	561967	7.098282	up	1E-161
CUST_18568_P1414333748	TC81188	791844	8.102504	up	5E-80
CUST_18545_P1414333748	TC81165	562300	8.203242	up	3E-29
CUST_9781_P1414333748	TC72399	335153	8.338406	up	0.87
CUST_18756_P1414333748	TC81376	568277	8.891761	up	5.1
CUST_25465_P1414333748	TC88088	378866	9.601078	up	8E-41
CUST_18253_P1414333748	TC80873	448856	9.79651	up	3E-18
CUST_13769_P1414333748	TC76388	30164	9.964179	up	1E-118
CUST_3350_P1414333748	TC65968	335651	10.55945	up	1E-91
CUST_22061_P1414333748	TC84681	100149146	11.85712	up	2E-08
CUST_13554_P1414333748	TC76173	360208	12.32283	up	1E-45
CUST_13778_P1414333748	TC76397	399485	12.66512	up	0
CUST_15385_P1414333748	TC78004	794359	14.7858	up	2.1
CUST_10269_P1414333748	TC72887	100002992	15.39041	up	5E-72
CUST_13741_P1414333748	TC76360	447944	16.22088	up	5E-48
CUST_9255_P1414333748	TC71873	100002992	17.01027	up	2E-61

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Supplementary Table 1. Continued.

Probe set ID	Gene symbol	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_10385_P1414333748	TC73003	566212	19.37271	up	1E-32
CUST_10625_P1414333748	TC73243	445095	19.63056	up	3E-13
CUST_4992_P1414333748	TC67610	571762	20.18644	up	0.000005
CUST_23029_P1414333748	TC85649	798565	21.4341	up	2.5
CUST_1346_P1414333748	TC63964	30645	22.5728	up	1E-19
CUST_9825_P1414333748	TC72443	447944	22.64274	up	9E-49
CUST_5142_P1414333748	TC67760	568241	23.03652	up	1E-23
CUST_10262_P1414333748	TC72880	30295	24.81935	up	1E-39
CUST_22285_P1414333748	TC84905		29.23207	up	
CUST_10563_P1414333748	TC73181		30.93148	up	
CUST_13047_P1414333748	TC75666	567046	43.01942	up	1E-129
CUST_8380_P1414333748	TC70998	30597	44.31414	up	4E-60
CUST_2220_P1414333748	TC64838	100003133	54.35656	up	4E-95
CUST_8260_P1414333748	TC70878	555765	56.45492	up	2E-66
CUST_4214_P1414333748	TC66832	30537	81.46389	up	1.4
CUST_16620_P1414333748	TC79240	798780	81.97932	up	4.9
CUST_3131_P1414333748	TC65749	567046	91.01174	up	2E-85
CUST_16558_P1414333748	TC79178	81538	101.2286	up	8E-66
CUST_7166_P1414333748	TC69784	100270763	111.2159	up	0.015
CUST_19136_P1414333748	TC81756	100270760	114.0142	up	0.00002
CUST_19435_P1414333748	TC82055	100006521	171.2856	up	3.6
CUST_2337_P1414333748	TC64955	555679	323.2781	up	1E-27

**Supplementary Table 2.** List of down-regulated genes in the gills of medaka exposed to carbonate alkalinity stress ( $P < 0.05$ , fold change  $> 1.5$ ).

Probe set ID	Gene symbol	Regulated	dre	Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_12773_P1414333748	TC75392	368614	1.500758		down	7E-49	
CUST_11348_P1414333748	TC73966	572007	1.501107		down	1E-65	
CUST_21751_P1414333748	TC84371	550512	1.503499		down	2.5	
CUST_8734_P1414333748	TC71352	795467	1.505		down	0.52	
CUST_102_P1414333748	TC62720	561345	1.50689		down	0	
CUST_15774_P1414333748	TC78393	568268	1.507406		down	1.1	
CUST_6337_P1414333748	TC68955	100037360	1.507414		down	1E-46	
CUST_12488_P1414333748	TC75107	100004938	1.507481		down	2E-36	
CUST_6202_P1414333748	TC68820	560793	1.507673		down	2.9	
CUST_20371_P1414333748	TC82991	795916	1.508803		down	1.6	
CUST_4391_P1414333748	TC67009	407712	1.509123		down	1E-74	
CUST_3677_P1414333748	TC66295	557384	1.50929		down	1E-78	
CUST_22421_P1414333748	TC85041	559570	1.509292		down	4E-43	
CUST_18487_P1414333748	TC81107	407714	1.509481		down	4E-15	
CUST_8158_P1414333748	TC70776	140634	1.510386		down	0	
CUST_5100_P1414333748	TC67718	387258	1.511475		down	8E-81	
CUST_155_P1414333748	TC62773	406483	1.511524		down	1E-150	
CUST_14710_P1414333748	TC77329	378854	1.51178		down	1E-75	
CUST_18427_P1414333748	TC81047	794337	1.51288		down	0.99	
CUST_23823_P1414333748	TC86444	565496	1.514627		down	3E-17	
CUST_25446_P1414333748	TC88069	394109	1.515162		down	3E-16	
CUST_13140_P1414333748	TC75759	403051	1.515968		down	6.4	
CUST_19996_P1414333748	TC82616	323883	1.516608		down	9.6	
CUST_18166_P1414333748	TC80786	796880	1.517501		down	1.9	
CUST_4625_P1414333748	TC67243	548602	1.517751		down	2E-76	
CUST_10527_P1414333748	TC73145	556869	1.518174		down	3E-25	
CUST_21180_P1414333748	TC83800	555941	1.518304		down	4.4	
CUST_15985_P1414333748	TC78604	563784	1.52038		down	1E-42	
CUST_15418_P1414333748	TC83163	393351	1.521574		down	1E-77	
CUST_5488_P1414333748	TC78037	445088	1.525749		down	4E-12	
CUST_3454_P1414333748	TC66072	797438	1.525956		down	8E-72	
CUST_17188_P1414333748	TC79808	555940	1.526588		down	6E-49	
CUST_21053_P1414333748	TC83673	557223	1.526929		down	1E-56	
CUST_26281_P1414333748	TC88904	321298	1.527314		down	4E-07	
CUST_20619_P1414333748	TC83239	100151365	1.527653		down	1E-16	
CUST_19957_P1414333748	TC82577	100101653	1.52812		down	0.014	
CUST_11763_P1414333748	TC74382	394161	1.528431		down	3E-11	
			1.529327		down	1.3	

