Thesis for the Degree of Doctor of Engineering

Analysis of environmental stress inducible genes of abalone, *Haliotis discus hannai* using expressed sequence tags and cDNA microarray

by

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(EST와 cDNA microarray를 이용한 참전복, *Haliotis discus hannai* 의 환경 스트레스 관련 유전자 분석)

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Analysis of environmental stress inducible genes of abalone, *Haliotis discus hannai* using expressed sequence tags and cDNA microarray

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EST와 cDNA microarray를 이용한 참전복, *Haliotis discus hannai* 의 환경 스트레스 관련 유전자 분석

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요 약

전복류는 전 세계적으로 100여종이 분포하며, 식용으로 기호성이 높기 때문에 생물학적 연구가 많이 이루어져 왔다. 그 중에서도 양식 환경에서 질병이나 스트레스에 대한 내성, 성장 등에 관심이 모아지고 있다. 해양생물의 생존 및 성장에 가장 큰 영향을 미치는 환경 요인으로는 수온과 염분을 들 수 있다. 해양생물 가운데 전복은 운동성이 제한되어 있어 수온이나 염분 농도의 변화는 대량 폐사를 유발할 수 있다.

최근 인간을 비롯한 많은 생물체에서 발현유전자 조각 (EST; expressed sequence tag)의 분석으로 유전자 발현양상에 대한 많은 연구가 이루어지고 있으나 EST 데이터 베이스의 대부분은 육상동물, 특히 포유류에 국한되어 있고, 전복을 비롯한 패류에 대한 EST자료는 극히 소수에 불과하다. 따라서 본 연구는 참전복, *Haliotis discus hannai*의 7 조직(아가미, 소화관, 간췌장, 표피, 근육, 정소, 난소)으로부터 EST를 확보하였으며, 환경 스트레스로 작용하는 수온과 염분 농도를 변화 시킨 후 유전자의 발현 양상을 조사하였다. 그 후 microarray 방법을 통하여 환경 스트레스 노출 시 발현의 정도를 분석하고 특성을 연구하였다.

전복의 조직별 유전자 발현의 특성을 밝히기 위하여, 생물정보학을 이용하여 전복의 주요 조직으로부터 확보된 1,393개의 EST를 분석한 결과 cluster는 135개, singleton은 951개로 구성되어 있으며, 발현 중복율은 22%로 나타났다. 또한 BLASTX를 이용하여 유전자 검색 결과 1,278개 (91.7%)의 EST가 이미 알려진 유전자와 상동성을 가지는 것으로 나타났고, 나머지 115개 (8.3%)의 EST는 이제까지 알려지지 않은 유전자로 밝혀졌다.

전복의 주요 조직에서 밝혀진 발현 유전자 정보를 바탕으로, 전복의 수온과 염분 스트레스 시 전복에서 유도되는 유전자들을 탐색하기 위하여 고수온, 저수온, 저염분에 노출 시킨 후 suppression subtractive hybridization (SSH) 방법을 통하여 EST를 확보하였다. 총 1316개의 EST가 분석되었으며, 998개 (75.8%)의 EST가 이미 알려진 유전자와 상동성을 가지는 것으로 나타났고, 나머지 318개 (24.2%)의 EST는 알려지지 않은 유전자로 밝혀졌다. 밝혀진 유전자들을 분석한 결과 HSC, HSP family, major histocompatibility complex (MHC) class IIa chain, CD45, IRF7 등 다수의 클론들은 스트레스와 면역에 관련된 유전자와 상동성을 나타내었다.

전복의 환경변화 노출 시에 발현되는 스트레스 관련 유전자의 발현 차이를 조사하기 위하여 cDNA microarray 분석을 수행하였다. 우선 전복의 7개 조직으로부터 확보한 1,457개의 cDNA clone과 환경스트레스 노출에 의해 확보된 1,536개의 cDNA clone을 이용하여 3K cDNA microarray를 구축한 후, 수온 또는 염

분 스트레스에 대한 발현 정도를 조사하였다. 그 결과 스트레스와 면역에 관련된 많은 인자들이 유의적인 차이를 보이고 있었으며, 기존에 알려진 유전자와 상동성을 지니지 않은 유전자들 가운데도 스트레스 조건에 따른 유의적인 차이를 보이는 유전자들이 조사되었다.

본 연구는 전복의 정상 조직의 발현유전자들의 특성을 조사하고, 환경 스트레스 노출 하에서 발현 차이를 EST 와 cDNA microarray 방법을 통하여 분석하였다. 분석된 결과들은 상대적으로 부족한 패류의 환경 스트레스 (수온, 염분)와 면역관련 유전자들에 대한 기초적 자료로 이용될 뿐만 아니라 유전자 발현양상의 특성을 조사함으로써 개체 및 조직 특이적 분석, 스트레스 특이적 유전자 표지 개발, 그리고 전체 cDNA 와 발현조절 부위 분리, 기능 분석에 응용 할 수 있다. 뿐만 아니라 스트레스 특이적 유전자와 유용 형질 유전자에 대한 탐색과 개발은 분자육종의 기초가 되는 유전자 지도 작성에 이용할 경우 신품종의 효율적 선발이나 유전자원의 평가와 같은 여러 분야에 활용이 가능하다.

따라서 본 연구 결과를 통해 전복의 환경스트레스 조건하에서 특이적으로 발현되는 유전자들의 정보를 확보하고 특성을 연구함으로써 패류 유전체학의 유용한 정보로 이용될 뿐만 아니라 향후 내병성, 내환경성 전복의 품종 개발을 통해 전복 양식에 밑바탕이 될 것으로 기대 된다.

Chapter I.

General introduction

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General information on abalone culture

Abalone is one of the most prized sea delicacies worldwide. Farming of abalone was begun in the late 1950's and early 1960's in Japan and China, respectively, and developed very rapidly in the 1990s, and culture of abalone is now widespread in many countries including East Asia (especially in China, Japan, and Korea), Latin America (especially Chile) and South East Asia.

About one hundred abalone species are found through the world. Of them, six species are found along the coasts of Korea. They are *Haliotis diversicolor supertexta, H. diversicolor diversicolor, H. discus hannai, H. discus discus, H. sieboldii,* and *H. gigantea*. *H. discus hannai* is a commercially important shellfish species in East Asia especially in China, Japan and Korea. This species has several desirable characteristics for aquaculture including tolerance to low water temperatures, establishment of seedling production, and ability to withstand high stocking density [1-3].

Farming of *H. discus hannai* in Korea has been developed since 1970 and aquaculture production is currently increasing rapidly. It is likely to maintain this increasing trend in the production because of the decrease in wild abalone captures due to overexploitation, water pollution, and other causes. However, intensive culture of abalone in enclosed spaces like tanks, cages, and ponds make the shellfish more susceptible to stress and disease [4, 5]. Environmental stress is responsible for the outbreak mass mortalities and has caused vast economic losses.

Environmental stress on abalone

Stress response is the mechanism by which animals can maintain homeostasis when exposed to physical (e.g., temperature or salinity) or biological (e.g., food quantity and quality, competition for space or sexual partners, presence of pathogens) changes induced by natural or anthropogenic perturbations. Studies on organisms from various phyla demonstrated a link between stress and increased mortality, apparently due to reduced disease resistance [6-10].

In shellfish farming, production may be subjected to various limiting factors including mortality outbreaks. For several decades now, various shellfish livestock have presented abnormal mortality outbreaks during the summer period, which is called summer mortality. The representative case of summer mortality is Pacific oyster, *Crassostrea gigas*. Since the beginning of the 1960s, abnormal episoces of *C. gigas* mortality have increased in the world, as reported in Japan and North America since the 1940s [11-13]. Cheney et al. have hypothesised that summer mortality outbreaks are the result of multiple factors, including elevated temperatures and physiological stress associated with sexual maturation, aquaculture practices, pathogens or pollutants [14].

In Korea, due to various stress during the summer period, large numbers of shellfish such as abalone, oyster, shortnek clam, and red shell in farming areas died during last 20 years and the aquaculture industry is in bad shape. Despite of the increasing seriousness of mass mortality, research on the stress response of abalone is limited.

Since 1970, mass mortality of the abalone *H. discus hannai* has occurred in the southern coast of Korea during the rainy season of summer. Mortalities of abalone are the result of multiple factors or stressors including elevated temperature and hyposalinity by poured floodwaters in the rainy season. Environmental stress such as temperature or salinity changes can affect the defense mechanisms of marine invertebrate organisms [15].

Molecular techniques applicable in the study of environmental stress

Now, new families of "omics" technologies that provide a massive and reliable analysis of cellular component such as RNA, DNA, and protein are introduced. These technologies are very powerful tools for the identifying novel genes and further studying the correlation of these genes and proteins, which enables researchers to sufficiently understand the response of organisms to environmental stress or infectious disease [16].

Techniques used for these thechonlogies include expressed sequence tages (ESTs) analysis, suppressive subtractive hybridization (SSH), differential display reverse transcriptase polymerase chain reaction (DD RT-PCR), serial analysis of gene expression (SAGE), and ultimately, microarray [17]. These PCR based techniques make it possible to screen massively differential expressed gene by environmental stress, simultaneously. Nevertheless, the importance of obtaining cDNA sequences, studies for their expression pattern analysis and physiological properties of each gene or protein should not be overlooked.

Objectives of the study

This study aimed to build up a wide array of genes expressed in abalone, *H. discus hannai* and to analyze environmental stress inducible genes of abalone by ESTs and cDNA microarray.

In order to obtain cDNA sequences from this species, cDNA libraries were constructed from major seven tissues in abalone *H. discus hannai* and then 1,393 ESTs were analyzed. After exposure to environmental stress, differential expressed genes were cloned using SSH methods and 1,316 ESTs were analyzed. Additionaly, 3K cDNA microarray was designed and produced, which targeted primarily toward environmental stress-related genes. The changes of expression levels of stress-related genes or some unkown genes induced by environmental stress were evaluated to better understand the abalone defense system using cDNA microarray analysis.

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Chapter II.

EST-based survey of genes in seven different tissues of abalone, *Haliotis discus hannai*

Chapter II. EST-based survey of genes in seven different tissues of abalone, *Haliotis discus hannai*

ABSTRACT

Analysis of expressed sequence tags (ESTs) is an efficient approach for gene discovery, expression profiling, and development of resources useful for functional genomics. To analysis the transcriptome of abalone, EST analysis was conducted using seven cDNA libraries made from gill, gut, heptopancreas, skin, muscle, testis, and ovary of abalone. Redundant ESTs were assembled into overlapping contigs by using the assembly program ICAtools software. The results showed that the 1,393 ESTs were composed of 135 clusters and 951 singletons, suggesting that the overall redundancy of the library was 22%. of the 1,393 clones, 1,278 clones (91.7%) were identified as known genes by BLAST searches and 115 clones (8.3%) did not match to any previously described genes. Based on major function of their encoded proteins, the identified clones were classified into 16 broad categories. Sequence analysis of the ESTs revealed the presence of microsatellite-containing genes that may be valuable for further gene mapping studies. This study contributes to the identification of many EST clones that could be useful for genetics and developmental biology of abalone.

Key words : abalone, *Haliotis discus hannai*, expressed sequence tag (EST), expression profile, marker.

INTRODUCTION

Identification of the genes expressed within the cell of a given tissue is a basic step in the determination of gene function and the analysis of tissue physiology. One efficient approach in the characterization of gene transcript involves partial sequencing of cDNA clones from cDNA libraries obtaining expressed sequence tags, which are commonly refferred to as ESTs [1]. EST analysis not only enables the identification of genes transcribed within specific tissues, but also reveals the expression profiles of the tissues from which the cDNA library was constructed. The second primary use of ESTs involves their application as molecular reagents for comparative functional genomic studies, using cDNA microarray technology [2]. ESTs can also be employed in the development of polymorphism markers [3].

Abalone is one of the most widely cultivated shellfish in East Asia including China, Japan, and Korea. Research on abalone to date has focused mainly on environmental factors and their relation to aquaculture such as culture techniques, tank design, and nutrition [4]. A better understanding on various genes of abalone and the differences with the genes of other organisms will provide an insight into abalone physiology at molecular level, leading to the introduction of genes with desired characters such as disease resistance and rapid growth. However, the number of shellfish-related ESTs stored in public databases remains minuscule relative to the amount of mammalian sequences, and relatively few tissue-specific cDNA libraries are available [5]. The expression profiles inherent to tissue-specific cDNA libraries sometimes provide novel and different information, which can also provide more profound functional meaning.

In the present study, as a component of a transcriptome analysis of abalone, seven cDNA libraries were constructed from gill, gut, heptopancreas, skin, muscle, testis and ovary of adult abalone, and 1,393 expressed sequence tag (EST) clones were analyzed.

MATERIALS AND METHODS

Animals and tissue preparation

Abalones, *Haliotis discus hannai* were suplied by Jeju Fisheries Research Institute (Jeju, Republic of Korea) and acclimatized to laboratory conditions. The seven tissues including gill, gut, heptopancreas, skin, muscle, testis, and ovary were collected and cut into small pieces as possible. Pooled tissues were rapidly frozen with liquid nitrogen and ground with a mortar and pestle.

Construction of abalone cDNA libraries

mRNAs were isolated from seven tissues using a PolyA Tract mRNA isolation kit (Promega). cDNA synthesis was carried out using a cDNA synthesis kit with oligo-(dT) primer. The cDNA library was constructed in Uni-ZAP XR vectors according to the manufacturer's instruction (Stratagene). All primary libraries were amplified and aliquots of each amplified library were stored at both 4°C and -80°C.

Plasmid preparation and sequencing

Conversion of the recombinant Uni-ZAP XRs into pBluescript phagemids was carried out by *in vivo* excision according to the manufacturer's instruction (Stratagene). Plasmid DNA was prepared by the alkaline lysis method [6] using the Qiagen Spin Column Mini-plasmid kits. Single-pass sequencing of 5′

-termini of selected cDNA clones in phagemid form was performed using the ABI 3730 automatic DNA sequencer (PE Applied Biosystems) and the ABI Prism Big Dye Terminatior Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

Bioinformatic analysis

Bioinformatic analysis was conducted to determine gene identities using GeneMaster 3.0 software (Ensoltek). Briefly, vector sequence was removed and database search was limited to ESTs >100bp in length. ESTs were then assembled in clusters of contigous sequences (contig) using ICAtools program [7]. Gene annotation procedures and homology searches of the sequenced ESTs have been locally done by BLASTX for amino acid similarity comparisons [8]. Matches were considered to be significant only when the probability (P) was less than 1×10^{-3}using BLASTX with all parameters at the defaults. All ESTs that were not identified as orthologues of known genes were designated as unknown EST clones and hypothetical proteins were considered as known EST clones.

Identification of microsatellite containing cDNAs

During the complication of EST sequences, genes that containing microsatellites were identified and their microsatellites were characterized in terms of complexity and repeat number. Clones containing microsatellites were identified by determination of a minimal number of repeats in the microsatellite sequences: dinucleotide, eight repeats; trinucleotide, five repeats; tetranucleotide, three repeats. Single nucleotide repeats were not included since they are not very useful for polymorphic markers. Some cDNA clones contained more than one type of repeat, in which case these clones were categorized according to the longest repeats.

RESULTS AND DISCUSSION

Summary of EST clones in cDNA libraries from seven tissues of abalone

cDNA libraries were constructed from seven different tissues of abalone including gill, gut, hepatopancreas, skin, muscle, testis, and ovary, consisting of 1.0×10^6 pfu/ml from polyadenylated fractions of RNA isolated from abalone tissue. The number of clones in the constructed cDNA libraries were believed to be sufficient to encompass the predominantly expressed mRNA within each tissues. The average length of the insert cDNA fragment was found to be 1.7 kb, in a range from 0.5 to 3 kb. Total of 1,457 clones were randomly selected from the seven cDNA libraries. The nucleotide sequences of these clones were determined using T3 primer, and then homologous sequences were searched in GenBank database. A summary of the identified genes is shown in Table 1. The initial ESTs were grouped into total 1,086 consensus sequeces, comprised of 158 clusters (gill, 31; gut, 24; hepatopancreas, 9; skin, 15; muscle, 19; testis, 13 and ovary, 24) and 794 singletons (gill, 196; gut, 214; hepatopancreas, 77; skin, 120; muscle, 115; testis, 119 and ovary, 110), suggesting that the libraries had an overall redundancy of 31.7%. BLASTX comparisons established that 1,278 (91.7%) of the clones were orthologues of known genes (157 (12.3%) of known genes were identified as hypothetical protein) and the remaining 115 (8.3%) clones were not identified via similarity comparisons ($E \pm 1 \times e^{-3}$). Among the 1,086 EST clones, six unique genes were identified as homologues of previously reported abalone ESTs, and 1080 (99.5%) genes were identified as orthologues of known genes from other organisms. These results suggest that EST analysis constitutes a powerful technique for the rapid discovery of large numbers of useful genes in shellfish.

Table 1. General characteristics of seven tissues ESTs taken from abalone

	Gill	Gut	Hepato pancreas	Skin	Muscle	Testis	Ovary	Sum
Number of clones sequenced	288	288	101	171	204	199	206	1,457
Number of clones analysed	287	284	99	158	196	173	196	1,393
Average insert size (kb)	1.6±0.6	1.7±0.4	2.0±0.5	1.3±0.6	1.6±0.4	1.8±0.4	1.7±0.3	1.7±0.5
EST clusters	31	24	9	15	19	13	24	158
Unigene	227	238	86	135	134	132	134	952
Redundancy (%)	20.9%	16.2%	15.2%	14.6%	31.6%	23.7%	31.6%	31.7%
ESTs with E value $< 1\times e^{-3}$ (matched)(%)	268 (90.9%)	263 (92.6%)	90 (90.9%)	141 (89.2%)	173 (88.3%)	158 (91.3%)	185 (94.4%)	1278 (91.7%)
ESTs with E value $\geq 1\times e^{-3}$ (unknown)(%)	19 (6.6%)	21 (7.4%)	9 (9.1%)	17 (10.8%)	23 (11.7%)	15 (8.7%)	11 (5.6%)	115 (8.3%)

Expression profile in seven tissues of abalone

The expression profiles of the known genes identified in seven tissues of abalone are provided in Fig. 1. Among 952 identified genes, 794 genes (83.4%) were sequenced only once; 115 genes (12.1%) 2-4 times; 43 (4.5%) 5 times or more. The percentage of singletons was acceptable, although redundancy tends to increase with increasing numbers of sequenced clones. The most abundantly expressed genes in the seven tissues were as follows: in gill, peroxidasin (4.4%), actin A3 (4.0%), actin (4.0%), and guanine nucleotide-binding protein 7 (3.1%); in gut, serine protease (3.8%), cytochrome c oxidase subunit Ⅰ (2.9%), translation elongation factor 1-alpha (2.1%), and actin A3 (2.1%); in hepatopancreas, ferritin-like protein 4 (4.7%) and elongation factor 1 alpha 3 (3.5%); in skin, actin A3 (4.4%), cytochrome c oxidase subunit Ⅰ (3.0%), and laminin receptor precursor (3.0%); in muscle, cytoskeletal beta actin (14.9%), actin A1 (12.7%), myosin essential light chain (7.5%), and aminodeoxychorismate lyase (5.2%); in testis, arginine kinase (12.9%), putative alpha-tubulin (5.3%), and elongation factor 1 alpha (4.5%); in ovary, vitellin coat protein 41 (6.7%) and 42 (6.0%), vitellogenin structural genes (yolk protein genes) family member (vit-1) (6.0%), and alpha tubulin (5.2%) (Fig. 1).