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_5560_P1414333748	TC68178	335098	1.529608	down	5E-64
CUST_7466_P1414333748	TC70084	445495	1.53172	down	5E-53
CUST_24983_P1414333748	TC87606	406707	1.53236	down	1.7
CUST_24394_P1414333748	TC87017	562388	1.532893	down	3.9
CUST_9028_P1414333748	TC71646	100136848	1.533946	down	0.11
CUST_432_P1414333748	TC63050	393342	1.534664	down	3E-58
CUST_23112_P1414333748	TC85732	324479	1.534921	down	0.93
CUST_17707_P1414333748	TC80327	791762	1.53535	down	8E-30
CUST_9549_P1414333748	TC72167	796417	1.535418	down	3E-07
CUST_1640_P1414333748	TC64258	794311	1.536041	down	1E-176
CUST_22466_P1414333748	TC85086	560971	1.536745	down	5.9
CUST_4398_P1414333748	TC67016	562881	1.536853	down	8E-78
CUST_2876_P1414333748	TC65494	570542	1.537185	down	2
CUST_16_P1414333748	TC62634	140425	1.537394	down	0
CUST_11024_P1414333748	TC73642	503523	1.53759	down	0.79
CUST_20607_P1414333748	TC83227	402977	1.53774	down	1E-83
CUST_20156_P1414333748	TC82776	565945	1.53818	down	5E-30
CUST_11451_P1414333748	TC74070	100038777	1.53862	down	3E-15
CUST_5721_P1414333748	TC68339	565695	1.539454	down	10
CUST_9891_P1414333748	TC72509	406308	1.540701	down	1E-66
CUST_11120_P1414333748	TC73738	100007824	1.541089	down	0.08
CUST_23859_P1414333748	TC86481	386723	1.541357	down	3E-08
CUST_14786_P1414333748	TC77405	588048	1.541704	down	2.7
CUST_16713_P1414333748	TC79333	100150473	1.542646	down	1.9
CUST_823_P1414333748	TC63441	573010	1.543499	down	3E-88
CUST_18967_P1414333748	TC81587	60634	1.544497	down	3E-68
CUST_1092_P1414333748	TC63710	557315	1.545126	down	0
CUST_2204_P1414333748	TC64822	562881	1.546003	down	7E-90
CUST_548_P1414333748	TC63166	368305	1.54672	down	2E-82
CUST_8755_P1414333748	TC71373	402969	1.546774	down	2E-08
CUST_19131_P1414333748	TC81751	567986	1.549161	down	8E-51
CUST_10325_P1414333748	TC72943	559552	1.552631	down	8.7
CUST_10467_P1414333748	TC73085	568509	1.553898	down	7E-62
CUST_21464_P1414333748	TC84084	100004879	1.553963	down	2E-92
CUST_10399_P1414333748	TC73017	100037375	1.554731	down	4E-97
CUST_4354_P1414333748	TC66972	100005261	1.554935	down	6E-80
CUST_7941_P1414333748	TC70559	386614	1.556656	down	6E-90
CUST_12159_P1414333748	TC74778	567751	1.557472	down	0