Distribution of the identified clones in seven tissues of abalone

Based on the major functions of their encoded proteins, the identified clones were classified into 16 broad categories, as follows: biogenesis of cellular components, cell cycle/ DNA processing, cell fate, cell rescue/ defense/ virulence, cell type differentiation, cellular communication/signal transduction mechanism, cellular transport/ transport facilitation/ transport routes, development (systemic), energy, interaction with the cullular environment, metabolism, protein activity regulation, protein fate (folding, modification, destination), protein synthesis, protein with binding function/ cofactor requirement (structural or catalytic), and transcription. The distribution of identified clones from the cDNA libraries of seven tissues is shown in Fig. 2.

Libraries derived from seven tissues of abalone show some differences in overall patterns of gene expression that reflect their different function in abalone. However, notable differences were not detected. Clones associated with cell cycle/ DNA processing, cellular transport/ transport facilitation/ transport routes, metabolism, and protein synthesis were all represented in high percentages. In other hand, a reverse trend was also noted; namely, clones associated with cellular communication/signal transduction mechanism, development (systemic), and protein activity regulation were represented at relatively low percentages.

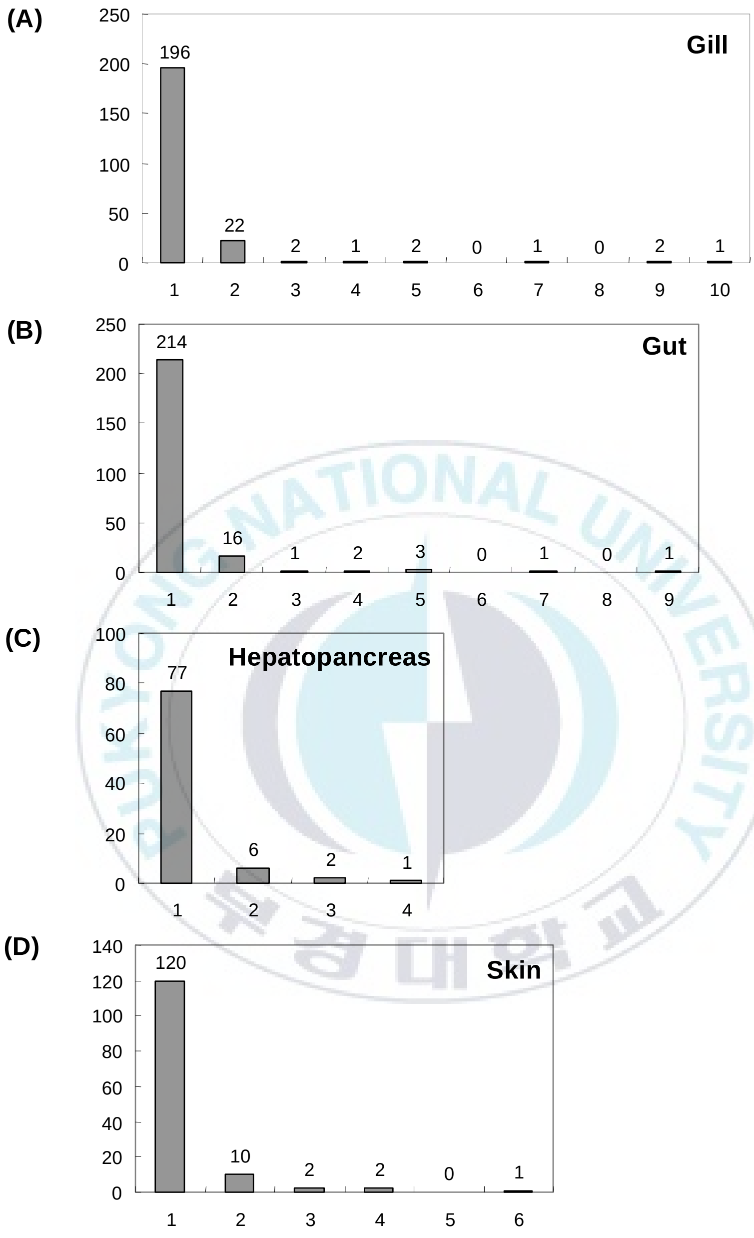
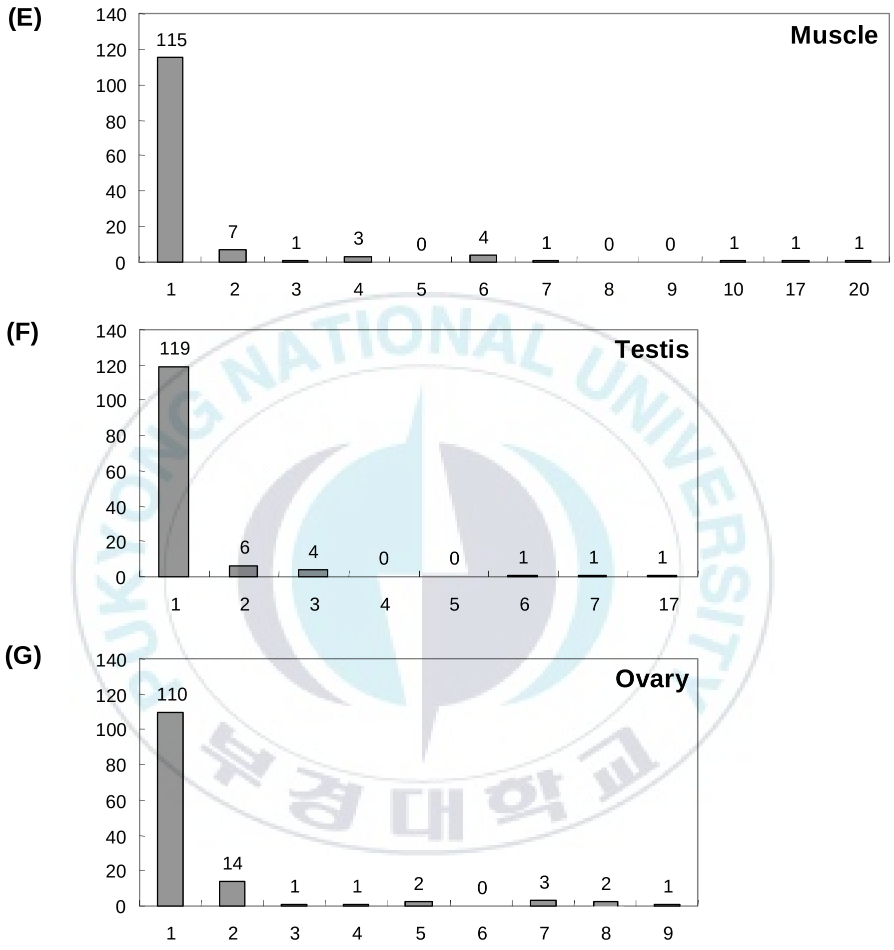


Fig. 1. Expression profiles and sequencing redundancy in the analysis of ESTs from the seven tissues of abalone. (redundancy, X axis; hits per gene, Y axis)

Fig. 1. (*continued*)



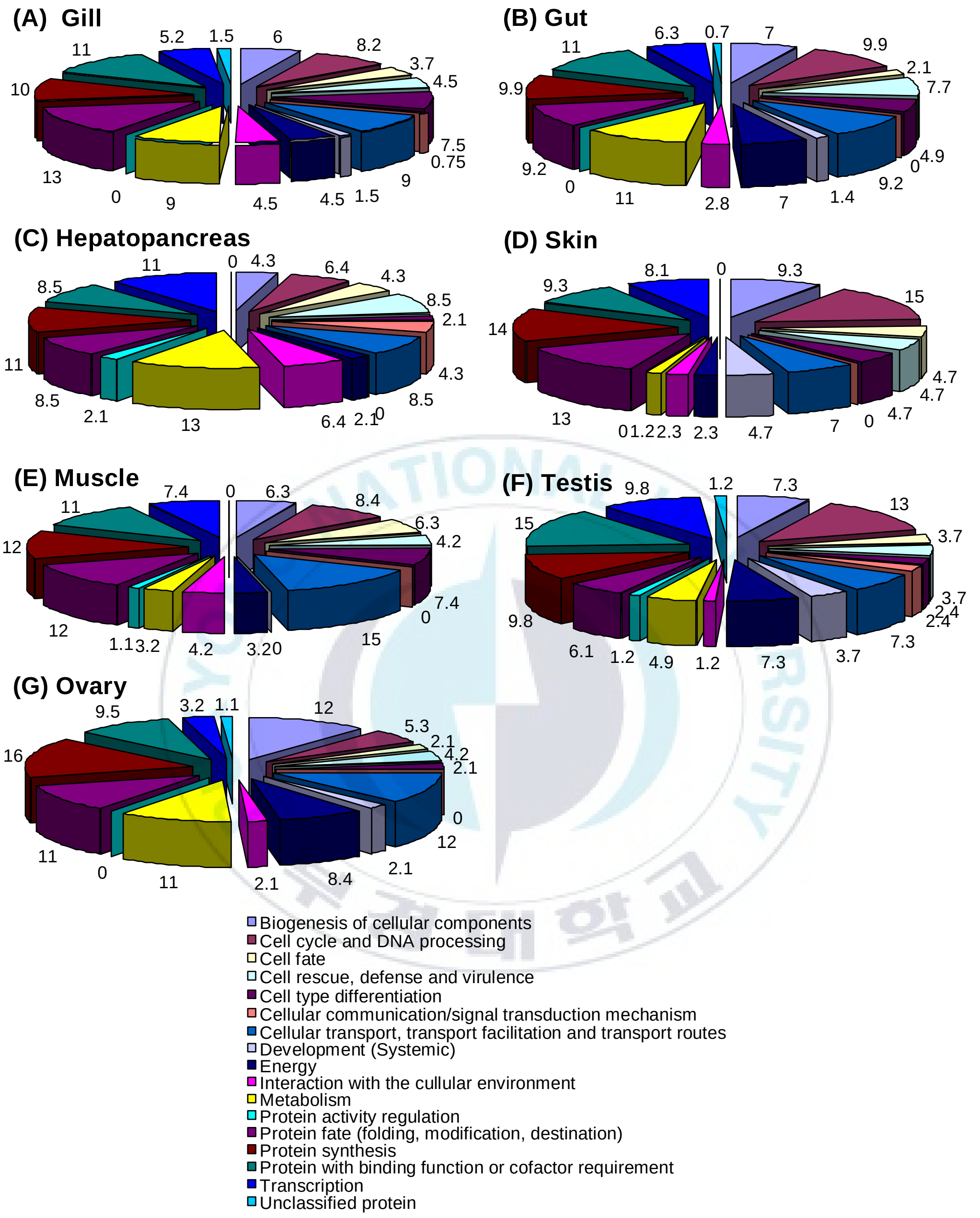


Fig. 2. Functional categorization of the seven tissues cDNA libraries taken from abalone.

Genes potentially involved in defense mechanisms
- Group I: Immune and stress related genes

Another object of this study was the identification of cDNA clones in abalone tissues that may be involved in immune and stress response activities. Database searches identified putative immune- or stress response-related genes from the abalone cDNA clones. The putative amino acids sequence deduced from one cDNA clone, DGT-207, was identified as the macrophage migration inhibitory factor (MIF). MIF was initially described as an immune activity isolated from the supernatants of T lymphocytes and inhibited the random migration of macrophages [9]. Expression of MIF was associated with physiological stress or as a consequence of systemic inflammatory conditions such as endotoxemia [10]. The putative amino acids sequence deduced from one cDNA clone, GIL71, was identified as the bcl2-associated athanogene, a cell death-inhibitory protein. During apoptosis, mitochondrial swelling allows cytochrome c to leak into the cytosol, a process which results in subsequent DNA fragmentation. Bcl2 interacts with the mitochondrial outer membrane, which affects a blockage of mitochondrial swelling [11].

Several ESTs (DGT-152, GIL-143, GIL-165, and SKN117) with high homology to various heat shock proteins (HSPs) were identified in seven libraries. HSPs are well known as stress proteins because various forms of stress enhance their transcriptional activation and biosynthesis in organisms, ranging from bacteria to humans [12]. In recent years, cDNAs encoding HSP70 and

HSP90 were described from abalone (HSP70; GeneBank accession no. DQ324856)[13] and tube abalone, *Haliotis tuberculata* (HSP70; EMBL accession no.; AM283516.1, HSP90; EMBL accession no.; AM283515.1)[14]. Our libraries also contain clones matched with HSP70 (GIL165) and HSP90 (DGT152).

- *Group II: Recognition proteins*

In the gut library, clone DGT-151 matched with immune recognition molecules β-1,3 glucan binding protein (β-GBP). β-GBP is also knwon as lipoprotein 1 (LP1), which is associated with lipid transport to ovary [15-21].

A related EST (RM-162) from the muscle library showed similarity to a scavenger receptor, expressed by human endothelial cells, which mediates the binding and degradation of acetylated low density lipoprotein (LDL) [22]. This EST contained a cysteine-rich region that is homologous to the scavenger receptor cysteine-rich (SRCR) domains characteristic of the SRCR superfamily. In addition to binding to modified LDL, these receptors can also bind bacterial endotoxins as well as intact gram-negative bacteria [23, 24].

- *Group III : Proteinases and proteinase inhibitors*

With respect to invertebrate immune function, the role of proteinases and proteinase inhibitors has been well characterized in the regulation of melanization, mediated by the prophenoloxidase system, and hemolymph coagulation [25, 26]. In support of their protective role in molluscan immunity, antibodies against various cathepsins, and cysteine proteinases, have been demonstrated the presence of these proteins in the granules of hemocytes from *Mytilus edulis* [27]. Proteinase inhibitors also support host defense mechanisms by affording protection from a variety of proteinases associated with pathogenic virulence [26]. A comparative study demonstrated the presence of proteinase inhibitors in the hemolymph of *Crassostrea spp.* [28]. In addition to inhibiting a variety of activities attributable to the mechanistic classes of proteinases, plasma from *Crassostrea spp.* inhibited extracellular proteinases produced by *Perkinsus marinus* and *Vibrio vulnificus* [28]. The production of extracellular serine proteinases by *Perkinsus marinus* is an important factor in the virulence of this parasite. Interestingly, *C. gigas*, which is resistant to *Perkinsus*, appears to possess proteinase inhibitors with greater activity compare to those in *C. virginica* [28, 29].

In support of these previous observations, two cysteine proteinases were identified from seven tissues cDNA libraries. A single EST (GIL-257) in the gill library showed similarity to cathepsin B. Two clones (VHP-215 and GIL-269) of cathepsin L were identified from the hepatopancreas and gill libraries, respectively.

One clone (VHP-242) of multicystatin (cysteine proteinase inhibitor) was identified in the hepatopancreas library. Cystatins, along with proteinases, were demonstrated to play a role in coagulation and complement activation [30, 31, 32]. Interestingly, these molecules were expressed from gill and hepatopancreas of abalone. It could be speculated that gill and hepatopancreas are the site for the production of immune related molecules.

- Group IV: Other immune effectors

Several other immune and potential immune transcripts were identified in the cDNA libraries from seven tissues of abalone. Among them, transcripts of metal binding protein are sequenced. Two major metal binding proteins of ferritin (VHP227) and metallothionein 1 (GOF031) were found from hepatopancreas and ovary, respectively. Homologues to ferritin were quite common in the hepatopancreas cDNA libraries. Ferritin, a major intracellular iron binding protein, protects cells (both prokaryotic and eukaryotic) from oxidative stresses [33, 34] and can sequester free iron from bacterial pathogens. Metallothioneins, also metal-binding proteins, bind heavy metals and are involved in detoxification processes. Metallothioneins are scavengers of reactive oxygen intermediates and are upregulated during immune responses in vertebrates, as well as in invertebrates. An abalone cyclophilin was also identified (RM-084). Cyclophilins have diverse regulatory functions in mammalian cells, but it is interesting to note that they can be involved in viral attachment to cells [35] and in the stress response to oxygen depletion [36].

Isolation of EST clones containing microsatellite

A number of the sequenced cDNA clones were found to harbor microsatellite sequences (Table 2). They were located in 5 ' or 3 ' untranslated regions (UTRs) with a high frequency (data not shown). Microsatellites are generally thought to occur primarily in noncoding DNA. However, surveys of other cDNA libraries have revealed that up to 8% of clones may harbor microsatellites [37]. These microsatellites, if they are polymorphic, may prove useful for genomic mapping. This study determined that the targeting of microsatellite regions within cDNAs is an efficient way to develop type I polymorphic markers representing genes of known function. Due to evolutionary conservation, the mutation rates within gene-coding sequences are lower than those detected in non-coding genomic sequences. As a result, the identification of type I polymorphic markers is usually more difficult to be identified. Therefore, by tagging the highly polymorphic microsatellites to known genes, the efficiency of the development of type I markers can be significantly enhanced. Since the primary objective of this study was to develop EST resources, polymorphism of these microsatellite clones was not attempted to characterize.

These EST clones isolated from this study, might be used as probe to develop molecular markers for tissue-specific or cell-specific, to determine full-length of cDNA or gene interest, and to utilize for gene expression or gene function of abalone.

Table 2. EST-containing MS cDNA clones from the seven tissues cDNA libraries of abalone

Clone Name	Nucleotide repeat unit	Microsatellite repeats	Gene name
GIL99.ab1	Dinucleotide	(GT)5	ENSANGP00000010305
SKN011-SK.ab1	Dinucleotide	(AT)5/(TG)4	AMP-dependent synthetase and ligase
GIL-219.ab1	Pentanucleotide	(GTGAC)3	hypothetical protein XP_780435
DGT-230.ab1	Dinucleotide	(GT)5	agrin isoform 1
VHP-286.ab1	Tetranucleotide	(ACAA)4	putative transposase b homolog
SKN101.ab1	Trinucleotide	(TCA)4	hypothetical protein AN4541.2
VHP-209.ab1	Dinucleotide	(CT)5	hypothetical protein
GIL74.ab1	Trinucleotide	(GCA)4	ENSANGP00000003404
DGT11.ab1	Trinucleotide	(GAT)6	LOC400590 protein
GIL140.ab1	Trinucleotide	(GAA)4	AFR490Cp
DGT112.ab1	Dinucleotide	(AG)5	unnamed protein product
GOF091-sk.ab1	Tetranucleotide	(ATTG)3	unknown
GIL108.ab1	Tetranucleotide	(CTCA)4	unknown
GOF086-SK.ab1	Dinucleotide	(GA)6	vitelline envelope sperm lysin receptor
GIL82.ab1	Trinucleotide	(CAA)4	unknown
DGT-213.ab1	Trinucleotide	(CCA)4	ependymin-related protein
DGT152.ab1	Trinucleotide	(GAA)4	heat shock protein 90
RM091.ab1	Tetranucleotide	(TGAG)4	unknown
RM042-SK.ab1	Tetranucleotide	(AGTG)3	hypothetical protein
GIL-210.ab1	Dinucleotide	(TC)5	MGC81140 protein
GOM067-sk.ab1	Trinucleotide	(CAG)4	ENSANGP00000003404
DGT183.ab1	Tetranucleotide	(ACCA)3	GA17076-PA
GIL5.ab1	Tetranucleotide	(GTGC)3/(GAGT)3	ferrisiderophore, outer membrane receptor
DGT20.ab1	Dinucleotide	(GA)5	Rho-related BTB domain-containing protein 1
SKN202.ab1	Trinucleotide	(CAC)4	unknown
SKN008-SK.ab1	Dinucleotide	(AT)5	Serpentine Receptor, class T family member (srt-7)
GOF049-sk.ab1	Trinucleotide	(TAT)4	keratin associated protein 10-2
VHP-221.ab1	Dinucleotide	(GA)5	hypothetical protein MGG_ch7g1038
GIL230.ab1	Dinucleotide	(TA)7/(TA)13/ (TA)7	unknown

Table 3. List of identified ESTs from the gill cDNA library of abalone

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
GIL-193.ab1	RL22_TRIGR 60S ribosomal protein L22 (Development-specific protein 217)		4.5P13732	
GIL-194.ab1	KRP-A	*Aplysia californica*	CAA48558.1	89
GIL-195.ab1	Zgc:101545	*Danio rerio*	AAH86701.1	92
GIL-197.ab1	similar to retinoblastoma binding protein 4 isoform 6	*Canis familiaris*	XP_864405.1	76
GIL-198.ab1	eukaryotic translation initiation factor 3, subunit 6 interacting protein	*Gallus gallus*	NP_001012553.1	64
GIL-199.ab1	ADP-ribosylation factor	*Cryptosporidium hominis TU502*	XP_666445.1	47
GIL-200.ab1	Tis11 family protein	*Crassostrea virginica*	AAB69448.1	54
GIL-202.ab1	cytochrome c oxidase subunit I	*Haliotis rubra*	YP_026069.1	84
GIL-203.ab1	importin beta 1	*Aplysia californica*	AAY81965.1	79
GIL-204.ab1	ribosomal protein L11	*Ixodes scapularis*	AAY66938.1	78
GIL-205.ab1	integral membrane protein	*Plasmodium berghei strain ANKA*	XP_674371.1	40
GIL-206.ab1	Birc2-prov protein	*Xenopus laevis*	AAH77368.1	32
GIL-207.ab1	Atp5a1-prov protein	*Xenopus tropicalis*	AAH91067.1	80
GIL-208.ab1	mFLJ00070 protein	*Mus musculus*	BAD21376.1	29
GIL-210.ab1	MGC81140 protein	*Xenopus laevis*	AAH68707.1	28
GIL-211.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_703995.1	30
GIL-212.ab1	-		_	0
GIL-213.ab1	unnamed protein product	*Spisula solidissima*	CAA33513.1	37
GIL-214.ab1	hypothetical protein cihA8B16	*Ciona intestinalis*	AAP91745.1	28
GIL-215.ab1	hypothetical protein XP_511394	*Pan troglodytes*	XP_511394.1	54
GIL-217.ab1	hypothetical protein, conserved	*Trypanosoma brucei*	AAZ12294.1	42
GIL-218.ab1	similar to CG33525-PF, isoform F	*Strongylocentrotus purpuratus*	XP_780930.1	49
GIL-219.ab1	hypothetical protein XP_780435	*Strongylocentrotus purpuratus*	XP_785528.1	29
GIL-220.ab1	Conserved hypothetical protein	*Streptococcus pneumoniae R6*	AAK98929.1	34
GIL-221.ab1	hypothetical protein AN1024.2	*Aspergillus nidulans FGSC A4*	XP_658628.1	31
GIL-222.ab1	ZK829.7	*Caenorhabditis elegans*	NP_502269.1	37
GIL-224.ab1	guanine nucleotide-binding protein	*Petromyzon marinus*	AAM88904.1	82
GIL-225.ab1	hypothetical protein	*Guillardia theta*	NP_113184.1	34
GIL-226.ab1	Rho1 GTPase	*Paracoccidioides brasiliensis*	AAQ93069.2	42
GIL-227.ab1	similar to seryl-aminoacyl-tRNA synthetase 2	*Strongylocentrotus purpuratus*	XP_785096.1	26
GIL-228.ab1	conserved hypothetical protein	*Pseudomonas aeruginosa*	AAP22559.1	33
GIL-229.ab1	MGC79564 protein	*Xenopus tropicalis*	AAH79933.1	83
GIL-230.ab1	-		_	0
GIL-231.ab1	hemocyanin type 1	*Haliotis tuberculata*	CAB76379.1	78
GIL-232.ab1	putative ubiquitin-conjugating enzyme	*Homalodisca coagulata*	AAT01083.1	91
GIL-233.ab1	Hypothetical protein LOC406376	*Danio rerio*	AAH65987.1	88
GIL-234.ab1	-		_	0
GIL-235.ab1	ENSANGP00000003537	*Anopheles gambiae str. PEST*	EAA09204.2	37
GIL-236.ab1	CG10687-PA	*Drosophila melanogaster*	NP_609948.1	80
GIL-237.ab1	cyclin g-associated kinase, putative	*Plasmodium falciparum 3D7*	NP_701816.1	50
GIL-238.ab1	hypothetical protein DDB0220669	*Dictyostelium discoideum*	XP_641585.1	34

Table 3. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I^a
GIL-239.ab1	unnamed protein product	*Aspergillus oryzae*	BAE64514.1	33
GIL-240.ab1	hypothetical protein	*Plasmodium berghei strain ANKA*	XP_678539.1	23
GIL-243.ab1	similar to RIKEN cDNA 2810451A06	*Strongylocentrotus purpuratus*	XP_788624.1	38
GIL-244.ab1	ENSANGP00000027568	*Anopheles gambiae str. PEST*	EAL41525.1	70
GIL-247.ab1	actin A3	*Haliotis iris*	AAX19288.1	100
GIL-248.ab1	Hypothetical protein LOC406766	*Danio rerio*	AAH71537.1	47
GIL-249.ab1	exocyst complex component 7 isoform b	*Homo sapiens*	NP_056034.2	59
GIL-250.ab1	unnamed protein product	*Aspergillus oryzae*	BAE63051.1	25
GIL-251.ab1	Ghitm-prov protein	*Xenopus laevis*	AAH41226.1	67
GIL-252.ab1	similar to Paf1/RNA polymerase II complex component	*Mus musculus*	XP_922595.1	42
GIL-253.ab1	ferritin GF2	*Crassostrea gigas*	AAP83794.1	81
GIL-254.ab1	ENSANGP00000020368	*Anopheles gambiae str. PEST*	EAA08459.1	29
GIL-256.ab1	Zgc:92282	*Danio rerio*	AAH75983.1	67
GIL-257.ab1	cathepsin B-like proteinase	*Triatoma sordida*	AAT48984.1	56
GIL-258.ab1	hypothetical protein TP02_0615	*Theileria parva strain Muguga*	XP_765181.1	36
GIL-259.ab1	Hypothetical protein CBG06905	*Caenorhabditis briggsae*	CAE62742.1	27
GIL-260.ab1	similar to ankyrin repeat domain protein 17 isoform b, partial	*Strongylocentrotus purpuratus*	XP_786997.1	40
GIL-261.ab1	Leukocyte cell derived chemotaxin 1	*Danio rerio*	AAH66426.1	22
GIL-262.ab1	RE01051p	*Drosophila melanogaster*	AAV36923.1	28
GIL-263.ab1	ENSANGP00000001657	*Anopheles gambiae str. PEST*	EAA06779.3	25
GIL-264.ab1	CG11387-PA, isoform A	*Drosophila melanogaster*	NP_524764.1	28
GIL-265.ab1	hypothetical protein	*Brassica napus*	BAC98910.1	30
GIL-266.ab1	similar to microsomal glutathione S-transferase 3	*Strongylocentrotus purpuratus*	XP_793267.1	55
GIL-267.ab1	FGF receptor	*Halocynthia roretzi*	AAG27717.1	29
GIL-268.ab1	Hypothetical protein CBG15123	*Caenorhabditis briggsae*	CAE69098.1	28
GIL-269.ab1	similar to Cathepsin L precursor (Cysteine proteinase 1)	*Strongylocentrotus purpuratus*	XP_780580.1	64
GIL-270.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF93389.1	45
GIL-271.ab1	hypothetical protein XP_790877	*Strongylocentrotus purpuratus*	XP_795970.1	35
GIL-273.ab1	similar to ubiquitin-like 7 (bone marrow stromal cell-derived)	*Strongylocentrotus purpuratus*	XP_782544.1	34
GIL-274.ab1	hypothetical protein XP_787841	*Strongylocentrotus purpuratus*	XP_792934.1	51
GIL-277.ab1	similar to Wnt inhibitory factor 1 precursor (WIF-1)	*Gallus gallus*	XP_422145.1	27
GIL-282.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF96443.1	95
GIL-283.ab1	NifU-like protein	*Branchiostoma belcheri tsingtaunese*	AAQ83894.1	74
GIL-286.ab1	peritrophin 1	*Mamestra configurata*	AAP33177.1	23
GIL-288.ab1	hypothetical chloroplast RF1	*Zygnema circumcarinatum*	AAX45877.1	29
GIL1.ab1	hypothetical protein DDB0186385	*Dictyostelium discoideum*	XP_638358.1	37
GIL10.ab1	acetyl coenzyme A-transferase	*Artemia franciscana*	AAP68833.1	62
GIL100.ab1	ribosomal protein S2	*Chlamys farreri*	AAM94271.1	90

Table 3. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I^{a}
GIL102.ab1	similar to CG17941-PA	*Strongylocentrotus purpuratus*	XP_785445.1	40
GIL103.ab1	similar to CG4170-PA, isoform A	*Apis mellifera*	XP_392925.2	30
GIL104.ab1	hexokinase II	*Oryza sativa (japonica cultivar-group)*	XP_477001.1	43
GIL105.ab1	Zgc:101858	*Danio rerio*	AAH81417.1	40
GIL106.ab1	malate dehydrogenase precursor	*Nucella lapillus*	AAF27650.1	74
GIL107.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF92618.1	89
GIL108.ab1	-		_	0
GIL109.ab1	hypothetical protein XP_923464	*Mus musculus*	XP_928557.1	29
GIL110.ab1	HyTSRp1 protein	*Hydra vulgaris*	CAJ65510.2	44
GIL111.ab1	hypothetical protein ECH_0700	*Ehrlichia chaffeensis str. Arkansas*	YP_507505.1	30
GIL112.ab1	maltose permease	*Pichia angusta*	AAX92669.1	41
GIL113.ab1	hypothetical protein LOC320871	*Mus musculus*	NP_796254.1	36
GIL114.ab1	ribosomal protein L4	*Danio rerio*	NP_998272.1	82
GIL115.ab1	cytoplasmic intermediate filament protein A	*Aplysia californica*	CAA42839.1	47
GIL116.ab1	cytochrome b5	*Crassostrea gigas*	CAD92095.1	62
GIL117.ab1	NADH dehydrogenase subunit 2	*Haliotis rubra*	YP_026068.1	64
GIL118.ab1	Achalasia, adrenocortical insufficiency, alacrimia (Allgrove, triple-A)	*Homo sapiens*	AAH00659.1	42
GIL119.ab1	unnamed protein product	*Mus musculus*	BAC29012.1	77
GIL12.ab1	-		_	0
GIL120.ab1	inhibitor of apoptosis protein 3	*Orgyia pseudotsugata multicapsid nucleopolyhedrovirus*	NP_046191.1	36
GIL122.ab1	hypothetical protein XP_697260	*Danio rerio*	XP_702352.1	29
GIL123.ab1	Phosphoserine aminotransferase 1	*Danio rerio*	AAH64289.1	63
GIL124.ab1	MGC52542 protein	*Xenopus laevis*	AAH41183.1	45
GIL125.ab1	120-kDa protein	*Sarcophaga peregrina*	BAA92143.1	29
GIL126.ab1	-		_	0
GIL127.ab1	Translation initiation factor IF-2	*Prochlorococcus marinus subsp. pastoris str. CCMP1986*	CAE19953.1	29
GIL128.ab1	Na-dependent Cl/HCO3 exchanger	*Loligo pealei*	AAN75454.1	54
GIL129.ab1	-		_	0
GIL13.ab1	similar to Multiple EGF-like-domain protein 5 precursor (Multiple epidermal growth factor-like domains 9)	*Bos taurus*	XP_613349.2	27
GIL130.ab1	Lysophospholipase, putative	*Paramecium tetraurelia*	YP_054235.1	23
GIL131.ab1	similar to signal peptide, CUB domain, EGF-like 3	*Canis familiaris*	XP_538874.2	42
GIL132.ab1	putative chitin synthase	*ericoid mycorrhizal fungus PSIV*	CAC95227.1	27
GIL133.ab1	hypothetical protein CHGG_08236	*Chaetomium globosum CBS 148.51*	EAQ86983.1	30
GIL134.ab1	Hypothetical protein CBG06318	*Caenorhabditis briggsae*	CAE62259.1	32
GIL135.ab1	MGC83180 protein	*Xenopus laevis*	AAH71044.1	35
GIL137.ab1	-		_	0

Table 3. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
GIL138.ab1	immobilization antigen LA	*Tetrahymena thermophila*	AAG38116.1	24
GIL139.ab1	similar to CG9285-PA, isoform A, partial	*Strongylocentrotus purpuratus*	XP_795581.1	62
GIL14.ab1	KARG_NORMA Arginine kinase (AK)		P51544	73
GIL140.ab1	AFR490Cp	*Ashbya gossypii ATCC 10895*	AAS53861.1	38
GIL141.ab1	similar to WD repeat domain 41	*Strongylocentrotus purpuratus*	XP_782810.1	46
GIL142.ab1	COG3025	*Rubrivivax gelatinosus PM1*	ZP_00242551.1	32
GIL143.ab1	heat shock protein gp96	*Strongylocentrotus purpuratus*	AAO21341.1	72
GIL145.ab1	protein Y94A7B.2 - Caenorhabditis elegans	*Caenorhabditis elegans*	F89402	37
GIL146.ab1	probable Major facilitator superfamily (MFS) transporter	*Psychrobacter arcticus 273-4*	AAZ19359.1	38
GIL147.ab1	ribosomal protein P1	*Biomphalaria glabrata*	AAZ39530.1	56
GIL148.ab1	-		_	0
GIL150.ab1	GA21175-PA	*Drosophila pseudoobscura*	EAL26100.1	35
GIL152.ab1	similar to tubulin, beta, 2	*Strongylocentrotus purpuratus*	XP_791790.1	80
GIL154.ab1	-		_	0
GIL155.ab1	protoheme IX farnesyltransferase	*Bdellovibrio bacteriovorus*	CAE77947.1	33
GIL156.ab1	CHH_CANPG Crustacean hyperglycemic hormone (CHH)		8.6P81032	
GIL157.ab1	ribosomal protein L7	*Crassostrea gigas*	CAD89885.1	77
GIL158.ab1	cytochrome b	*Haliotis rubra*	YP_026076.1	88
GIL159.ab1	Oxidoreductase, N-terminal:Oxidoreductase, C-terminal	*Chlorobium phaeobacteroides DSM 266*	ZP_00529298.1	38
GIL16.ab1	Asparagine synthetase	*Danio rerio*	AAH67140.1	55
GIL160.ab1	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	*Danio rerio*	AAH64889.1	47
GIL162.ab1	hypothetical protein PFL1085w	*Plasmodium falciparum 3D7*	NP_701579.1	38
GIL165.ab1	heat shock protein 70	*Crassostrea virginica*	CAB89802.1	52
GIL168.ab1	asparagine synthetase	*Gallus gallus*	NP_001026148.1	69
GIL169.ab1	acyl-coenzyme A: cholesterol acyltransferase	*Homo sapiens*	AAC37532.2	52
GIL17.ab1	DNL1_XENLA DNA ligase I (Polydeoxyribonucleotide synthase)	*ATP*	P51892	73
GIL171.ab1	-		_	0
GIL172.ab1	mu class glutathione S-transferase	*Crassostrea gigas*	CAD90167.1	60
GIL173.ab1	LIM protein	*Mytilus edulis*	ABB73031.1	61
GIL174.ab1	67kD laminin receptor precursor	*Xenopus laevis*	AAW62261.1	79
GIL175.ab1	similar to tripartite motif-containing 2	*Strongylocentrotus purpuratus*	XP_780295.1	29
GIL176.ab1	ENSANGP00000014400	*Anopheles gambiae str. PEST*	EAA14482.2	36
GIL177.ab1	similar to Coagulation factor XII precursor (Hageman factor) (HAF) isoform 4	*Bos taurus*	XP_888407.1	42
GIL178.ab1	CG10231-PA	*Drosophila melanogaster*	NP_609885.1	25
GIL179.ab1	olfactory receptor 152	*Mus musculus*	NP_666857.1	25
GIL18.ab1	LOC570288 protein	*Danio rerio*	AAH90688.1	53
GIL180.ab1	maturase	*Haworthia attenuata var. britteniana*	CAD54547.2	40
GIL181.ab1	alpha-tubulin 1	*Branchiostoma floridae*	AAM73981.1	98
GIL182.ab1	tryptophan 2,3-dioxygenase	*Chlamys farreri*	AAY32933.1	46