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_9135_P1414333748	TC71753	394038	1.557741	down	0.6
CUST_16244_P1414333748	TC78863	402969	1.557933	down	8E-46
CUST_9081_P1414333748	TC71699	334444	1.559474	down	0.19
CUST_25127_P1414333748	TC87750	100002200	1.561187	down	6E-69
CUST_25015_P1414333748	TC87638	436647	1.561651	down	4E-25
CUST_387_P1414333748	TC63005	352930	1.561693	down	4
CUST_10556_P1414333748	TC73174	100005469	1.561905	down	3E-96
CUST_21403_P1414333748	TC84023	324345	1.562205	down	1E-151
CUST_5977_P1414333748	TC68595	497649	1.562759	down	6E-48
CUST_18688_P1414333748	TC81308	557591	1.562923	down	0.32
CUST_14929_P1414333748	TC77548	569131	1.563679	down	1E-73
CUST_22403_P1414333748	TC85023	447884	1.563875	down	2.2
CUST_19333_P1414333748	TC81953	564820	1.564298	down	2E-26
CUST_23885_P1414333748	TC86507	100151150	1.564781	down	0.018
CUST_21164_P1414333748	TC83784	571932	1.565351	down	0.99
CUST_23197_P1414333748	TC85817	571576	1.565357	down	0.08
CUST_16696_P1414333748	TC79316	100002548	1.565831	down	2.7
CUST_2678_P1414333748	TC65296	30200	1.566707	down	4E-58
CUST_17018_P1414333748	TC79638	100034473	1.566997	down	5
CUST_13861_P1414333748	TC76480	100148963	1.567567	down	1E-95
CUST_8560_P1414333748	TC71178	368234	1.567575	down	6E-94
CUST_8738_P1414333748	TC71356	767760	1.568447	down	4.2
CUST_24490_P1414333748	TC87113	325361	1.568457	down	8E-89
CUST_18516_P1414333748	TC81136	140819	1.568857	down	1.6
CUST_24183_P1414333748	TC86805	569232	1.569071	down	8.8
CUST_4340_P1414333748	TC66958	793524	1.569539	down	2E-39
CUST_15382_P1414333748	TC78001	324254	1.569747	down	0
CUST_25947_P1414333748	TC88570	100006858	1.570356	down	3E-16
CUST_21178_P1414333748	TC83798	100149581	1.572226	down	1.2
CUST_4369_P1414333748	TC66987	360135	1.572779	down	4E-22
CUST_21081_P1414333748	TC66987	792402	1.575282	down	1E-120
CUST_12376_P1414333748	TC83701	492676	1.575321	down	5E-08
CUST_3708_P1414333748	TC74995	557525	1.575498	down	7E-19
CUST_9231_P1414333748	TC66326	406640	1.575776	down	3E-76
CUST_3399_P1414333748	TC71849	100005732	1.57623	down	1E-31
CUST_21285_P1414333748	TC66017	100005854	1.576737	down	1E-62
CUST_13846_P1414333748	TC83905	30663	1.577976	down	5.9
	TC76465	100002909	1.578185	down	9.9

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated	dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_17679_P1414333748	TC80299		571519	1.578477	down	1.7
CUST_8870_P1414333748	TC71488		335339	1.579639	down	1E-20
CUST_25220_P1414333748	TC87843			1.579703	down	
CUST_3377_P1414333748	TC65995		406204	1.581227	down	2
CUST_24952_P1414333748	TC87575		415146	1.581352	down	1E-108
CUST_9101_P1414333748	TC71719	100000160		1.581881	down	2E-50
CUST_9736_P1414333748	TC72354		323464	1.58451	down	2E-50
CUST_16880_P1414333748	TC79500		567560	1.586906	down	1E-115
CUST_17977_P1414333748	TC80597		58326	1.587972	down	9E-72
CUST_6876_P1414333748	TC69494		100002245	1.588279	down	1E-10
CUST_9226_P1414333748	TC71844		100005469	1.588698	down	7E-31
CUST_5969_P1414333748	TC68587		794293	1.589298	down	1E-39
CUST_11201_P1414333748	TC73819	100002060		1.589753	down	1E-58
CUST_3364_P1414333748	TC65982		1590715	1.590715	down	
CUST_23271_P1414333748	TC85891		566173	1.590941	down	2E-09
CUST_14492_P1414333748	TC77111		321064	1.591632	down	1.3
CUST_22643_P1414333748	TC85263		436759	1.592226	down	3E-21
CUST_10707_P1414333748	TC73325	100149074		1.594445	down	0.39
CUST_6135_P1414333748	TC68753		566323	1.59577	down	4E-55
CUST_3191_P1414333748	TC65809		59626	1.598231	down	0
CUST_902_P1414333748	TC63520		791180	1.598486	down	7E-44
CUST_3293_P1414333748	TC65911		554137	1.59852	down	0.47
CUST_4163_P1414333748	TC66781		393378	1.599387	down	1E-163
CUST_6259_P1414333748	TC68877		550452	1.59965	down	2E-89
CUST_16657_P1414333748	TC79277			1.600546	down	
CUST_14164_P1414333748	TC76783		559432	1.600932	down	1.9
CUST_22367_P1414333748	TC84987		541535	1.601112	down	2.4
CUST_13061_P1414333748	TC75680		619192	1.602797	down	1.9
CUST_25011_P1414333748	TC87634		793168	1.604705	down	0
CUST_9739_P1414333748	TC72357		567913	1.605353	down	0.91
CUST_6450_P1414333748	TC69068		394184	1.605558	down	2E-51
CUST_21544_P1414333748	TC84164	100006460		1.606122	down	2E-11
CUST_2118_P1414333748	TC64736			1.607248	down	
CUST_13395_P1414333748	TC76014		794311	1.607688	down	5E-75
CUST_7906_P1414333748	TC70524		321056	1.608974	down	4E-38
CUST_6587_P1414333748	TC69205		567021	1.60952	down	0.75
CUST_26021_P1414333748	TC88644			1.611201	down	
CUST_15592_P1414333748	TC78211		402977	1.611229	down	4E-54

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre	Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_13362_P1414333748	TC75981	100148257		1.611669	down	0.12
CUST_19047_P1414333748	TC81667	406711		1.611765	down	2.4
CUST_26339_P1414333748	TC88963	568740		1.613024	down	1E-108
CUST_1510_P1414333748	TC64128	406698		1.613092	down	6.3
CUST_23342_P1414333748	TC85962	563662		1.613127	down	3.3
CUST_11964_P1414333748	TC74583	791696		1.61536	down	4
CUST_26220_P1414333748	TC88843	573122		1.615626	down	6E-50
CUST_20587_P1414333748	TC83207	564174		1.616826	down	2.1
CUST_3970_P1414333748	TC66588	100004210		1.61713	down	0.6
CUST_18254_P1414333748	TC80874	797126		1.617417	down	2E-26
CUST_9047_P1414333748	TC71665	793037		1.617468	down	5E-27
CUST_24251_P1414333748	TC86873	492467		1.619075	down	0.00002
CUST_15398_P1414333748	TC78017	83496		1.620426	down	2E-44
CUST_104_P1414333748	TC62722	58049		1.621008	down	1E-139
CUST_26045_P1414333748	TC88668	572359		1.62209	down	1.7
CUST_16301_P1414333748	TC78920	492676		1.622166	down	1E-07
CUST_6_P1414333748	TC62624	566945		1.622807	down	0
CUST_25077_P1414333748	TC87700	323473		1.624997	down	1
CUST_2732_P1414333748	TC65350	436604		1.625042	down	1E-133
CUST_4888_P1414333748	TC67506	100003704		1.625282	down	5
CUST_16731_P1414333748	TC79351	406251		1.627036	down	6E-94
CUST_13448_P1414333748	TC76067	562149		1.627245	down	6.4
CUST_15203_P1414333748	TC77822	562881		1.627868	down	3E-63
CUST_3892_P1414333748	TC66510	449806		1.628319	down	5E-56
CUST_26352_P1414333748	TC88976	566397		1.629629	down	1E-147
CUST_5098_P1414333748	TC88729	556647		1.630373	down	3E-35
CUST_26106_P1414333748	TC85066	558900		1.630547	down	1E-107
CUST_22446_P1414333748	TC78154	557836		1.630906	down	8E-84
CUST_15535_P1414333748	TC69437	550339		1.632265	down	5E-59
CUST_6819_P1414333748	TC66474	558271		1.632485	down	4E-73
CUST_3856_P1414333748	TC76332	553423		1.632539	down	1E-110
CUST_13713_P1414333748	TC69603	100148910		1.63376	down	2E-35
CUST_6985_P1414333748	TC66499	570552		1.634652	down	1E-112
CUST_3881_P1414333748	TC88004	619245		1.637146	down	5E-48
CUST_25381_P1414333748	TC75217	799300		1.637452	down	7.8
CUST_12598_P1414333748	TC71182	368243		1.639168	down	5E-88
CUST_8564_P1414333748	TC82337	30291		1.640986	down	2E-56
CUST_19717_P1414333748				1.641855	down	1E-106

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_8715_P1414333748	TC71333	436647	1.642191	down	1E-147
CUST_170_P1414333748	TC62788	58051	1.642575	down	1E-104
CUST_7579_P1414333748	TC70197	368636	1.642899	down	2.3
CUST_20602_P1414333748	TC83222	359835	1.643681	down	6E-91
CUST_10369_P1414333748	TC72987	394121	1.644135	down	5E-83
CUST_15599_P1414333748	TC78218	541324	1.644822	down	3E-12
CUST_25242_P1414333748	TC87865	565101	1.649206	down	4.4
CUST_24294_P1414333748	TC86916	553474	1.650361	down	2E-50
CUST_13272_P1414333748	TC75891	100005067	1.651631	down	2.8
CUST_6753_P1414333748	TC69371	562897	1.652567	down	4E-24
CUST_17584_P1414333748	TC80204	317641	1.652632	down	0.42
CUST_13949_P1414333748	TC76568	556307	1.652987	down	1E-39
CUST_20649_P1414333748	TC83269	565241	1.654118	down	2.5
CUST_13640_P1414333748	TC76259	791762	1.654581	down	4E-29
CUST_12032_P1414333748	TC74651	100002893	1.655285	down	1E-131
CUST_15232_P1414333748	TC77851	558610	1.656859	down	1E-58
CUST_20798_P1414333748	TC83418	100036547	1.657558	down	0.37
CUST_16499_P1414333748	TC79119	30037	1.658171	down	2E-76
CUST_14423_P1414333748	TC77042	402969	1.658204	down	5E-35
CUST_1292_P1414333748	TC72105	796128	1.660161	down	0.00001
CUST_17431_P1414333748	TC63910	100073329	1.660363	down	0.049
CUST_20768_P1414333748	TC83388	393710	1.661737	down	2E-50
CUST_18219_P1414333748	TC80839	555932	1.662463	down	6.4
CUST_19715_P1414333748	TC82335	445393	1.663171	down	8E-16
CUST_22241_P1414333748	TC84861	100149919	1.663771	down	
CUST_25245_P1414333748	TC87868	567452	1.666444	down	0.002
CUST_3349_P1414333748	TC65967	406385	1.66752	down	4E-52
CUST_16105_P1414333748	TC78724	406385	1.667611	down	0.56
CUST_18916_P1414333748	TC81536	406751	1.66789	down	0.88
CUST_12702_P1414333748	TC75321	436935	1.668822	down	2E-38
CUST_13377_P1414333748	TC75996	30264	1.670872	down	4E-28
CUST_17838_P1414333748	TC80458	100150703	1.670899	down	4.9
CUST_21030_P1414333748	TC83650	100001337	1.671015	down	1E-100
CUST_26241_P1414333748	TC88864	560644	1.671347	down	1E-101
CUST_7069_P1414333748	TC69687	566323	1.672497	down	2E-96
CUST_24458_P1414333748	TC87081	792404	1.673011	down	4E-61
CUST_1245_P1414333748	TC63863	566796	1.674683	down	1E-89