Table 3. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
GIL183.ab1	-		_	0
GIL184.ab1	conserved hypothetical protein	*Rhodoferax ferrireducens DSM 15236*	ZP_00693303.1	26
GIL186.ab1	plancitoxin I	*Acanthaster planci*	BAD13432.1	43
GIL187.ab1	-		_	0
GIL188.ab1	Ctsql2 protein	*Rattus norvegicus*	AAH97257.1	35
GIL189.ab1	bicaudal D homolog 1 isoform 1	*Homo sapiens*	NP_001705.2	56
GIL19.ab1	phosphohistidine phosphatase	*Mus musculus*	NP_083569.1	53
GIL190.ab1	similar to CG12002-PA, isoform A	*Danio rerio*	XP_698715.1	43
GIL191.ab1	ST1C2_HUMAN Sulfotransferase 1C2 (SULT1C) (SULT1C#2)		6.00E-30O75897	
GIL192.ab1	Hypothetical protein MGC76172	*Xenopus tropicalis*	AAH68215.1	43
GIL2.ab1	similar to proteasome subunit beta 7 isoform 1	*Danio rerio*	XP_682902.1	79
GIL20.ab1	stromal cell derived factor 2-like protein	*Branchiostoma belcheri tsingtaunese*	AAT34988.1	38
GIL22.ab1	GTPase, IMAP family member 7	*Mus musculus*	AAH26200.1	24
GIL23.ab1	similar to Asparaginyl-tRNA synthetase, cytoplasmic (Asparagine--tRNA ligase) (AsnRS)	*Bos taurus*	XP_873087.1	60
GIL24.ab1	COG0596: Predicted hydrolases or acyltransferases (alpha/beta hydrolase superfamily)	*Burkholderia pseudomallei S13*	ZP_00497519.1	33
GIL25.ab1	similar to Neurogenic locus notch homolog protein 2 precursor	*Strongylocentrotus purpuratus*	XP_793179.1	33
GIL26.ab1	isochorismate synthase	*Bacillus subtilis subsp. subtilis str. 168*	CAB15189.1	33
GIL28.ab1	-		_	0
GIL29.ab1	-		_	0
GIL3.ab1	similar to leucine rich repeat containing 49, partial	*Bos taurus*	XP_875201.1	39
GIL30.ab1	actin	*Haliotis discus hannai*	AAQ92368.1	99
GIL32.ab1	Hypothetical protein CBG00049	*Caenorhabditis briggsae*	CAE57199.1	42
GIL33.ab1	similar to huntingtin-associated protein interacting protein (duo)	*Gallus gallus*	XP_422102.1	31
GIL36.ab1	calponin homolog	*Schistosoma japonicum*	AAD11976.1	62
GIL37.ab1	serine/threonine protein phosphatase	*Trypanosoma cruzi strain CL Brener*	XP_808861.1	38
GIL38.ab1	hypothetical protein FG05827.1	*Gibberella zeae PH-1*	XP_386003.1	30
GIL39.ab1	unnamed protein product	*Mus musculus*	BAC41141.1	30
GIL4.ab1	conserved hypothetical protein	*Streptococcus pneumoniae*	CAI33513.1	27
GIL41.ab1	SJCHGC02792 protein	*Schistosoma japonicum*	AAW25342.1	73
GIL43.ab1	-		_	0
GIL44.ab1	SLC25A3 protein	*Homo sapiens*	AAH51367.1	67
GIL46.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF91219.1	46

Table 3. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
GIL47.ab1	similar to vomeronasal 1 receptor, E12	*Mus musculus*	XP_893239.1	42
GIL48.ab1	hypothetical protein	*Plasmodium chabaudi chabaudi*	XP_743225.1	34
GIL49.ab1	putative carbohydrate kinase	*Yersinia pestis biovar Medievalis str. 91001*	AAS64055.1	33
GIL5.ab1	TonB dependent, hydroxamate-type ferrisiderophore, outer membrane receptor	*Rhodobacter sphaeroides 2.4.1*	ABA77581.1	36
GIL50.ab1	-		_	0
GIL51.ab1	similar to Golgi autoantigen, golgin subfamily A member 1 (Golgin-97)	*Strongylocentrotus purpuratus*	XP_783013.1	44
GIL56.ab1	hypothetical protein	*Azoarcus sp. EbN1*	CAI10525.1	34
GIL57.ab1	similar to mitochondrial ATP synthase lipid binding protein precursor	*Apis mellifera*	XP_623661.1	40
GIL58.ab1	variable region-containing chitin-binding protein 1	*Branchiostoma floridae*	AAN62848.1	36
GIL59.ab1	similar to eukaryotic translation initiation factor 5A	*Strongylocentrotus purpuratus*	XP_785877.1	66
GIL6.ab1	139R protein	*Yaba-like disease virus*	CAC21377.1	38
GIL60.ab1	repressor	*Neurospora crassa*	CAA32753.1	31
GIL61.ab1	INVO_PONPY Involucrin		P14708	36
GIL65.ab1	SJCHGC01867 protein	*Schistosoma japonicum*	AAW25067.1	25
GIL66.ab1	TCB2_CAEBR Transposable element Tcb2 transposase		6.00E-14Q04202	
GIL69.ab1	Hypothetical protein CBG21335	*Caenorhabditis briggsae*	CAE73785.1	38
GIL70.ab1	polyubiquitin	*Artemia franciscana*	CAA52416.1	93
GIL71.ab1	similar to bcl2-like, partial	*Strongylocentrotus purpuratus*	XP_787479.1	33
GIL72.ab1	similar to KIAA1958	*Danio rerio*	XP_698239.1	32
GIL74.ab1	ENSANGP00000003404	*Apis mellifera*	XP_624359.1	81
GIL75.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF92450.1	38
GIL77.ab1	MGC83093 protein	*Xenopus laevis*	AAH74470.1	50
GIL78.ab1	lipase class 3-like	*Oryza sativa (japonica cultivar-group)*	XP_465669.1	35
GIL79.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02734.1	79
GIL8.ab1	phosphoribosyl pyrophosphate synthase isozyme 4	*Spinacia oleracea*	CAB43602.1	47
GIL80.ab1	similar to EQYK340	*Gallus gallus*	XP_413861.1	25
GIL81.ab1	similar to Dipeptidyl-peptidase II precursor (DPP II) (Dipeptidyl aminopeptidase II) (Quiescent cell proline dipeptidase) (Dipeptidyl peptidase 7)	*Strongylocentrotus purpuratus*	XP_797434.1	54
GIL82.ab1	-		_	0
GIL83.ab1	cingulin	*Xenopus laevis*	AAF20208.1	32
GIL84.ab1	spermatogenesis associated 4	*Ciona intestinalis*	NP_001029005.1	62
GIL87.ab1	unnamed protein product	*Aspergillus oryzae*	BAE63146.1	36
GIL89.ab1	NADH dehydrogenase subunit 2	*Lagonosticta virata*	AAQ97038.1	25

Table 3. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
GIL92.ab1	type II protein geranylgeranyltransferase subunit beta	*Plasmodium yoelii yoelii str. 17XNL*	XP_729872.1	29
GIL93.ab1	tonoplast intrinsic protein		AAB17284.1	50
GIL95.ab1	Signal sequence receptor, alpha	*Rattus norvegicus*	AAH98008.1	46
GIL96.ab1	-		_	0
GIL97.ab1	chitin synthase	*Atrina rigida*	AAY86556.1	28
GIL99.ab1	ENSANGP00000010305	*Anopheles gambiae str. PEST*	EAA12308.2	37
GIL-278.ab1	Hypothetical protein LOC606663	*Danio rerio*	AAI00057.1	56
GIL-279.ab1	similar to jumonji domain containing 1B	*Apis mellifera*	XP_392473.2	77
GIL-281.ab1	TPA: TPA_exp: HDC17201	*Drosophila melanogaster*	DAA03187.1	32

[a] Identity(%)

Table 4. List of identified ESTs from the gut cDNA library of abalone

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
DGT-195.ab1	SJCHGC04784 protein	*Schistosoma japonicum*	AAW26731.1	42
DGT-196.ab1	cytochrome c oxidase subunit III	*Haliotis rubra*	YP_026066.1	76
DGT-198.ab1	-		_	0
DGT-199.ab1	similar to ribosomal protein L11	*Apis mellifera*	XP_393094.2	94
DGT-200.ab1	hypothetical protein 44.t00024	*Entamoeba histolytica HM-1:IMSS*	XP_654768.1	35
DGT-201.ab1	Na(+)-translocating NADH-quinone reductase subunit B	*Alteromonas macleodii 'Deep ecotype'*	ZP_01111024.1	33
DGT-202.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG01328.1	50
DGT-203.ab1	putative envelope protein	*Enterobacteria phage lambda*	NP_597781.1	100
DGT-204.ab1	hypothetical protein jk0583	*Corynebacterium jeikeium K411*	YP_250360.1	36
DGT-205.ab1	ferritin-like protein	*Pinctada fucata*	AAQ12076.1	83
DGT-206.ab1	Arp3	*Danio rerio*	AAM63548.1	79
DGT-207.ab1	macrophage migration inhibitory factor II	*Branchiostoma belcheri tsingtaunese*	AAT77698.1	52
DGT-208.ab1	unnamed protein product	*Haliotis rufescens*	CAA50572.1	77
DGT-209.ab1	hypothetical protein	*Trypanosoma cruzi strain CL Brener*	XP_812821.1	37
DGT-210.ab1	hypothetical protein UM03624.1	*Ustilago maydis 521*	XP_759771.1	22
DGT-211.ab1	putative zinc-binding protein	*Oryza sativa (japonica cultivar-group)*	XP_464434.1	42
DGT-212.ab1	ribosomal protein L4 variant	*Homo sapiens*	BAD92214.1	79
DGT-213.ab1	ependymin-related protein	*Branchiostoma belcheri tsingtaunese*	AAQ19608.1	30
DGT-214.ab1	hypothetical protein	*Strongylocentrotus purpuratus*	XP_795237.1	28
DGT-215.ab1	similar to notch homolog 5, partial	*Strongylocentrotus purpuratus*	XP_798650.1	32
DGT-216.ab1	Elongator protein 3/MiaB/NifB	*Clostridium beijerincki NCIMB 8052*	ZP_00909118.1	33
DGT-217.ab1	similar to Carboxypeptidase A1 precursor isoform 1	*Canis familiaris*	XP_532424.2	26
DGT-218.ab1	multidrug translocase MdfA, chloramphenicol resistance pump Cmr	*Legionella pneumophila subsp. pneumophila str. Philadelphia 1*	AAU27487.1	33
DGT-219.ab1	hypothetical protein MGG_ch7g450	*Magnaporthe grisea 70-15*	EAQ71043.1	30
DGT-220.ab1	cysteine peptidase 2 cathepsin-B-like	*Lonomia obliqua*	AAV91452.1	64
DGT-222.ab1	recombination activating protein 1	*Rhampholeon kerstenii*	AAT36607.1	32
DGT-223.ab1	unnamed protein product	*Mus musculus*	BAB27678.2	72
DGT-224.ab1	similar to leukotriene B4 12-hydroxydehydrogenase	*Strongylocentrotus purpuratus*	XP_781982.1	46
DGT-225.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09510.1	23
DGT-226.ab1	cytochrome c oxidase subunit I	*Haliotis rubra*	YP_026069.1	84
DGT-229.ab1	conserved hypothetical protein	*Anabaena variabilis ATCC 29413*	ABA20289.1	28
DGT-230.ab1	similar to agrin isoform 1	*Canis familiaris*	XP_536713.2	37
DGT-231.ab1	-		_	0
DGT-232.ab1	similar to COMM domain containing 2	*Strongylocentrotus purpuratus*	XP_781013.1	66

Table 4. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
DGT-233.ab1	actin	*Haliotis discus hannai*	AAQ92368.1	91
DGT-234.ab1	similar to Butyrate response factor 1 (TIS11B protein)	*Strongylocentrotus purpuratus*	XP_782811.1	48
DGT-235.ab1	ENSANGP00000022619	*Anopheles gambiae str. PEST*	EAA43623.1	64
DGT-236.ab1	Heat shock protein htpG	*Fusobacterium nucleatum subsp. nucleatum ATCC 25586*	AAL94527.1	32
DGT-237.ab1	translation elongation factor 1-alpha	*Ancylostoma ceylanicum*	ABB53348.1	96
DGT-238.ab1	ENSANGP00000021821	*Anopheles gambiae str. PEST*	EAA03959.3	74
DGT-239.ab1	Tubulin, alpha 1	*Mus musculus*	AAH83344.1	100
DGT-241.ab1	novel hemicentin protein	*Danio rerio*	CAI11663.1	48
DGT-242.ab1	MGC81227 protein	*Xenopus laevis*	AAH68740.1	69
DGT-243.ab1	Hypothetical protein LOC553722	*Danio rerio*	AAH95794.1	46
DGT-245.ab1	LP10960p	*Drosophila melanogaster*	AAM49985.1	27
DGT-247.ab1	similar to Sorbitol dehydrogenase (L-iditol 2-dehydrogenase)	*Gallus gallus*	XP_413719.1	53
DGT-248.ab1	similar to CG15544-PA	*Apis mellifera*	XP_623992.1	28
DGT-250.ab1	-		_	0
DGT-251.ab1	similar to Angiotensin-converting enzyme, testis-specific isoform precursor (ACE-T)	*Strongylocentrotus purpuratus*	XP_798005.1	56
DGT-252.ab1	putative peroxidase	*Oryza sativa (japonica cultivar-group)*	XP_467718.1	31
DGT-253.ab1	heat shock 90 kDa protein	*Priapulus caudatus*	ABB29634.1	70
DGT-254.ab1	PREDICTED: similar to COP9 signalosome subunit 6, partial	*Strongylocentrotus purpuratus*	XP_790338.1	72
DGT-255.ab1	similar to cubilin isoform 4	*Mus musculus*	XP_914901.1	21
DGT-256.ab1	unknown protein; 28005-31097	*Arabidopsis thaliana*	AAG52285.1	43
DGT-258.ab1	pyridoxine biosynthesis protein	*Dehalococcoides ethenogenes 195*	AAW40334.1	76
DGT-259.ab1	CD98hc amino acid transporter protein	*Aedes aegypti*	AAP76306.1	41
DGT-260.ab1	hypothetical protein PFF0340c	*Plasmodium falciparum 3D7*	XP_966059.1	32
DGT-261.ab1	axonemal dynein light chain p33	*Strongylocentrotus purpuratus*	NP_999680.1	83
DGT-262.ab1	hemocyanin type 1	*Haliotis tuberculata*	CAB76379.1	84
DGT-263.ab1	hypothetical protein PB2503_03532	*Parvularcula bermudensis HTCC2503*	ZP_01016573.1	31
DGT-264.ab1	R04B5.5	*Caenorhabditis elegans*	NP_505591.1	55
DGT-265.ab1	SPCC18B5.08c	*Schizosaccharomyces pombe*	CAB52155.1	37
DGT-266.ab1	SJCHGC02321 protein	*Schistosoma japonicum*	AAX25813.2	38
DGT-267.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF98179.1	42
DGT-268.ab1	centrin-related-protein,putative	*Paramecium tetraurelia*	CAI38942.1	36
DGT-269.ab1	hypothetical protein DDB0219680	*Dictyostelium discoideum*	XP_635304.1	44
DGT-270.ab1	dopamine beta-hydroxylase	*Equus caballus*	BAA82274.1	28
DGT-271.ab1	similar to methyltransferase Cyt19	*Gallus gallus*	XP_421735.1	37
DGT-273.ab1	60S ribosomal protein L13A	*Mytilus edulis*	ABA46793.1	80
DGT-275.ab1	unnamed protein product	*Homo sapiens*	BAD18552.1	25
DGT-276.ab1	peroxiredoxin V protein	*Branchiostoma belcheri tsingtaunese*	AAM18076.1	58