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_23798_P1414333748	TC86419	794352	1.674853	down	7
CUST_20205_P1414333748	TC82825	550587	1.675736	down	1E-11
CUST_1995_P1414333748	TC64613	323641	1.676104	down	1E-136
CUST_23020_P1414333748	TC85640	325084	1.676798	down	9E-12
CUST_20813_P1414333748	TC83433	100149782	1.677879	down	0.74
CUST_406_P1414333748	TC63024	791178	1.678596	down	5E-22
CUST_15572_P1414333748	TC78191	402927	1.680007	down	6.8
CUST_13596_P1414333748	TC76215	406567	1.68129	down	1E-110
CUST_21460_P1414333748	TC84080	562668	1.684298	down	1E-135
CUST_4514_P1414333748	TC67132	403066	1.684523	down	0.098
CUST_16785_P1414333748	TC79405	393674	1.685281	down	3.3
CUST_2270_P1414333748	TC64888	447819	1.685575	down	1E-47
CUST_8236_P1414333748	TC70854	555204	1.685882	down	8E-26
CUST_22439_P1414333748	TC85059	323904	1.686017	down	0.84
CUST_7347_P1414333748	TC69965	567159	1.686369	down	3E-09
CUST_12297_P1414333748	TC74916	561829	1.687102	down	2E-35
CUST_21602_P1414333748	TC84222	550539	1.687107	down	2E-22
CUST_4889_P1414333748	TC67507	100148465	1.687473	down	4.1
CUST_20913_P1414333748	TC83533	570960	1.688324	down	1E-115
CUST_14916_P1414333748	TC77535	100004729	1.689386	down	1E-97
CUST_10246_P1414333748	TC72864	550487	1.690206	down	9E-51
CUST_22432_P1414333748	TC85052	566844	1.692857	down	1.6
CUST_24977_P1414333748	TC87600	394112	1.694949	down	2.2
CUST_1138_P1414333748	TC63756	799977	1.695219	down	3E-75
CUST_18586_P1414333748	TC81206	795080	1.696043	down	1.1
CUST_5211_P1414333748	TC67829	58113	1.696541	down	4E-31
CUST_17362_P1414333748	TC79982	553266	1.697222	down	1E-140
CUST_9030_P1414333748	TC71648	335396	1.697995	down	3E-48
CUST_6389_P1414333748	TC69007	559351	1.699787	down	4E-20
CUST_3030_P1414333748	TC65648	541513	1.700771	down	2E-73
CUST_1363_P1414333748	TC63981	405783	1.708118	down	1E-112
CUST_22415_P1414333748	TC85035	797363	1.70947	down	1.9
CUST_16126_P1414333748	TC78745	791158	1.710635	down	1E-54
CUST_14933_P1414333748	TC77552	503778	1.712038	down	3E-16
CUST_2207_P1414333748	TC64825	403010	1.713258	down	4E-51
CUST_11289_P1414333748	TC73907	404618	1.716173	down	2E-74
CUST_7746_P1414333748	TC70364	793897	1.71653	down	0.91
CUST_23559_P1414333748	TC86180	100148465	1.718706	down	

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_563_P1414333748	TC63181	30524	1.718735	down	3E-67
CUST_15996_P1414333748	TC78615	565082	1.720023	down	0.00002
CUST_23045_P1414333748	TC85665	569695	1.721992	down	0.18
CUST_9182_P1414333748	TC71800	796299	1.722633	down	0.007
CUST_15752_P1414333748	TC78371	100148555	1.722793	down	1.4
CUST_14850_P1414333748	TC77469	393368	1.722838	down	3.3
CUST_3336_P1414333748	TC65954	538671	1.724824	down	0.002
CUST_9577_P1414333748	TC72195	572199	1.728391	down	3.2
CUST_21925_P1414333748	TC84545	554050	1.731338	down	3E-51
CUST_18401_P1414333748	TC81021	541408	1.731947	down	1E-60
CUST_17118_P1414333748	TC79738	393735	1.733699	down	2E-40
CUST_21498_P1414333748	TC84118	30485	1.733869	down	5E-50
CUST_26204_P1414333748	TC88827	335206	1.735262	down	1E-175
CUST_11657_P1414333748	TC74276	58150	1.735942	down	3E-25
CUST_14657_P1414333748	TC77276	565101	1.736824	down	2.5
CUST_2402_P1414333748	TC65020	402807	1.737235	down	7E-33
CUST_17662_P1414333748	TC80282	571282	1.738348	down	2E-27
CUST_19750_P1414333748	TC82370	565271	1.738749	down	3.3
CUST_20002_P1414333748	TC82622	541515	1.738933	down	1E-126
CUST_339_P1414333748	TC62957	336503	1.742508	down	6E-82
CUST_13328_P1414333748	TC75947	100005118	1.742887	down	2.2
CUST_4261_P1414333748	TC66879	567630	1.743149	down	4E-94
CUST_3978_P1414333748	TC66596	562028	1.746201	down	9E-82
CUST_21378_P1414333748	TC83998	407676	1.748036	down	0.002
CUST_2915_P1414333748	TC65533	768152	1.750694	down	5E-75
CUST_22812_P1414333748	TC85432	405846	1.751635	down	8E-55
CUST_10363_P1414333748	TC72981	791191	1.752998	down	0
CUST_22000_P1414333748	TC84620	562639	1.755348	down	5E-39
CUST_14866_P1414333748	TC77485	569473	1.756362	down	2E-35
CUST_19019_P1414333748	TC81639	561879	1.756529	down	7E-24
CUST_2102_P1414333748	TC64720	83496	1.763657	down	7E-49
CUST_9884_P1414333748	TC72502	436994	1.764281	down	1.5
CUST_6749_P1414333748	TC69367	327396	1.765452	down	1E-135
CUST_15541_P1414333748	TC78160	368848	1.766634	down	1E-25
CUST_5491_P1414333748	TC68109	100001119	1.767805	down	3.1
CUST_14591_P1414333748	TC77210	566751	1.770433	down	5E-46
CUST_18410_P1414333748	TC81030	538967	1.774834	down	3E-24
CUST_4071_P1414333748	TC66689	100149728	1.775267	down	1.4