Table 4. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
DGT-277.ab1	similar to 5 nucleotidase, partial	*Strongylocentrotus purpuratus*	XP_797421.1	48
DGT-278.ab1	undecaprenyl-PP-N-acetylmuramic acid-pentapeptide N-acetylglucosamine transferase	*Enterococcus faecalis*	AAC45636.1	48
DGT-279.ab1	BAG-family molecular chaperone regulator-3; BAG-3	*Homo sapiens*	AAD16122.2	41
DGT-281.ab1	Zgc:56585 protein	*Danio rerio*	AAH66622.1	49
DGT-282.ab1	similar to regucalcin	*Canis familiaris*	XP_538011.2	63
DGT-283.ab1	-		_	0
DGT-285.ab1	-		_	0
DGT-286.ab1	actin	*Haliotis discus hannai*	AAQ92368.1	100
DGT-287.ab1	-		_	0
DGT-288.ab1	acrosin	*Rattus norvegicus*	NP_036622.1	58
DGT1.ab1	actin A3	*Haliotis iris*	AAX19288.1	100
DGT10.ab1	hypothetical protein XP_511394	*Pan troglodytes*	XP_511394.1	54
DGT100.ab1	Hypothetical protein LOC406376	*Danio rerio*	AAH65987.1	86
DGT101.ab1	-		_	0
DGT103.ab1	HesB-like domain protein	*Plasmodium falciparum 3D7*	NP_473335.1	27
DGT104.ab1	similar to transposable element tcb1 transposase (2C227)	*Strongylocentrotus purpuratus*	XP_788852.1	30
DGT106.ab1	QM protein	*Pinctada fucata*	AAN85578.1	85
DGT107.ab1	unknown protein	*Oryza sativa (japonica cultivar-group)*	AAV44016.1	40
DGT108.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	AAN36826.1	23
DGT109.ab1	similar to CG4785-PA	*Strongylocentrotus purpuratus*	XP_795990.1	54
DGT110.ab1	similar to solute carrier family 2 (facilitated glucose transporter), member 13	*Danio rerio*	XP_687870.1	37
DGT112.ab1	unnamed protein product	*Aspergillus oryzae*	BAE56181.1	30
DGT113.ab1	LOC447981 protein	*Xenopus tropicalis*	AAH76693.1	77
DGT114.ab1	Rpsa protein	*Mus musculus*	AAH92041.1	68
DGT116.ab1	RE46519p	*Drosophila melanogaster*	AAR30186.1	34
DGT117.ab1	-		_	0
DGT118.ab1	Rab7	*Aiptasia pulchella*	AAQ23388.1	92
DGT119.ab1	S-adenosylhomocysteine hydrolase	*Aurelia aurita*	AAX09927.1	72
DGT12.ab1	KARG_NORMA Arginine kinase (AK)		P51544	76
DGT120.ab1	-		_	0
DGT121.ab1	similar to ENSANGP00000020783	*Apis mellifera*	XP_395511.2	27
DGT122.ab1	chitobiase	*Arthrobacter sp.*	CAB72127.1	32
DGT123.ab1	hypothetical protein XP_785342	*Strongylocentrotus purpuratus*	XP_790435.1	35
DGT124.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG04980.1	29
DGT125.ab1	SPAC3A11.11c	*Schizosaccharomyces pombe*	CAB16386.1	45
DGT126.ab1	hypothetical protein PB000759.00.0	*Plasmodium berghei strain ANKA*	XP_670334.1	28
DGT127.ab1	thioester-containing protein	*Euphaedusa tau*	BAE44110.1	57
DGT129.ab1	CG32685-PC	*Drosophila melanogaster*	NP_727393.1	36

Table 4. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
DGT130.ab1	triple gene block	*grapevine Rupestris stem pitting associated virus*	AAC62911.1	50
DGT132.ab1	cytochrome c oxidase subunit II	*Haliotis rubra*	YP_026070.1	76
DGT133.ab1	similar to gamma-glutamyltranspeptidase precursor	*Staphylococcus aureus subsp. aureus Mu50*	BAB56371.1	29
DGT134.ab1	cytosolic malate dehydrogenase	*Homo sapiens*	NP_005908.1	71
DGT135.ab1	C38C10.2	*Caenorhabditis elegans*	NP_499065.1	48
DGT136.ab1	unnamed protein product	*Mus musculus*	BAE27593.1	39
DGT137.ab1	tektin 3	*Xenopus tropicalis*	NP_001016475.1	55
DGT138.ab1	similar to CG7466-PA, partial	*Apis mellifera*	XP_625133.1	55
DGT139.ab1	ethylene-responsive protein, putative	*Arabidopsis thaliana*	AAM66054.1	38
DGT14.ab1	glutathione S-transferase sigma	*Bombyx mori*	BAD91107.1	43
DGT141.ab1	hemocyanin type 1	*Haliotis tuberculata*	CAB76379.1	79
DGT142.ab1	similar to reduced expression 2 isoform 9	*Mus musculus*	XP_905490.1	33
DGT143.ab1	-		_	0
DGT144.ab1	NEQ325	*Nanoarchaeum equitans Kin4-M*	AAR39173.1	38
DGT149.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_702713.1	28
DGT15.ab1	-		_	0
DGT151.ab1	lipopolysaccharide- and beta-1,3-glucan-binding protein	*Chlamys farreri*	AAP82240.1	63
DGT152.ab1	heat shock protein 90	*Chlamys farreri*	AAR11781.1	88
DGT154.ab1	hypothetical protein	*Nocardia farcinica IFM 10152*	BAD60035.1	28
DGT155.ab1	hypothetical protein D1044.3 - Caenorhabditis elegans	*Caenorhabditis elegans*	T15881	31
DGT156.ab1	LOC495363 protein	*Xenopus laevis*	AAH84827.1	42
DGT157.ab1	similar to regucalcin, partial	*Strongylocentrotus purpuratus*	XP_780550.1	51
DGT158.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_704759.1	45
DGT159.ab1	mFLJ00114 protein	*Mus musculus*	BAD21383.1	54
DGT16.ab1	similar to 3-hydroxyacyl-CoA dehydrogenase type II (Type II HADH) (Scully protein) isoform 1	*Strongylocentrotus purpuratus*	XP_780014.1	71
DGT160.ab1	GRAAL protein	*Drosophila melanogaster*	CAB64653.1	30
DGT161.ab1	Na-dependent Cl/HCO3 exchanger	*Loligo pealei*	AAN75454.1	67
DGT162.ab1	similar to CG9291-PB, isoform B	*Strongylocentrotus purpuratus*	XP_788561.1	88
DGT163.ab1	similar to Betaine--homocysteine S-methyltransferase	*Strongylocentrotus purpuratus*	XP_785340.1	38
DGT164.ab1	cobalamin-5-phosphate synthase CobS	*Thiobacillus denitrificans ATCC 25259*	YP_316468.1	37
DGT165.ab1	-		_	0
DGT166.ab1	-		_	0
DGT168.ab1	selenoprotein W1	*Danio rerio*	AAO86696.1	62
DGT169.ab1	similar to notch homolog 5	*Canis familiaris*	XP_539440.2	26
DGT17.ab1	MGC80936 protein	*Xenopus laevis*	AAH73439.1	75

Table 4. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
DGT167.ab1	similar to Guanine nucleotide-binding protein G(olf), alpha subunit (Adenylate cyclase-stimulating G alpha protein, olfactory type)	*Pan troglodytes*	XP_512019.1	31
DGT171.ab1	ENSANGP00000020389	*Anopheles gambiae str. PEST*	EAA01312.2	55
DGT172.ab1	-		_	0
DGT173.ab1	putative long-chain fatty acid--CoA ligase	*Rhodopseudomonas palustris CGA009*	CAE26892.1	33
DGT174.ab1	Aldh1-A protein	*Xenopus laevis*	AAH77256.1	73
DGT175.ab1	similar to olfactory receptor, family 8, subfamily S, member 1	*Bos taurus*	XP_870538.1	32
DGT177.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF98538.1	52
DGT178.ab1	moj29	*Drosophila arizonae*	AAZ42765.1	75
DGT179.ab1	GH01128p	*Drosophila melanogaster*	AAM12266.1	30
DGT18.ab1	cellulase EGX	*Ampullaria crossean*	AAP31839.1	44
DGT180.ab1	similar to likely ortholog of mouse klotho lactase-phlorizin hydrolase related protein	*Canis familiaris*	XP_544736.2	44
DGT181.ab1	similar to Deoxycytidylate deaminase (dCMP deaminase) isoform 1	*Canis familiaris*	XP_849027.1	59
DGT182.ab1	LOC443666 protein	*Xenopus laevis*	AAH73564.1	30
DGT183.ab1	GA17076-PA	*Drosophila pseudoobscura*	EAL24720.1	82
DGT184.ab1	similar to Zinc finger protein 93 (Zinc finger protein HTF34)	*Strongylocentrotus purpuratus*	XP_797334.1	39
DGT186.ab1	hypothetical protein AN9416.2	*Aspergillus nidulans FGSC A4*	XP_868798.1	48
DGT187.ab1	PCK2 protein	*Xenopus laevis*	AAH44042.1	78
DGT188.ab1	aquaporin	*Pyrocoelia rufa*	AAL09065.1	41
DGT189.ab1	phospholipid-transporting P-type ATPase	*Entamoeba histolytica HM-1:IMSS*	XP_649745.1	24
DGT190.ab1	methionine adenosyltransferase	*Haliotis rufenscens*	AAZ30689.1	92
DGT192.ab1	ENSANGP00000013237	*Anopheles gambiae str. PEST*	EAA04177.2	30
DGT20.ab1	similar to Rho-related BTB domain-containing protein 1 isoform 4	*Bos taurus*	XP_886685.1	24
DGT22.ab1	elongation factor 1 alpha	*Haliotis rufenscens*	AAZ30688.1	93
DGT23.ab1	-		_	0
DGT24.ab1	hypothetical protein, conserved	*Theileria annulata*	CAI75758.1	28
DGT25.ab1	ZK829.7	*Caenorhabditis elegans*	NP_502269.1	38
DGT26.ab1	ribosomal protein S5	*Crassostrea gigas*	CAD91445.1	90
DGT27.ab1	-		_	0
DGT28.ab1	similar to transposable element tcb1 transposase (2C227)	*Strongylocentrotus purpuratus*	XP_788852.1	44
DGT29.ab1	ATPase, H+ transporting, lysosomal accessory protein 1	*Danio rerio*	AAH85541.1	47
DGT3.ab1	phosphoesterase	*Bacillus thuringiensis serovar konkukian str. 97-27*	AAT59453.1	38

Table 4. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
DGT30.ab1	tropomyosin 2	*Haliotis asinina*	AAP85232.1	80
DGT33.ab1	Hypothetical LOC304860	*Rattus norvegicus*	AAH82004.1	36
DGT34.ab1	alpha-tubulin 1	*Myxine glutinosa*	AAM73997.1	99
DGT37.ab1	-		_	0
DGT38.ab1	MGC80281 protein	*Xenopus laevis*	AAH72175.1	41
DGT4.ab1	plancitoxin I	*Acanthaster planci*	BAD13432.1	40
DGT40.ab1	hypothetical protein UM06345.1	*Ustilago maydis 521*	XP_762492.1	71
DGT41.ab1	similar to protein tyrosine phosphatase, receptor type, Q isoform 1 precursor	*Danio rerio*	XP_683017.1	37
DGT42.ab1	-		_	0
DGT43.ab1	hemocyanin	*Haliotis tuberculata*	CAC20588.1	89
DGT44.ab1	ENSANGP00000015804	*Anopheles gambiae str. PEST*	EAA01581.2	47
DGT45.ab1	similar to CG12132-PA	*Mus musculus*	XP_911461.1	25
DGT46.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_703995.1	30
DGT47.ab1	hypothetical protein CHGG_04568	*Chaetomium globosum CBS 148.51*	EAQ87949.1	21
DGT48.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10060.1	35
DGT49.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_473201.1	44
DGT5.ab1	MGC107844 protein	*Xenopus tropicalis*	NP_001015715.1	29
DGT50.ab1	hypothetical protein TP03_0696	*Theileria parva strain Muguga*	XP_762820.1	34
DGT51.ab1	keratin associated protein 5-2	*Homo sapiens*	NP_001004325.1	52
DGT52.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF92929.1	41
DGT53.ab1	ENSANGP00000012047	*Anopheles gambiae str. PEST*	EAA00609.3	31
DGT54.ab1	cytochrome c oxidase subunit I	*Haliotis rubra*	YP_026069.1	84
DGT55.ab1	ATP synthase F0 subunit 6	*Haliotis rubra*	YP_026072.1	84
DGT56.ab1	similar to WD repeat domain 34	*Strongylocentrotus purpuratus*	XP_788363.1	48
DGT58.ab1	galaxin	*Galaxea fascicularis*	BAC41519.1	37
DGT59.ab1	SJCHGC04882 protein	*Schistosoma japonicum*	AAW25230.1	44
DGT60.ab1	ENSANGP00000010271	*Anopheles gambiae str. PEST*	EAA08121.2	43
DGT61.ab1	Plasmodium chabaudi chabaudi elongation factor G	*Plasmodium chabaudi chabaudi*	XP_746107.1	27
DGT62.ab1	guanine nucleotide-binding protein	*Petromyzon marinus*	AAM88904.1	83
DGT63.ab1	chloride intracellular channel protein	*Bombyx mori*	AAS91556.1	34
DGT64.ab1	asparaginyl-tRNA synthetase	*Pan troglodytes*	XP_512149.1	74
DGT65.ab1	putative serine/threonine kinase	*Oryza sativa (japonica cultivar-group)*	XP_479261.1	32
DGT66.ab1	putative ATP-dependent dsDNA exonuclease	*Yersinia pseudotuberculosis IP 32953*	CAH20155.1	41
DGT67.ab1	glr3907	*Gloeobacter violaceus PCC 7421*	BAC91848.1	38
DGT68.ab1	similar to Agmatinase, mitochondrial precursor	*Strongylocentrotus purpuratus*	XP_795606.1	64
DGT69.ab1	similar to WD repeat domain 41	*Strongylocentrotus purpuratus*	XP_782810.1	37

Table 4. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^{a}**
DGT7.ab1	similar to transposable element tcb1 transposase (2C227)	*Strongylocentrotus purpuratus*	XP_788852.1	43
DGT70.ab1	RH01388p	*Drosophila melanogaster*	AAO39468.1	30
DGT71.ab1	similar to membrane frizzled-related protein	*Strongylocentrotus purpuratus*	XP_795691.1	47
DGT72.ab1	unnamed protein product	*Kluyveromyces lactis*	XP_453121.1	27
DGT73.ab1	var1 ribosomal protein	*Saccharomyces servazzii*	CAD23422.1	26
DGT74.ab1	ribosomal protein L	*Argopecten irradians*	AAN05596.1	85
DGT75.ab1	-		_	0
DGT76.ab1	H3 histone, family 3A	*Mus musculus*	AAH88835.1	100
DGT77.ab1	cytosol non-specific dipeptidase	*Lactobacillus acidophilus NCFM*	AAV41940.1	41
DGT78.ab1	-		_	0
DGT79.ab1	hypothetical protein SERP1032	*Staphylococcus epidermidis RP62A*	YP_188609.1	28
DGT8.ab1	similar to Wnt inhibitory factor 1 precursor (WIF-1)	*Gallus gallus*	XP_418688.1	28
DGT80.ab1	similar to heme binding protein 2	*Strongylocentrotus purpuratus*	XP_797226.1	53
DGT81.ab1	GA22070-PA	*Drosophila pseudoobscura*	EAL26285.1	56
DGT82.ab1	hypothetical protein CaO19_10195	*Candida albicans SC5314*	XP_720203.1	33
DGT84.ab1	sigma-54 dependent transcriptional regulator	*Vibrio sp. MED222*	ZP_01063316.1	24
DGT85.ab1	similar to sperm associated antigen 8 isoform 2	*Strongylocentrotus purpuratus*	XP_781437.1	44
DGT87.ab1	Pole2 protein	*Danio rerio*	AAH76287.1	33
DGT90.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_704222.1	43
DGT91.ab1	similar to CG6383-PA	*Strongylocentrotus purpuratus*	XP_786539.1	37
DGT93.ab1	pannexin 5	*Aplysia californica*	AAX24144.1	30
DGT94.ab1	Splicing factor, arginine/serine-rich 2 (SC-35)	*Xenopus tropicalis*	AAH64167.1	80
DGT95.ab1	-		_	0
DGT96.ab1	AT22870p	*Drosophila melanogaster*	AAL90142.1	51
DGT97.ab1	60S acidic ribosomal protein P0	*Ixodes scapularis*	AAY66850.1	75
DGT98.ab1	cytochrome b	*Haliotis rubra*	YP_026076.1	87
DGT99.ab1	hypothetical protein Tb11.02.4690	*Trypanosoma brucei TREU927*	XP_828759.1	34

[a] Identity(%)

Table 5. List of identified ESTs from the hepatopancreas cDNA library of abalone

Clone no.	Putative identification	Closest species	Accession no.	I[a]
VHP-193.ab1	DNA-binding response regulator, putative	*Campylobacter jejuni RM1221*	AAW36204.1	31
VHP-194.ab1	GH28017p	*Drosophila melanogaster*	AAL39380.1	25
VHP-195.ab1	hypothetical protein	*Pongo pygmaeus*	CAH91930.1	65
VHP-196.ab1	LOC447981 protein	*Xenopus tropicalis*	AAH76693.1	81
VHP-197.ab1	selenium dependent salivary glutathione peroxidase	*Ixodes scapularis*	AAY66814.1	47
VHP-198.ab1	glr4144	*Gloeobacter violaceus PCC 7421*	BAC92085.1	35
VHP-199.ab1	60S acidic ribosomal protein P0	*Ixodes scapularis*	AAY66850.1	76
VHP-200.ab1	actin A1	*Haliotis iris*	AAX19286.1	98
VHP-201.ab1	hypothetical protein FG10911.1	*Gibberella zeae PH-1*	XP_391087.1	26
VHP-202.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_703995.1	30
VHP-203.ab1	-		_	0
VHP-204.ab1	ribosomal protein S3a	*Crassostrea gigas*	CAD91420.1	83
VHP-205.ab1	similar to transposable element tcb1 transposase (2C227)	*Strongylocentrotus purpuratus*	XP_788852.1	34
VHP-206.ab1	similar to NOD3 protein	*Danio rerio*	XP_693781.1	34
VHP-207.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_705104.1	36
VHP-208.ab1	CPK27; ATP binding / calcium ion binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase	*Arabidopsis thaliana*	NP_192379.2	31
VHP-209.ab1	hypothetical protein Tc00.1047053507395.40	*Trypanosoma cruzi strain CL Brener*	XP_805930.1	31
VHP-210.ab1	Myc homolog	*Crassostrea virginica*	AAB34577.1	32
VHP-211.ab1	similar to Keratin, type II cytoskeletal 8 (Cytokeratin 8) (Cytokeratin endo A)	*Canis familiaris*	XP_852709.1	24
VHP-212.ab1	similar to deleted in malignant brain tumors 1	*Strongylocentrotus purpuratus*	XP_784537.1	26
VHP-213.ab1	endo-1,3-beta-D-glucanase	*Mizuhopecten yessoensis*	AAW34372.1	50
VHP-214.ab1	hypothetical protein LOC393259	*Danio rerio*	NP_956583.1	50
VHP-215.ab1	similar to Cathepsin L precursor (Cysteine proteinase 1)	*Strongylocentrotus purpuratus*	XP_780580.1	60
VHP-216.ab1	-		_	0
VHP-217.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF96443.1	90
VHP-218.ab1	LOC446918 protein	*Xenopus laevis*	AAI06213.1	41
VHP-219.ab1	Myc homolog	*Crassostrea virginica*	AAB34577.1	40
VHP-220.ab1	alginate lyase	*Haliotis discus hannai*	BAC87758.1	79
VHP-221.ab1	hypothetical protein MGG_ch7g1038	*Magnaporthe grisea 70-15*	EAQ71631.1	24
VHP-224.ab1	hypothetical protein	*Phthorimaea operculella granulovirus*	NP_663208.1	39
VHP-225.ab1	alkaline phosphatase		AAA27331.1	30
VHP-226.ab1	P-553	*Borrelia hermsii*	AAX19498.1	24
VHP-227.ab1	ferritin-like protein	*Pinctada fucata*	AAQ12076.1	70