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_15268_P1414333748	TC77887	558967	1.77621	down	5E-16
CUST_17959_P1414333748	TC80579	565381	1.7779	down	0.004
CUST_12584_P1414333748	TC75203	140744	1.781496	down	0
CUST_13692_P1414333748	TC76311	100037359	1.784087	down	3E-49
CUST_25684_P1414333748	TC88307	100149292	1.78644	down	1E-26
CUST_19178_P1414333748	TC81798	563261	1.789043	down	7.8
CUST_14847_P1414333748	TC77466	559776	1.791053	down	5E-68
CUST_24669_P1414333748	TC87292	406541	1.792346	down	2E-08
CUST_22851_P1414333748	TC85471	100002048	1.794504	down	0.0006
CUST_16739_P1414333748	TC79359	100147922	1.797453	down	0.48
CUST_1949_P1414333748	TC64567	259260	1.797586	down	4E-97
CUST_7235_P1414333748	TC69853	447930	1.798961	down	5E-88
CUST_22552_P1414333748	TC85172	793359	1.799635	down	2.5
CUST_17705_P1414333748	TC80325	100149692	1.800421	down	3.3
CUST_18259_P1414333748	TC80879	266755	1.809698	down	2E-63
CUST_895_P1414333748	TC63513	566878	1.810097	down	1E-128
CUST_17370_P1414333748	TC79990	445226	1.810782	down	4E-30
CUST_15312_P1414333748	TC77931	63999	1.811025	down	1E-118
CUST_18704_P1414333748	TC81324	798285	1.812533	down	4E-50
CUST_7853_P1414333748	TC70471	58150	1.812981	down	5E-20
CUST_1512_P1414333748	TC64130	794268	1.813902	down	2.5
CUST_13433_P1414333748	TC76052	557384	1.815609	down	0.000002
CUST_10958_P1414333748	TC73576	550539	1.81751	down	1E-133
CUST_10378_P1414333748	TC72996	799300	1.820684	down	1E-147
CUST_4413_P1414333748	TC67031	100126009	1.820992	down	1.4
CUST_15061_P1414333748	TC77680	795086	1.821038	down	8E-39
CUST_1239_P1414333748	TC63857	558079	1.82353	down	1E-56
CUST_10196_P1414333748	TC72814	550232	1.825767	down	4E-37
CUST_22777_P1414333748	TC85397	559364	1.826879	down	0.5
CUST_21025_P1414333748	TC83645	100005977	1.829043	down	3E-10
CUST_8764_P1414333748	TC71382	393996	1.829973	down	8E-66
CUST_2264_P1414333748	TC64882	100149528	1.83702	down	0.009
CUST_21723_P1414333748	TC84343	100006692	1.839352	down	0.97
CUST_22816_P1414333748	TC85436	559882	1.840279	down	2.4
CUST_20349_P1414333748	TC82969	563501	1.84036	down	1E-15
CUST_14212_P1414333748	TC76831	563056	1.84154	down	2E-22
CUST_15971_P1414333748	TC78590	571540	1.842294	down	0.79
CUST_19893_P1414333748	TC82513	566752	1.842719	down	0.00004

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_6543_P1414333748	TC69161	408256	1.843168	down	0
CUST_3348_P1414333748	TC65966	567403	1.84803	down	1E-66
CUST_12709_P1414333748	TC75328	568527	1.8489	down	1E-118
CUST_23207_P1414333748	TC85827	550487	1.849414	down	0.000009
CUST_5759_P1414333748	TC68377	445065	1.849992	down	1E-112
CUST_26053_P1414333748	TC88676	100151417	1.852929	down	4.9
CUST_12847_P1414333748	TC75466	799300	1.853354	down	1E-156
CUST_23840_P1414333748	TC86462	564899	1.856256	down	0.85
CUST_13461_P1414333748	TC76080	556600	1.856811	down	2E-34
CUST_21788_P1414333748	TC84408	792995	1.857125	down	0.73
CUST_10185_P1414333748	TC72803		1.858557	down	
CUST_2830_P1414333748	TC65448	259189	1.85865	down	0
CUST_22893_P1414333748	TC85513	568887	1.86128	down	1E-50
CUST_21942_P1414333748	TC84562	558936	1.862395	down	0.00001
CUST_4486_P1414333748	TC67104	570305	1.863085	down	5E-22
CUST_19575_P1414333748	TC82195	447942	1.867014	down	7E-33
CUST_5540_P1414333748	TC68158	494472	1.868059	down	6E-37
CUST_18332_P1414333748	TC80952	796111	1.874961	down	2E-81
CUST_13428_P1414333748	TC76047	557237	1.877265	down	3E-73
CUST_10913_P1414333748	TC73531	799670	1.879834	down	4E-16
CUST_2946_P1414333748	TC65564	564388	1.88018	down	7E-78
CUST_20972_P1414333748	TC83592	568600	1.883107	down	0.36
CUST_25736_P1414333748	TC88359	563408	1.883304	down	2.7
CUST_21314_P1414333748	TC83934	798864	1.885481	down	2
CUST_720_P1414333748	TC63338	327588	1.8883	down	7E-76
CUST_9932_P1414333748	TC72550	406541	1.890299	down	1E-119
CUST_7561_P1414333748	TC70179	431717	1.89508	down	1E-109
CUST_21944_P1414333748	TC84564	569041	1.899331	down	2.6
CUST_19143_P1414333748	TC81763	335573	1.902289	down	9E-45
CUST_8832_P1414333748	TC71450	795895	1.908901	down	0.15
CUST_18617_P1414333748	TC81237	654774	1.909009	down	2E-32
CUST_16309_P1414333748	TC78928	494070	1.91133	down	1E-74
CUST_10811_P1414333748	TC73429	406741	1.911742	down	4E-47
CUST_3456_P1414333748	TC66074	792527	1.914314	down	4E-74
CUST_8286_P1414333748	TC70904	571079	1.91474	down	6E-42
CUST_16901_P1414333748	TC79521	100001900	1.919204	down	2E-25
CUST_7791_P1414333748	TC70409	494472	1.919416	down	4E-28
CUST_1959_P1414333748	TC64577	560629	1.927921	down	1E-169