Table 5. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I^a
VHP-228.ab1	YadA-like, C-terminal:Haemagluttinin motif:Hep_Hag	*Burkholderia vietnamiensis G4*	ZP_00423121.1	23
VHP-229.ab1	NADH dehydrogenase subunit 2	*Haliotis rubra*	YP_026068.1	66
VHP-230.ab1	hypothetical protein	*Plasmodium berghei strain ANKA*	XP_676960.1	31
VHP-231.ab1	hypothetical protein XP_525377	*Pan troglodytes*	XP_525377.1	33
VHP-232.ab1	unnamed protein product	*Aspergillus oryzae*	BAE61882.1	46
VHP-233.ab1	C-type LECtin family member (clec-49)	*Caenorhabditis elegans*	NP_507829.1	44
VHP-234.ab1	hypothetical protein DDB0205241	*Dictyostelium discoideum*	XP_641211.1	40
VHP-236.ab1	Y-box factor-like protein	*Lymnaea stagnalis*	AAT97092.1	81
VHP-237.ab1	collagen pro alpha-chain	*Haliotis discus*	BAA75669.1	87
VHP-239.ab1	similar to hydrolase (3B446)	*Strongylocentrotus purpuratus*	XP_796058.1	41
VHP-240.ab1	GA14168-PA	*Drosophila pseudoobscura*	EAL32817.1	25
VHP-242.ab1	multicystatin	*Vigna unguiculata*	AAQ62561.1	33
VHP-243.ab1	V (tail component;256)	*bacteriophage lambda*	AAA96545.1	93
VHP-246.ab1	Staphylococcal nuclease domain containing 1	*Xenopus tropicalis*	AAH63211.1	62
VHP-247.ab1	hypothetical protein PTD2_08194	*Pseudoalteromonas tunicata D2*	ZP_01133610.1	30
VHP-248.ab1	Rab7	*Aiptasia pulchella*	AAQ23388.1	89
VHP-249.ab1	hypothetical protein	*Plasmodium berghei strain ANKA*	XP_675556.1	33
VHP-250.ab1	GA11607-PA	*Drosophila pseudoobscura*	EAL32579.1	56
VHP-251.ab1	hypothetical protein XP_794737	*Strongylocentrotus purpuratus*	XP_799830.1	38
VHP-252.ab1	defender against apopototic cell death 1	*Argopecten irradians*	AAX56947.1	81
VHP-254.ab1	TPA: TPA_exp: spermidine/spermine N-1 acetyltransferase 2	*Sus scrofa*	DAA01468.1	47
VHP-255.ab1	ribosomal 5S rRNA E-loop binding protein Ctc/L25/TL5	*Acidiphilium cryptum JF-5*	ZP_01145877.1	36
VHP-256.ab1	COG0841: Cation/multidrug efflux pump	*Yersinia mollaretii ATCC 43969*	ZP_00826169.1	30
VHP-257.ab1	D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase family protein	*Cyanobacteria bacterium Yellowstone A-Prime*	YP_475388.1	31
VHP-258.ab1	Myc homolog	*Crassostrea virginica*	AAB34577.1	68
VHP-259.ab1	cytochrome c oxidase subunit I	*Haliotis diversicolor supertexta*	AAQ84296.1	83
VHP-261.ab1	-		_	0
VHP-262.ab1	novel protein similar to vertebrate eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein) (EEF1D)	*Danio rerio*	CAI21007.1	31
VHP-263.ab1	mature-parasite-infected erythrocyte surface antigen		4.00E-08AAA29651.1	
VHP-264.ab1	eukaryotic translation initiation factor 3, subunit 6 interacting protein	*Gallus gallus*	NP_001012553.1	67
VHP-265.ab1	-		_	0
VHP-266.ab1	ENSANGP00000019033	*Anopheles gambiae str. PEST*	EAA06842.2	29
VHP-268.ab1	phosphoribosylpyrophosphate synthetase	*Trypanosoma cruzi strain CL Brener*	XP_807546.1	27

Table 5. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
VHP-270.ab1	hypothetical protein AcidDRAFT_3587	*Solibacter usitatus Ellin6076*	ZP_00523593	29
VHP-271.ab1	-		_	0
VHP-272.ab1	IMP dehydrogenase/GMP reductase:Thrombospondin type 3 repeat	*Desulfuromonas acetoxidans DSM 684*	ZP_00551385.1	29
VHP-274.ab1	similar to ENSANGP00000026584	*Apis mellifera*	XP_623167.1	58
VHP-275.ab1	COG5295: Autotransporter adhesin	*Cytophaga hutchinsonii*	ZP_00310324.1	31
VHP-277.ab1	putative sphingosine-1-phosphate lyase	*Symbiobacterium thermophilum IAM 14863*	BAD40259.1	51
VHP-278.ab1	Myc homolog	*Crassostrea virginica*	AAB34577.1	38
VHP-279.ab1	Phosphoserine aminotransferase 1	*Danio rerio*	AAH64289.1	65
VHP-280.ab1	-		_	0
VHP-281.ab1	similar to alcohol dehydrogenase 5	*Strongylocentrotus purpuratus*	XP_784441.1	73
VHP-282.ab1	nitrate reductase 1 alpha subunit	*Shigella flexneri 2a str.*	AAP16726.1	100
VHP-283.ab1	putative sphingosine-1-phosphate lyase	*Symbiobacterium thermophilum IAM 14863*	BAD40259.1	55
VHP-284.ab1	-		_	0
VHP-286.ab1	putative transposase b homolog	*Pyrococcus furiosus DSM 3638*	AAL81139.1	33
VHP-287.ab1	elongation factor 1 alpha	*Haliotis rufenscens*	AAZ30688.1	91
VHP-288.ab1	SJCHGC05731 protein	*Schistosoma japonicum*	AAX28066.2	44
VHP039-2.ab1	sensory box/GGDEF family protein	*Legionella pneumophila subsp. pneumophila str. Philadelphia 1*	AAU28700.1	26
VHP058-2.ab1	-		_	0
VHP103-2.ab1	-		_	0
VHP143-2.ab1	hypothetical protein XP_776172	*Strongylocentrotus purpuratus*	XP_781265.1	39

[a] Identity(%)

Table 6. List of identified ESTs from the skin cDNA library of abalone

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
SKN001.ab1	hypothetical protein LOC84226	*Homo sapiens*	NP_115642.2	24
SKN002.ab1	-		_	0
SKN003.ab1	unnamed protein product TRANSMEMBRANE	*Mus musculus*	BAB27678.2	73
SKN004.ab1	SERINE/THREONINE-PROTEIN KINASE D PKNDb	*Mycobacterium bovis AF2122/97*	CAD93815.1	24
SKN005.ab1	CG34043-PA	*Drosophila melanogaster*	NP_001033893.1	33
SKN006.ab1	cytochrome c oxidase subunit I	*Haliotis diversicolor supertexta*	AAQ84296.1	83
SKN007.ab1	hypothetical protein DDB0190674	*Dictyostelium discoideum*	XP_646407.1	36
SKN008.ab1	Serpentine Receptor, class T family member (srt-7)	*Caenorhabditis elegans*	NP_503842.1	33
SKN009.ab1	similar to CG16965-PA	*Bos taurus*	XP_589347.2	39
SKN010-2.ab1	60S ribosomal protein L37A	*Cryptochiton stelleri*	AAC15655.1	87
SKN010.ab1	60S ribosomal protein L37A	*Cryptochiton stelleri*	AAC15655.1	80
SKN011.ab1	AMP-dependent synthetase and ligase	*Nitrosomonas europaea ATCC 19718*	CAD86261.1	45
SKN013-2.ab1	67kD laminin receptor precursor	*Xenopus laevis*	AAW62261.1	79
SKN014.ab1	Putative transcriptional regulator, LytR family	*Lactobacillus sakei subsp. sakei 23K*	YP_394795.1	30
SKN015.ab1	protein disulfide isomerase		AAA85099.1	65
SKN016.ab1	unnamed protein product	*Oryza sativa (japonica cultivar-group)*	NP_913011.1	34
SKN017.ab1	nardilysin (N-arginine dibasic convertase)	*Pan troglodytes*	XP_513403.1	43
SKN018.ab1	similar to guanylate cyclase OlGC-R2	*Danio rerio*	XP_688499.1	59
SKN019.ab1	Hypothetical protein LOC283078	*Homo sapiens*	AAH36207.1	42
SKN020.ab1	SLACK-A isoform	*Rattus norvegicus*	AAX16016.1	29
SKN021.ab1	-		_	0
SKN022.ab1	SJCHGC02792 protein	*Schistosoma japonicum*	AAW25342.1	74
SKN023.ab1	similar to arylsulfatase I	*Danio rerio*	XP_691165.1	31
SKN024.ab1	Cobalamin synthesis protein/P47K	*Burkholderia sp. 383*	ABB10033.1	32
SKN025.ab1	-		_	0
SKN026.ab1	23S rRNA methyltransferase/RumA	*Thermoanaerobacter ethanolicus ATCC 33223*	ZP_00779347.1	34
SKN027.ab1	guanine nucleotide-binding protein	*Petromyzon marinus*	AAM88904.1	84
SKN028.ab1	cytochrome b	*Haliotis rubra*	YP_026076.1	88
SKN029.ab1	hypothetical protein MYPE8400	*Mycoplasma penetrans HF-2*	NP_758228.1	26
SKN031.ab1	similar to fucosidase, alpha-L- 2, plasma	*Strongylocentrotus purpuratus*	XP_796773.1	37
SKN032.ab1	hypothetical protein cgd4_750	*Cryptosporidium parvum Iowa II*	XP_625677.1	36
SKN033.ab1	similar to ENSANGP00000014714	*Apis mellifera*	XP_624580.1	95
SKN034.ab1	putative alpha-tubulin	*Oikopleura dioica*	AAP80594.1	99
SKN035.ab1	hypothetical protein XP_511394	*Pan troglodytes*	XP_511394.1	64
SKN036.ab1	conserved hypothetical protein	*Agrobacterium tumefaciens str. C58*	AAL43459.1	31

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
SKN037.ab1	hypothetical protein	*uncultured Acidobacteria bacterium*	AAP58583.1	40
SKN038.ab1	unnamed protein product	*Kluyveromyces lactis*	XP_454119.1	37
SKN039.ab1	-		_	0
SKN040.ab1	-		_	0
SKN042.ab1	hypothetical protein CHGG_06887	*Chaetomium globosum CBS 148.51*	EAQ85634.1	30
SKN043.ab1	-		_	0
SKN045.ab1	putative protein tyrosine kinase	*Dictyostelium discoideum*	XP_644816.1	28
SKN046.ab1	caveolin-3	*Xenopus laevis*	AAM76916.1	40
SKN047.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_704864.1	24
SKN048.ab1	cubilin	*Rattus norvegicus*	NP_445784.1	28
SKN049.ab1	beta-tubulin	*Halocynthia roretzi*	BAA22382.1	91
SKN050.ab1	mucin	*Rattus norvegicus*	AAC53312.1	30
SKN052.ab1	hypothetical protein SAV7203	*Streptomyces avermitilis MA-4680*	NP_828379.1	47
SKN053.ab1	laminin receptor 1	*Danio rerio*	AAQ91246.1	74
SKN054.ab1	similar to RNA (guanine-9-) methyltransferase domain containing 1	*Strongylocentrotus purpuratus*	XP_799221.1	36
SKN055.ab1	similar to IQ motif containing D	*Strongylocentrotus purpuratus*	XP_784878.1	76
SKN056.ab1	similar to tubulin alpha 6	*Pan troglodytes*	XP_509043.1	85
SKN057.ab1	inhibitor of apoptosis 2 protein	*Glossina morsitans morsitans*	ABC25071.1	48
SKN058.ab1	unknown protein	*Synechocystis sp. PCC 6803*	BAD01833.1	25
SKN059.ab1	conserved hypothetical protein	*Pseudomonas putida F1*	ZP_00899862.1	42
SKN060.ab1	-		_	0
SKN061.ab1	NADH dehydrogenase subunit 2	*Haliotis rubra*	YP_026068.1	63
SKN063.ab1	hypothetical protein 470.t00006	*Entamoeba histolytica HM-1:IMSS*	XP_648359.1	35
SKN065-2.ab1	unnamed protein product	*Homo sapiens*	BAD18580.1	29
SKN066.ab1	-		_	0
SKN067.ab1	Mcm5-prov protein	*Xenopus laevis*	AAH47250.1	75
SKN069.ab1	Y47D3B.4	*Caenorhabditis elegans*	NP_499469.2	23
SKN070.ab1	lysin		AAA91565.1	99
SKN071.ab1	lymphocyte antigen 86	*Sus scrofa*	AAR22525.2	28
SKN072.ab1	similar to Tubulin alpha-3 chain (Alpha-tubulin 3)	*Strongylocentrotus purpuratus*	XP_795276.1	89
SKN073.ab1	ENSANGP00000021588	*Anopheles gambiae str. PEST*	EAA10553.2	44
SKN074.ab1	ENSANGP00000017945	*Anopheles gambiae str. PEST*	EAA00085.2	26
SKN075.ab1	-		_	0
SKN076-2.ab1	similar to novel gene similar to human and rodent IER5 (immediately early response 5) isoform 2	*Danio rerio*	XP_706732.1	32
SKN076.ab1	-		_	0
SKN077.ab1	unknown	*Aeromonas hydrophila*	AAR06617.1	35
SKN079-2.ab1	reverse transcriptase-like protein	*Takifugu rubripes*	AAD19348.1	50

Table 6. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
SKN079.ab1	similar to CG6454-PB, isoform B	*Strongylocentrotus purpuratus*	XP_782090.1	54
SKN080.ab1	hypothetical protein UM01098.1	*Ustilago maydis 521*	XP_757245.1	36
SKN081.ab1	actin	*Haliotis discus hannai*	AAQ92368.1	96
SKN082.ab1	unnamed protein product	*Rattus norvegicus*	CAA33143.1	88
SKN084.ab1	ENSANGP00000011567	*Anopheles gambiae str. PEST*	EAA00829.2	51
SKN086.ab1	glutamine-dependent NAD(+) synthetase	*Plasmodium yoelii yoelii str. 17XNL*	XP_730516.1	37
SKN088-2.ab1	salivary selenoprotein M precursor	*Ixodes scapularis*	AAY66722.1	47
SKN088.ab1	salivary selenoprotein M precursor	*Ixodes scapularis*	AAY66722.1	45
SKN089.ab1	inositol 5-phosphatase 2	*Dictyostelium discoideum*	AAN85438.1	25
SKN090.ab1	-		_	0
SKN091.ab1	ATP-dependent RNA helicase, putative	*Archaeoglobus fulgidus DSM 4304*	NP_068912.1	31
SKN092-2.ab1	membrane protein, putative	*Mycoplasma capricolum subsp. capricolum ATCC 27343*	ABC01140.1	36
SKN092.ab1	unnamed protein product	*Aspergillus oryzae*	BAE58689.1	53
SKN093.ab1	delta-like protein	*Schistocerca americana*	AAU14846.1	27
SKN094-2.ab1	similar to ENSANGP00000017088	*Apis mellifera*	XP_396071.2	82
SKN095.ab1	olfactory receptor Olfr779	*Mus musculus*	AAS99802.1	35
SKN096.ab1	immediate-early protein	*Serinus canaria*	AAA49537.1	48
SKN097.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03487.1	36
SKN099.ab1	PRAJA1BETA	*Homo sapiens*	AAM53040.1	37
SKN101.ab1	hypothetical protein AN4541.2	*Aspergillus nidulans FGSC A4*	XP_662145.1	31
SKN102.ab1	acidic ribosomal phosphoprotein P0	*Rattus norvegicus*	NP_071797.1	79
SKN103.ab1	-		_	0
SKN104.ab1	similar to CG1106-PB, isoform B	*Strongylocentrotus purpuratus*	XP_788777.1	60
SKN105-2.ab1	similar to RIKEN cDNA 1200009H11	*Gallus gallus*	XP_417954.1	35
SKN106.ab1	intermediate filament protein	*Biomphalaria glabrata*	AAZ39528.1	46
SKN108.ab1	MAS-related GPR, member A4	*Mus musculus*	NP_705744.1	44
SKN109.ab1	gp164	*Mycobacteriophage Omega*	NP_818464.1	32
SKN110.ab1	methionyl-tRNA synthetase	*Candidatus Blochmannia pennsylvanicus str. BPEN*	AAZ41101.1	43
SKN111.ab1	retrograde protein 51	*Lymnaea stagnalis*	AAT01542.1	50
SKN112.ab1	putative secreted major subunit of curlin, may bind calcium	*Pseudoalteromonas tunicata D2*	ZP_01133744.1	27
SKN113.ab1	hypothetical protein Chro.60269	*Cryptosporidium hominis TU502*	XP_668328.1	27
SKN114.ab1	-		_	0
SKN115.ab1	actin A3	*Haliotis iris*	AAX19288.1	99
SKN116.ab1	unknown protein	*Arabidopsis thaliana*	NP_564234.1	36
SKN117.ab1	heat shock protein gp96	*Xenopus laevis*	AAO21339.1	68
SKN118.ab1	ribosomal protein L7	*Loligo pealei*	AAU11097.1	77
SKN120.ab1	unknown protein	*Arabidopsis thaliana*	NP_850717.1	33
SKN121.ab1	similar to carboxylesterase	*Apis mellifera*	XP_397022.2	29
SKN122.ab1	similar to thyroid hormone receptor interactor 12	*Danio rerio*	XP_694020.1	63

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
SKN123.ab1	myosin regulatory light chain interacting protein	*Danio rerio*	AAQ97996.1	26
SKN124.ab1	actin	*Haliotis discus hannai*	AAQ92368.1	100
SKN125.ab1	open reading frame (196 AA)	*Mus musculus*	BAA00448.1	32
SKN127-2.ab1	similar to Actin-binding protein IPP (MIPP protein)	*Gallus gallus*	XP_422442.1	46
SKN128-2.ab1	hypothetical protein DDB0186385	*Dictyostelium discoideum*	XP_638358.1	37
SKN129.ab1	QM protein	*Pinctada fucata*	AAN85578.1	89
SKN130.ab1	hypothetical protein Tb09.211.3900	*Trypanosoma brucei TREU927*	XP_827531.1	41
SKN131.ab1	Major facilitator superfamily (MFS_1) transporter	*Burkholderia sp. 383*	ABB12598.1	33
SKN132.ab1	unnamed protein product	*Mus musculus*	BAE36777.1	27
SKN134.ab1	growth arrest and DNA damage-inducible protein 45	*Felis catus*	BAD60923.1	40
SKN135.ab1	Rpsa protein	*Mus musculus*	AAH92041.1	58
SKN136.ab1	pannexin 5	*Aplysia californica*	AAX24144.1	29
SKN137-2.ab1	-		_	0
SKN137.ab1	-		_	0
SKN138.ab1	-		_	0
SKN139.ab1	leucine-tRNA ligase	*Plasmodium chabaudi chabaudi*	XP_744802.1	38
SKN140.ab1	X-box binding protein 1	*Mytilus edulis*	ABA43316.1	57
SKN142.ab1	RPLP0 protein	*Homo sapiens*	AAH70194.1	87
SKN143.ab1	glycerol 3-phosphate dehydrogenase GfdA	*Aspergillus fumigatus Af293*	XP_749965.1	33
SKN197.ab1	Hypothetical protein CBG05788	*Caenorhabditis briggsae*	CAE61818.1	32
SKN198.ab1	ferritin GF2	*Crassostrea gigas*	AAP83794.1	80
SKN199.ab1	hypothetical protein, unknown function	*Leishmania major*	CAJ03287.1	32
SKN200.ab1	putative receptor-like kinase	*Oryza sativa (japonica cultivar-group)*	NP_909780.1	30
SKN202.ab1	-		_	0

[a] Identity(%)

Table 7. List of identified ESTs from the muscle cDNA library of abalone

Clone no.	Putative identification	Closest species	Accession no.	Identity
RM001.ab1	-		_	0
RM002.ab1	cytochrome c oxidase subunit II	*Haliotis rubra*	YP_026070.1	82
RM003.ab1	similar to 60S acidic ribosomal protein P0 (L10E) isoform 1	*Canis familiaris*	XP_535894.1	75
RM005-2.ab1	-		_	0
RM005.ab1	-		_	0
RM006.ab1	sec61 protein	*Ciona intestinalis*	NP_001027676.1	77
RM007.ab1	troponin T	*Mizuhopecten yessoensis*	BAA20456.1	62
RM008.ab1	similar to Peripheral-type benzodiazepine receptor-associated protein 1 (PRAX-1) (Peripheral benzodiazepine receptor interacting protein) (PBR-IP) (RIM binding protein 1) (RIM-BP1)	*Canis familiaris*	XP_548232.2	28
RM010.ab1	ENSANGP00000027068	*Anopheles gambiae str. PEST*	EAL41847.1	32
RM011.ab1	hypothetical protein 205.t00018	*Entamoeba histolytica HM-1:IMSS*	XP_650673.1	23
RM012.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97279.1	52
RM015.ab1	-		_	0
RM016.ab1	ubiquitin	*Biomphalaria glabrata*	AAG49540.1	99
RM017.ab1	-		_	0
RM019.ab1	-		_	0
RM021.ab1	myosin essential light chain	*Crassostrea gigas*	CAD91423.1	69
RM022.ab1	CG10228-PA	*Drosophila melanogaster*	NP_610999.3	26
RM024.ab1	olfactory receptor Olfr420	*Mus musculus*	AAP70939.1	35
RM027.ab1	40S ribosomal protein S9	*Ictalurus punctatus*	AAK95191.1	93
RM032.ab1	Alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1, 3-N-acetyl-galactosaminide alpha-2,6-sialyltransferase (NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc-alpha-2, 6-sialyltransferase) (ST6GalNAc IV) (Sialyltransferase 7D) (Sialyltransferase 3C)...	*Canis familiaris*	XP_851433.1	46
RM034.ab1	KARG_NORMA Arginine kinase (AK)		P51544	93
RM035.ab1	CO4A2_ASCSU Collagen alpha-2(IV) chain precursor		4.00E-51P27393	
RM036.ab1	putative sugar transporter	*Arabidopsis thaliana*	AAF27022.1	43
RM039.ab1	RPL18A protein	*Xenopus laevis*	AAH42256.1	73
RM040.ab1	60S ribosomal protein L13A	*Mytilus edulis*	ABA46793.1	68
RM041.ab1	-		_	0
RM042.ab1	MtrDRAFT_AC148971g2v1	*Medicago truncatula*	ABD28540.1	37
RM043.ab1	tropomyosin 1	*Haliotis asinina*	AAP85231.1	86

Table 7. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
RM044.ab1	hypothetical glycine-rich protein	*Mesorhizobium loti MAFF303099*	BAB48146.1	29
RM045.ab1	troponin I	*Mizuhopecten yessoensis*	BAA22853.1	57
RM046.ab1	calcium-dependent protein kinase	*Crassostrea gigas*	AAU93878.1	47
RM048.ab1	hypothetical protein	*Neurospora crassa N150*	XP_955763.1	35
RM049.ab1	unnamed protein product	*Homo sapiens*	BAC87430.1	23
RM051.ab1	MGC79134 protein	*Xenopus laevis*	AAH77247.1	86
RM052.ab1	-		_	0
RM053.ab1	Aminodeoxychorismate lyase	*Bradyrhizobium sp. BTAi1*	ZP_00861034.1	28
RM054-2.ab1	Hydrogenase-1 large chain	*Escherichia coli CFT073*	AAN79582.1	99
RM055.ab1	putative lysosomal trafficking regulator 2	*Oryza sativa (japonica cultivar-group)*	BAD27684.1	36
RM056.ab1	similar to bromodomain and WD repeat domain containing 2	*Strongylocentrotus purpuratus*	XP_791604.1	31
RM057.ab1	-		_	0
RM058-2.ab1	unnamed protein product	*Candida glabrata CBS138*	CAG60755.1	27
RM060.ab1	LysM domain protein	*Geobacter sulfurreducens PCA*	AAR36666.1	29
RM061.ab1	ribosomal protein P1	*Biomphalaria glabrata*	AAZ39530.1	55
RM062.ab1	-		_	0
RM063.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG01120.1	37
RM064-2.ab1	calponin-like protein	*Mytilus galloprovincialis*	BAB60813.1	50
RM064.ab1	JMJD2D protein	*Homo sapiens*	AAH74739.1	41
RM065-2.ab1	cytochrome b5	*Crassostrea gigas*	CAD92095.1	62
RM065.ab1	cytochrome b5	*mice, D2, liver microsomes, Peptide Partial, 97 aa, segment 1 of 2*	AAB22636.1	77
RM067-2.ab1	-		_	0
RM067.ab1	-		_	0
RM068-2.ab1	salivary selenoprotein M precursor	*Ixodes scapularis*	AAY66722.1	47
RM068.ab1	hypothetical protein UM04059.1	*Ustilago maydis 521*	XP_760206.1	50
RM070-2.ab1	-		_	0
RM070.ab1	-		_	0
RM071.ab1	-		_	0
RM072.ab1	ribosomal protein L24	*Crassostrea gigas*	CAD91424.1	82
RM073.ab1	plasminogen activator inhibitor 2 type A	*Rattus norvegicus*	CAA45864.1	42
RM074.ab1	RL4_URECA 60S ribosomal protein L4 (L1)		P49165	56
RM075-2.ab1	calponin homolog	*Schistosoma mansoni*	AAB47536.1	69
RM075.ab1	Serpentine Receptor, class H family member (srh-112)	*Caenorhabditis elegans*	NP_507363.1	34
RM076.ab1	tropomyosin	*Haliotis diversicolor*	AAG08987.1	98
RM077.ab1	CG7467-PC, isoform C	*Drosophila melanogaster*	NP_996228.1	40
RM078.ab1	hypothetical protein GSU0714	*Geobacter sulfurreducens PCA*	AAR34044.1	44
RM079.ab1	MYL5 protein	*Homo sapiens*	AAH40050.1	68
RM081.ab1	similar to Drosophila melanogaster CG1746	*Drosophila yakuba*	AAR09940.1	47
RM082.ab1	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase 2	*Homo sapiens*	AAH96821.1	45

Table 7. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
RM084.ab1	Peptidyl-prolyl cis-trans isomerase (PPIase) (Rotamase) (Cyclophilin) (Cyclosporin A-binding protein)	*Apis mellifera*	XP_393381.2	71
RM085.ab1	similar to GA14769-PA	*Apis mellifera*	XP_391958.2	68
RM086.ab1	polyprotein	*Human coxsackievirus B3*	CAC27393.1	28
RM087.ab1	hypothetical protein	*Oryza sativa (japonica cultivar-group)*	XP_465902.1	44
RM088.ab1	actin	*Haliotis rufescens*	AAB87082.1	95
RM089.ab1	actin A1	*Haliotis iris*	AAX19286.1	98
RM090.ab1	LIM protein	*Mytilus edulis*	ABB73031.1	52
RM091.ab1	-		_	0
RM092.ab1	GDP-mannose 4,6-dehydratase	*Rubrobacter xylanophilus DSM 9941*	ZP_00601579.1	43
RM093.ab1	hypothetical protein	*Plasmodium berghei strain ANKA*	XP_678635.1	31
RM094.ab1	ATP synthase F0 subunit 6	*Haliotis rubra*	YP_026072.1	84
RM095.ab1	saccharopine dehydrogenase (putative)	*Gallus gallus*	NP_001012893.1	54
RM096.ab1	peroxisomal membrane protein pex16, putative	*Cryptococcus neoformans var. neoformans JEC21*	AAW43797.1	30
RM100.ab1	similar to ENSANGP00000026584	*Apis mellifera*	XP_623167.1	59
RM101-2.ab1	thymosin beta 12	*perch, liver, Peptide, 43 aa*	AAB21004.1	88
RM102.ab1	COG2199: FOG: GGDEF domain	*Clostridium difficile QCD-32g58*	ZP_01032874.1	44
RM103.ab1	protein disulfide isomerase		AAA85099.1	68
RM104.ab1	hypothetical protein DEHA0B05918g	*Debaryomyces hansenii CBS767*	XP_457224.1	69
RM106.ab1	olfactory receptor Olfr779	*Mus musculus*	AAS99802.1	35
RM107.ab1	SJCHGC06220 protein	*Schistosoma japonicum*	AAX30635.2	51
RM108.ab1	DNA-directed RNA polymerase	*Glycine max*	CAA36734.1	31
RM109.ab1	unnamed protein product	*Aspergillus oryzae*	BAE65858.1	46
RM111.ab1	-		_	0
RM112.ab1	ARF_DUGJA ADP-ribosylation factor		P91924	91
RM113.ab1	-		_	0
RM114.ab1	GA15696-PA	*Drosophila pseudoobscura*	EAL25448.1	40
RM117.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_703251.1	31
RM118.ab1	hypothetical protein PY07113	*Plasmodium yoelii yoelii str. 17XNL*	XP_727906.1	30
RM120.ab1	MYSP_MYTGA Paramyosin		O96064	68
RM121.ab1	similar to ENSANGP00000003616	*Apis mellifera*	XP_624843.1	58
RM122.ab1	Tis11 family protein	*Crassostrea virginica*	AAB69448.1	64
RM124.ab1	myosin:SUBUNIT=regulatory light chain		1803425D	62
RM126.ab1	hypothetical protein ELI_06385	*Erythrobacter litoralis HTCC2594*	ABC63369.1	38
RM128.ab1	similar to KIAA1683 protein	*Homo sapiens*	XP_371125.2	23
RM130.ab1	lectin-like receptor kinase 1;1	*Medicago truncatula*	AAR11301.1	44
RM131.ab1	ENSANGP00000022107	*Anopheles gambiae str. PEST*	EAA07850.2	40

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
RM132.ab1	expressed protein	*Cryptococcus neoformans var. neoformans JEC21*	AAW46404.1	54
RM134.ab1	similar to forkhead box E1 (thyroid transcription factor 2)	*Canis familiaris*	XP_854932.1	35
RM135.ab1	KARG_NORMA Arginine kinase (AK)		P51544	100
RM136.ab1	myosin heavy chain	*Mytilus galloprovincialis*	CAB64662.1	69
RM137.ab1	hypothetical protein	*Yarrowia lipolytica*	XP_503715.1	30
RM138.ab1	-		_	0
RM140.ab1	60S ribosomal protein P2	*Cryptochiton stelleri*	AAC15656.1	48
RM141.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	AAN36787.1	80
RM144.ab1	adaptin-like protein, possible	*Cryptosporidium parvum*	CAD98311.1	54
RM145.ab1	-		_	0
RM148.ab1	collagen pro alpha-chain	*Haliotis discus*	BAA75669.1	42
RM149.ab1	Bardet-Biedl syndrome 4	*Homo sapiens*	NP_149017.2	65
RM156.ab1	elongation factor 1 alpha	*Haliotis rufenscens*	AAZ30688.1	91
RM158.ab1	polyprotein	*Poliovirus*	ABA29244.1	34
RM159.ab1	similar to Ubiquitin-like 1 activating enzyme E1A (SUMO-1 activating enzyme subunit 1) isoform 1	*Canis familiaris*	XP_533632.1	48
RM161.ab1	similar to CG9291-PB, isoform B	*Strongylocentrotus purpuratus*	XP_788561.1	88
RM162.ab1	similar to scavenger receptor class F, member 1 isoform 1 precursor	*Canis familiaris*	XP_853984.1	32
RM163.ab1	cytochrome c oxidase subunit III	*Haliotis rubra*	YP_026066.1	76
RM167.ab1	hypothetical protein ClimDRAFT_0912	*Chlorobium limicola DSM 245*	ZP_00512949.1	40
RM168.ab1	hypothetical protein XP_687931	*Danio rerio*	XP_693023.1	41
RM169.ab1	hypothetical protein PY01809	*Plasmodium yoelii yoelii str. 17XNL*	XP_729615.1	37
RM171.ab1	actin A1	*Haliotis iris*	AAX19286.1	97
RM172.ab1	protease	*Aeromonas veronii bv. sobria*	BAD22597.1	48
RM174.ab1	-		_	0
RM176.ab1	similar to histocompatibility 28	*Canis familiaris*	XP_537104.2	35
RM178.ab1	cytochrome c oxidase subunit I	*Haliotis rubra*	YP_026069.1	85
RM181.ab1	polyprotein	*Foot-and-mouth disease virus*	AAT01764.1	34
RM183.ab1	-		_	0
RM187.ab1	-		_	0
RM188.ab1	interleukin 18	*Canis familiaris*	NP_001003169.1	42
RM190.ab1	ENSANGP00000020389	*Anopheles gambiae str. PEST*	EAA01312.2	33

[a] Identity(%)

Table 8. List of identified ESTs from the testis cDNA library of abalone

Clone no.	Putative identification	Closest species	Accession no.	I[a]
GOM001.ab1	ribosomal protein L24e	*Hister sp. APV-2005*	CAJ17316.1	70
GOM002-2.ab1	rh187	*Cercopithecine herpesvirus 8*	YP_068276.1	32
GOM002.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF93381.1	54
GOM004.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97217.1	45
GOM006-2.ab1	hypothetical protein XP_776172	*Strongylocentrotus purpuratus*	XP_781265.1	41
GOM007.ab1	Peptidase M14, carboxypeptidase A	*Paracoccus denitrificans PD1222*	ZP_00631125.1	24
GOM008.ab1	similar to Tripartite motif protein 3 (RING finger protein 22) (RING finger protein HAC1) isoform 6	*Danio rerio*	XP_706631.1	33
GOM009.ab1	dendritic cell protein variant	*Homo sapiens*	BAD96284.1	50
GOM010.ab1	similar to echinoderm microtubule associated protein like 5	*Strongylocentrotus purpuratus*	XP_785437.1	52
GOM011.ab1	similar to CG10979-PA, partial	*Strongylocentrotus purpuratus*	XP_797107.1	46
GOM012.ab1	FK506-binding protein 6	*Homo sapiens*	AAH36817.1	38
GOM014.ab1	unnamed protein product	*Homo sapiens*	BAC86459.1	28
GOM015.ab1	similar to CG17870-PA, isoform A	*Apis mellifera*	XP_623302.1	60
GOM016.ab1	heat shock cognate	*Artemia franciscana*	AAL27404.1	85
GOM017.ab1	-		_	0
GOM018.ab1	putative alpha-tubulin	*Oikopleura dioica*	AAP80594.1	99
GOM019.ab1	-		_	0
GOM021.ab1	similar to histone H2A	*Mus musculus*	XP_890839.1	86
GOM023.ab1	OSJNBa0086P08.18	*Oryza sativa (japonica cultivar-group)*	NP_913586.1	35
GOM024.ab1	adaptor-related protein complex 2, sigma 1 subunit	*Mus musculus*	NP_941015.1	35
GOM025.ab1	guanine nucleotide-binding protein	*Petromyzon marinus*	AAM88904.1	81
GOM028.ab1	mini chromosome maintenance deficient 6	*Rattus norvegicus*	XP_344136.2	81
GOM029.ab1	ENSANGP00000029380	*Anopheles gambiae str. PEST*	EAL38972.1	30
GOM030.ab1	hypothetical protein XP_774971	*Strongylocentrotus purpuratus*	XP_780064.1	53
GOM031.ab1	similar to armadillo repeat containing 4	*Strongylocentrotus purpuratus*	XP_794287.1	54
GOM034.ab1	similar to Myb DNA binding domain-containing protein	*Rattus norvegicus*	XP_226205.3	37
GOM035.ab1	-		_	0
GOM037.ab1	hypothetical protein XP_778462	*Strongylocentrotus purpuratus*	XP_783555.1	52
GOM038.ab1	hypothetical protein GLP_577_38920_45159	*Giardia lamblia ATCC 50803*	XP_768963.1	56
GOM040.ab1	-		_	0
GOM041.ab1	unnamed protein product	*Macaca fascicularis*	BAE00904.1	66
GOM042-2.ab1	COG1368: Phosphoglycerol transferase and related proteins, alkaline phosphatase superfamily	*Pediococcus pentosaceus ATCC 25745*	ZP_00322570.1	36
GOM045.ab1	polyadenylate-binding protein nuclear 1	*Danio rerio*	NP_998424.1	56
GOM046.ab1	WW domain-containing binding protein 4	*Gallus gallus*	NP_001025995.1	42