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_5936_P1414333748	TC68554	30553	1.930931	down	4E-65
CUST_1319_P1414333748	TC63937	100006223	1.93477	down	1E-104
CUST_22732_P1414333748	TC85352	570521	1.93766	down	1.1
CUST_13877_P1414333748	TC76496	324302	1.939741	down	0.62
CUST_13261_P1414333748	TC75880	568277	1.95598	down	1E-46
CUST_21727_P1414333748	TC84347	553611	1.959863	down	1E-127
CUST_15995_P1414333748	TC78614	100000500	1.963202	down	7E-59
CUST_15192_P1414333748	TC77811	563405	1.97506	down	4E-07
CUST_14815_P1414333748	TC77434	555864	1.978631	down	2E-68
CUST_6556_P1414333748	TC69174	557290	1.981711	down	0.41
CUST_25370_P1414333748	TC87993	569375	1.991937	down	5
CUST_7386_P1414333748	TC70004	442923	1.995406	down	8E-21
CUST_133_P1414333748	TC62751	402923	1.997772	down	1E-34
CUST_21985_P1414333748	TC84605	569718	2.001262	down	0.49
CUST_21382_P1414333748	TC84002	794317	2.007159	down	3.4
CUST_24761_P1414333748	TC87384	447918	2.009841	down	
CUST_24259_P1414333748	TC86881	503524	2.020932	down	3E-83
CUST_16224_P1414333748	TC78843	553864	2.038635	down	5E-91
CUST_14490_P1414333748	TC77109	503756	2.04487	down	2E-78
CUST_3416_P1414333748	TC66034	322228	2.045937	down	0.85
CUST_7517_P1414333748	TC70135	556307	2.047111	down	0
CUST_8968_P1414333748	TC71586	798527	2.04954	down	9E-98
CUST_14070_P1414333748	TC76689	406539	2.056829	down	8E-40
CUST_17306_P1414333748	TC79926	799423	2.06083	down	1E-116
CUST_13849_P1414333748	TC76468	792049	2.061089	down	5E-33
CUST_21819_P1414333748	TC84439	10000526	2.064144	down	2E-73
CUST_3009_P1414333748	TC65627	30180	2.076064	down	0.14
CUST_25816_P1414333748	TC88439	550546	2.078895	down	1E-59
CUST_1400_P1414333748	TC64018	792884	2.08089	down	4E-34
CUST_3533_P1414333748	TC66151	406539	2.080438	down	0.31
CUST_25276_P1414333748	TC87899	100149104	2.087526	down	0
CUST_21044_P1414333748	TC83664	100003911	2.0931	down	1E-167
CUST_2763_P1414333748	TC65381	114426	2.094264	down	0.000004
CUST_15867_P1414333748	TC78486	447886	2.094932	down	7.2
CUST_23390_P1414333748	TC86010	30227	2.101706	down	8E-55
CUST_25457_P1414333748	TC88080	405882	2.112642	down	5.8
CUST_16039_P1414333748	TC78658	100302390	2.115402	down	0
			2.115722	down	0.00001

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre	Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_17576_P1414333748	TC80196	402937		2.126735	down	7E-14
CUST_23936_P1414333748	TC86558	678653		2.134481	down	0.63
CUST_15341_P1414333748	TC77960	677754		2.139874	down	2E-07
CUST_13727_P1414333748	TC76346	556600		2.143001	down	2E-34
CUST_22850_P1414333748	TC85470	100034505		2.144446	down	2.9
CUST_6761_P1414333748	TC69379	790934		2.150147	down	1E-174
CUST_14107_P1414333748	TC76726	561410		2.16338	down	1E-172
CUST_17990_P1414333748	TC80610	100005771		2.165096	down	2.1
CUST_23258_P1414333748	TC85878	565385		2.178103	down	3.8
CUST_20022_P1414333748	TC82642	30581		2.178618	down	1.8
CUST_8480_P1414333748	TC71098	322614		2.179084	down	2E-45
CUST_7587_P1414333748	TC70205	768181		2.181706	down	5E-31
CUST_15586_P1414333748	TC78205	568916		2.18833	down	0.12
CUST_23683_P1414333748	TC86304	100148822		2.193556	down	3E-09
CUST_18728_P1414333748	TC81348	562050		2.194112	down	1.5
CUST_5379_P1414333748	TC67997	555964		2.21711	down	1E-27
CUST_19034_P1414333748	TC81654	558344		2.239122	down	1.2
CUST_15728_P1414333748	TC78347	100001533		2.252594	down	3.2
CUST_19652_P1414333748	TC82272	561131		2.254313	down	0.008
CUST_23496_P1414333748	TC86117	195817		2.265824	down	4E-41
CUST_7500_P1414333748	TC70118	503565		2.272249	down	3.1
CUST_16919_P1414333748	TC79539	449799		2.272596	down	2E-21
CUST_23358_P1414333748	TC85978	337810		2.278912	down	5E-45
CUST_12075_P1414333748	TC74694	436684		2.279237	down	1E-38
CUST_1661_P1414333748	TC64279	100151464		2.291884	down	1E-30
CUST_9133_P1414333748	TC71751	266990		2.299422	down	5E-88
CUST_12719_P1414333748	TC75338	322614		2.309663	down	3E-66
CUST_22066_P1414333748	TC84686	100151566		2.310876	down	4E-17
CUST_25662_P1414333748	TC85285	797048		2.312804	down	1E-55
CUST_12506_P1414333748	TC88285	570445		2.318819	down	0.000005
CUST_18511_P1414333748	TC81131	100149681		2.34769	down	0.054
CUST_11122_P1414333748	TC73740	553699		2.353743	down	5E-38
CUST_12186_P1414333748	TC74805	447800		2.378395	down	2E-18
CUST_3830_P1414333748	TC66448	570890		2.391288	down	6E-95
CUST_24406_P1414333748	TC87029	322228		2.396752	down	2E-11
CUST_18216_P1414333748	TC80836	100148910		2.482806	down	2E-17
CUST_20572_P1414333748	TC83192	563749		2.483171	down	3E-18
				2.491016	down	4E-10

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_18290_P1414333748	TC80910	560406	2.503093	down	6E-16
CUST_118_P1414333748	TC62736	550387	2.508222	down	9E-46
CUST_8437_P1414333748	TC71055	100151566	2.511396	down	4E-16
CUST_8967_P1414333748	TC71585	555316	2.529342	down	3.3
CUST_4353_P1414333748	TC66971	100151566	2.541036	down	1E-15
CUST_22593_P1414333748	TC85213	404621	2.546969	down	4E-82
CUST_14301_P1414333748	TC76920	322614	2.567457	down	2E-82
CUST_25860_P1414333748	TC88483	100005183	2.578103	down	0.38
CUST_413_P1414333748	TC63031	334304	2.589383	down	1E-122
CUST_8787_P1414333748	TC71405	791783	2.600132	down	5E-20
CUST_22100_P1414333748	TC84720	566732	2.61195	down	0.079
CUST_19303_P1414333748	TC81923	100007517	2.643528	down	3.2
CUST_4517_P1414333748	TC67135	60310	2.679675	down	5E-52
CUST_21500_P1414333748	TC84120	100148876	2.824747	down	1E-131
CUST_19887_P1414333748	TC82507	561946	2.824747	down	0.49
CUST_6686_P1414333748	TC62774	30582	2.832216	down	0.41
CUST_156_P1414333748	TC69304	100151566	2.863484	down	4E-15
CUST_24434_P1414333748	TC87057	541438	2.917878	down	2E-33
CUST_2495_P1414333748	TC65113	30210	2.929962	down	0.34
CUST_23080_P1414333748	TC85700	558044	2.94691	down	0.34
CUST_25161_P1414333748	TC87784	568958	2.978133	down	0.000005
CUST_17923_P1414333748	TC80543	100148207	2.986765	down	2E-27
CUST_8206_P1414333748	TC70824	793066	2.999919	down	0.000005
CUST_4173_P1414333748	TC66791	100151566	3.004708	down	4E-15
CUST_5192_P1414333748	TC67810		3.017785	down	
CUST_16771_P1414333748	TC79391	799957	3.01974	down	0.77
CUST_20262_P1414333748	TC82882	327588	3.021119	down	1E-173
CUST_25887_P1414333748	TC88510	793454	3.028484	down	1E-123
CUST_25066_P1414333748	TC87689	558967	3.034088	down	1E-16
CUST_22471_P1414333748	TC85091	100000864	3.327359	down	3.2
CUST_12080_P1414333748	TC74699	562521	3.456668	down	0.073
CUST_23736_P1414333748	TC86357	100148871	3.459569	down	6E-07
CUST_6807_P1414333748	TC69425	393384	3.47668	down	4E-56
CUST_7686_P1414333748	TC70304	563531	3.485936	down	2E-12
CUST_23581_P1414333748	TC86202	799957	3.491002	down	1.5
CUST_569_P1414333748	TC63187	563353	3.527433	down	3.8
CUST_6382_P1414333748	TC69000	556746	3.559352	down	9E-42
CUST_8294_P1414333748	TC70912	405825	3.663846	down	5E-81

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Supplementary Table 2. Continued.

Probe set ID	Gene symbol	Regulated_dre Entrez Gene	[C32G vs C0G] fold change	[C32G vs C0G] regulation	dre e value
CUST_23873_PI414333748	TC86495	100001853	3.789431	down	0.66
CUST_11136_PI414333748	TC73754	503587	4.392991	down	6E-84
CUST_12551_PI414333748	TC75170	100037311	4.471607	down	0.11
CUST_23151_PI414333748	TC85771	568323	4.87506	down	7E-16
CUST_17433_PI414333748	TC80053	336655	4.900729	down	1E-28
CUST_16180_PI414333748	TC78799	566752	7.132075	down	0.0006
CUST_20448_PI414333748	TC83068	798497	9.709978	down	0.002