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
GOM047.ab1	hypothetical protein	*Oryza sativa (japonica cultivar-group)*	XP_450816.1	32
GOM048.ab1	ferritin GF2	*Crassostrea gigas*	AAP83794.1	81
GOM049.ab1	similar to hypothetical protein	*Rattus norvegicus*	XP_578172.1	21
GOM050.ab1	tripartite motif-containing 59	*Gallus gallus*	NP_001026491.1	34
GOM051.ab1	ENSANGP00000003474	*Anopheles gambiae str. PEST*	EAA13257.2	70
GOM052.ab1	fertilization protein		AAC37229.1	62
GOM053.ab1	hypothetical protein XP_397399	*Apis mellifera*	XP_397399.2	68
GOM056.ab1	similar to CG5186-PA, isoform A	*Strongylocentrotus purpuratus*	XP_783148.1	38
GOM057.ab1	LOC400590 protein	*Homo sapiens*	AAH62632.1	69
GOM058.ab1	similar to actin (41.8 kD) (act-2)	*Strongylocentrotus purpuratus*	XP_786585.1	98
GOM059.ab1	general transcription factor IIH, polypeptide 5	*Xenopus tropicalis*	NP_001017298.1	72
GOM061.ab1	hypothetical protein, conserved in P. falciparum	*Plasmodium falciparum 3D7*	NP_702673.1	39
GOM062.ab1	similar to Meiotic recombination protein REC8-like 1 (Cohesin Rec8p)	*Canis familiaris*	XP_547744.1	42
GOM063.ab1	putative alpha-tubulin	*Oikopleura dioica*	AAP80594.1	93
GOM064.ab1	centromere protein F, 350\/400ka (mitosin)	*Homo sapiens*	CAH73032.1	34
GOM066.ab1	hypothetical protein XP_795449, partial	*Strongylocentrotus purpuratus*	XP_800542.1	24
GOM067.ab1	similar to ENSANGP00000003404	*Apis mellifera*	XP_624359.1	83
GOM068.ab1	conserved hypothetical protein	*Prochlorococcus marinus str. MIT 9312*	ABB49340.1	39
GOM071.ab1	similar to CG10510-PA	*Strongylocentrotus purpuratus*	XP_790738.1	71
GOM072.ab1	similar to leucine-rich repeat containing protein family member (104.2 kD) (5M953)	*Strongylocentrotus purpuratus*	XP_787322.1	53
GOM073.ab1	mitochondrial DNA-directed RNA polymerase, putative	*Leishmania major*	CAJ04832.1	28
GOM076.ab1	similar to armadillo repeat containing 4	*Strongylocentrotus purpuratus*	XP_794287.1	82
GOM077.ab1	-		_	0
GOM079.ab1	GRIP and coiled-coil domain-containing 2 isoform b	*Homo sapiens*	NP_055450.1	28
GOM080.ab1	similar to ribophorin II	*Strongylocentrotus purpuratus*	XP_783899.1	41
GOM084.ab1	similar to carboxylesterase	*Apis mellifera*	XP_397022.2	29
GOM086.ab1	similar to HYLS1 protein	*Danio rerio*	XP_695583.1	26
GOM087-2.ab1	-		_	0
GOM089.ab1	thioredoxin peroxidase BgTPx	*Biomphalaria glabrata*	AAK26236.1	84
GOM090.ab1	elongation factor 1 alpha	*Axinella verrucosa*	CAE45763.1	88
GOM091.ab1	calcineurin A	*Mizuhopecten yessoensis*	BAA94542.1	88
GOM092.ab1	cytochrome c oxidase subunit III	*Haliotis rubra*	YP_026066.1	80
GOM093.ab1	ribosomal protein S10	*Branchiostoma belcheri tsingtaunese*	AAO31776.1	77
GOM094.ab1	similar to t-complex testis expressed 1	*Strongylocentrotus purpuratus*	XP_783725.1	83
GOM095.ab1	H3 histone, family 3A	*Mus musculus*	AAH88835.1	100

Table 8. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
GOM097-2.ab1	conserved hypothetical protein	*Agrobacterium tumefaciens str. C58*	AAL43459.1	31
GOM098.ab1	hypothetical protein XP_775895, partial	*Strongylocentrotus purpuratus*	XP_780988.1	39
GOM100-2.ab1	POL precursor	*Human immunodeficiency virus*	CAC19075.1	56
GOM101-2.ab1	hypothetical protein XP_683358	*Danio rerio*	XP_688450.1	51
GOM101.ab1	hypothetical protein XP_683358	*Danio rerio*	XP_688450.1	48
GOM102.ab1	KARG_NORMA Arginine kinase (AK)		P51544	77
GOM103.ab1	similar to A-kinase anchoring protein-associated sperm protein, partial	*Strongylocentrotus purpuratus*	XP_793854.1	81
GOM106-2.ab1	SJCHGC02792 protein	*Schistosoma japonicum*	AAW25342.1	73
GOM106.ab1	-		_	0
GOM107.ab1	ENSANGP00000002287	*Anopheles gambiae str. PEST*	EAA12220.3	30
GOM109-2.ab1	hypothetical protein, WD40 domain	*Paramecium tetraurelia*	YP_053986.1	30
GOM109.ab1	-		_	0
GOM110-2.ab1	lysin		AAA91565.1	100
GOM110.ab1	lysin		AAA91565.1	100
GOM111.ab1	Ribosomal protein L3	*Bos taurus*	AAI02254.1	84
GOM112.ab1	fibroblast growth factor 13	*Homo sapiens*	CAI42699.1	30
GOM113.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG08376.1	30
GOM114-2.ab1	similar to eukaryotic translation elongation factor 1 beta 2; eukaryotic translation elongation factor 1 beta 1	*Pan troglodytes*	XP_520983.1	76
GOM116.ab1	anaerobic glycerol-3-phosphate dehydrogenase, subunit A	*Salinibacter ruber DSM 13855*	YP_446536.1	26
GOM117.ab1	similar to toll interacting protein isoform 1	*Canis familiaris*	XP_540778.2	51
GOM119-2.ab1	-		_	0
GOM119.ab1	guanylyl cyclase	*Plasmodium falciparum 3D7*	NP_701254.1	31
GOM121.ab1	unknown	*Arabidopsis thaliana*	AAN60345.1	32
GOM122.ab1	similar to CDK5 regulatory subunit associated protein 1 isoform a isoform 1	*Strongylocentrotus purpuratus*	XP_780426.1	61
GOM123.ab1	beta-tubulin	*Crassostrea gigas*	AAU93877.1	87
GOM124.ab1	hypothetical protein MYPE6230	*Mycoplasma penetrans HF-2*	NP_758009.1	24
GOM125.ab1	similar to Hypothetical protein MGC27934, partial	*Gallus gallus*	XP_428973.1	48
GOM126.ab1	Hypothetical protein LOC381353	*Mus musculus*	AAH65698.1	26
GOM127.ab1	Sdhb-prov protein	*Xenopus laevis*	AAH43859.1	81
GOM128.ab1	MGC68964 protein	*Xenopus laevis*	AAH56029.1	52
GOM129.ab1	similar to cofactor required for Sp1 transcriptional activation, subunit 2, 150kDa	*Danio rerio*	XP_694175.1	51
GOM130.ab1	similar to Tumor protein p53 inducible nuclear protein 2	*Danio rerio*	XP_697918.1	43
GOM131.ab1	malate dehydrogenase precursor	*Nucella lapillus*	AAF27650.1	73
GOM132.ab1	Hypothetical protein Y76G2A.2	*Caenorhabditis elegans*	AAC78489.2	35
GOM133.ab1	-		_	0

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
GOM135.ab1	similar to A-kinase anchoring protein-associated sperm protein, partial	*Strongylocentrotus purpuratus*	XP_793854.1	76
GOM136.ab1	cytochrome c oxidase subunit I	*Haliotis diversicolor supertexta*	AAQ84296.1	92
GOM138.ab1	KARG_NORMA Arginine kinase (AK)		P51544	64
GOM139.ab1	RH43215p	*Drosophila melanogaster*	AAL68352.1	46
GOM140.ab1	similar to MOV10-like 1, partial	*Gallus gallus*	XP_427733.1	51
GOM141-2.ab1	similar to enkurin	*Strongylocentrotus purpuratus*	XP_784094.1	54
GOM141.ab1	Interferon-activatable protein 203 (Ifi-203) (Interferon-inducible protein p203)	*Mus musculus*	XP_910316.1	36
GOM143-2.ab1	similar to HORMA domain containing 1	*Strongylocentrotus purpuratus*	XP_784162.1	72
GOM143.ab1	conserved hypothetical protein	*Chlorobium phaeobacteroides BS1*	ZP_00532124.1	45
GOM144-2.ab1	-		_	0
GOM144.ab1	-		_	0
GOM145-2.ab1	similar to nicotinic acetylcholine receptor beta 4 subunit	*Danio rerio*	XP_696993.1	41
GOM147.ab1	similar to AAT1-alpha, partial	*Strongylocentrotus purpuratus*	XP_795692.1	70
GOM148.ab1	YBOXH_APLCA Y-box factor homolog (APY1)		P41824	79
GOM150.ab1	fertilization protein		AAC37229.1	63
GOM151.ab1	-		_	0
GOM152.ab1	F12K11.17	*Arabidopsis thaliana*	AAF24822.1	34
GOM154.ab1	40S ribosomal protein S9	*Corbicula fluminea*	AAT76788.1	90
GOM155.ab1	similar to mitochondrial ATP synthase lipid binding protein precursor	*Apis mellifera*	XP_623661.1	42
GOM156.ab1	Hypothetical protein B0546.1	*Caenorhabditis elegans*	AAB92010.1	60
GOM157-2.ab1	similar to Microsomal signal peptidase 12 kDa subunit (SPase 12 kDa subunit) (SPC12)	*Strongylocentrotus purpuratus*	XP_788906.1	65
GOM157.ab1	-		_	0
GOM158.ab1	-		_	0
GOM160.ab1	RPLP0 protein	*Homo sapiens*	AAH70194.1	82
GOM161.ab1	TPR-repeat-containing proteins	*Bacteroides thetaiotaomicron VPI-5482*	AAO77001.1	31
GOM163.ab1	similar to Thiosulfate sulfurtransferase (Rhodanese)	*Strongylocentrotus purpuratus*	XP_786526.1	30
GOM164-2.ab1	cytochrome b	*Haliotis rubra*	YP_026076.1	63
GOM164.ab1	cytochrome b	*Todarodes pacificus*	YP_112461.1	88

[a] Identity(%)

Table 9. List of identified ESTs from the ovary cDNA library of abalone

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
GOF00.ab1	CG11079-PC, isoform C	*Drosophila melanogaster*	NP_726312.1	46
GOF004.ab1	cytochrome c oxidase subunit I	*Haliotis discus discus*	AAQ84300.1	76
GOF006.ab1	similar to a disintegrin and metalloproteinase with thrombospondin motifs 9 preproprotein	*Canis familiaris*	XP_848393.1	34
GOF007.ab1	ornithine decarboxylase	*Sus scrofa*	AAS55915.1	59
GOF008.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	44
GOF009.ab1	similar to signal recognition particle 72kDa	*Strongylocentrotus purpuratus*	XP_797070.1	49
GOF011.ab1	unknown	*Schistosoma japonicum*	AAW27335.1	97
GOF012.ab1	snRNP-associated protein	*Danio rerio*	NP_991230.1	64
GOF013.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	37
GOF014.ab1	unnamed protein product	*Aspergillus oryzae*	BAE58095.1	31
GOF018.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	36
GOF022.ab1	KIAA0101	*Homo sapiens*	BAA03491.2	52
GOF023.ab1	vitelline coat protein 42	*Tegula pfeifferi*	BAB15930.1	42
GOF024.ab1	Trafficking protein particle complex 4	*Rattus norvegicus*	AAH98628.1	68
GOF027.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	45
GOF030.ab1	similar to KIAA1237 protein	*Gallus gallus*	XP_422104.1	53
GOF031.ab1	metallothionein	*Megathura crenulata*	AAM51554.1	70
GOF033.ab1	Hypothetical protein CBG22046	*Caenorhabditis briggsae*	CAE74333.1	56
GOF035.ab1	ribosomal protein L	*Argopecten irradians*	AAN05596.1	85
GOF036.ab1	chaperonin containing TCP1, subunit 4 (delta)	*Gallus gallus*	NP_996761.1	70
GOF038.ab1	GM25382p	*Drosophila melanogaster*	AAM50726.1	43
GOF039.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	53
GOF041.ab1	COG0477: Permeases of the major facilitator superfamily	*Microbulbifer degradans 2-40*	ZP_00314882.1	38
GOF042.ab1	KARG_NORMA Arginine kinase (AK)		P51544	74
GOF044.ab1	malate dehydrogenase precursor	*Nucella lapillus*	AAF27650.1	70
GOF045.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	44
GOF047.ab1	hypothetical protein	*Plasmodium chabaudi chabaudi*	XP_742690.1	26
GOF049.ab1	keratin associated protein 10-2	*Homo sapiens*	NP_941966.1	36
GOF050-2.ab1	hypothetical protein LOC415159	*Danio rerio*	NP_001002069.1	58
GOF052.ab1	Hypothetical protein CBG15191	*Caenorhabditis briggsae*	CAE69160.1	24
GOF053.ab1	TPA: TPA_exp: type V P-type ATPase isoform 3	*Mus musculus*	DAA05589.1	53
GOF054.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	32
GOF055.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_704796.1	28
GOF056.ab1	unnamed protein product	*Mus musculus*	BAE23538.1	39
GOF057.ab1	PDI	*Oxyuranus scutellatus scutellatus*	AAY33972.1	63
GOF058.ab1	-		_	0
GOF060.ab1	ribosomal protein S8	*Argopecten irradians*	AAN05595.1	72
GOF062.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	47
GOF064.ab1	similar to hypothetical protein MGC2817	*Pan troglodytes*	XP_527457.1	38

Table 9. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
GOF065.ab1	similar to mitochondrial ATP synthase lipid binding protein precursor	*Apis mellifera*	XP_623661.1	42
GOF067.ab1	type B DNA polymerase	*Bacillus cereus ATCC 14579*	AAP12349.1	26
GOF069.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	43
GOF070.ab1	similar to ARP3 actin-related protein 3 homolog isoform 2	*Strongylocentrotus purpuratus*	XP_801966.1	84
GOF072.ab1	ARP3 actin-related protein 3 homolog	*Xenopus tropicalis*	AAH64225.1	88
GOF073.ab1	shisa-4	*Xenopus laevis*	BAE53533.1	37
GOF074.ab1	protein disulfide isomerase		AAA85099.1	65
GOF077.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	33
GOF078.ab1	adenosylhomocysteinase	*Branchiostoma belcheri tsingtaunese*	AAQ96656.1	78
GOF079.ab1	actin	*Biomphalaria tenagophila*	AAK68714.1	98
GOF080.ab1	hypothetical protein	*Gallus gallus*	CAG32462.1	81
GOF081.ab1	dihydrolipoamide dehydrogenase precursor	*Cricetulus griseus*	AAN15202.1	80
GOF083.ab1	SJCHGC02792 protein	*Schistosoma japonicum*	AAW25342.1	73
GOF084.ab1	hypothetical protein LOC417699	*Gallus gallus*	NP_001025898.1	74
GOF085.ab1	similar to Serine/threonine phosphatase 4 regulatory subunit 1	*Bos taurus*	XP_587353.2	70
GOF086.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	40
GOF088.ab1	-		_	0
GOF089.ab1	alpha tubulin 2	*Patella vulgata*	CAA55978.1	90
GOF091.ab1	-		_	0
GOF093.ab1	Peptidylprolyl isomerase A	*Rattus norvegicus*	AAH59141.1	71
GOF094.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	39
GOF096.ab1	QM protein	*Plutella xylostella*	BAD26683.1	91
GOF099.ab1	vitelline coat protein 42	*Tegula pfeifferi*	BAB15930.1	50
GOF100.ab1	similar to transposable element tcb1 transposase (2C227)	*Strongylocentrotus purpuratus*	XP_788852.1	46
GOF101.ab1	variant-specific surface protein	*Giardia lamblia ATCC 50803*	XP_768389.1	34
GOF102.ab1	-		_	0
GOF103-2.ab1	Hypothetical protein SSO3103	*Sulfolobus solfataricus P2*	AAK43206.1	27
GOF104.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	37
GOF105.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	42
GOF106.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	53
GOF107.ab1	mitochondrial malate dehydrogenase precursor	*Monodonta lineata*	AAN23843.1	86
GOF110.ab1	-		_	0
GOF111.ab1	similar to Ubiquitin-activating enzyme E1 1	*Strongylocentrotus purpuratus*	XP_795302.1	40
GOF112.ab1	-		_	0
GOF113.ab1	similar to Probable G-protein coupled receptor 20	*Canis familiaris*	XP_539182.2	27
GOF114.ab1	Ribosomal protein S3	*Bos taurus*	AAI02091.1	92
GOF115.ab1	similar to MSTP052	*Danio rerio*	XP_694177.1	37
GOF116.ab1	similar to contactin associated protein 1	*Strongylocentrotus purpuratus*	XP_780441.1	24

Table 9. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
GOF117.ab1	similar to ENSANGP00000009968	*Apis mellifera*	XP_392432.2	73
GOF118.ab1	conserved hypothetical protein; putative methyltransferase	*Acinetobacter sp. ADP1*	CAG68478.1	59
GOF119.ab1	F46B3.1	*Caenorhabditis elegans*	NP_507973.1	37
GOF120.ab1	similar to ENSANGP00000024947	*Apis mellifera*	XP_623472.1	33
GOF123.ab1	Zgc:101545	*Danio rerio*	AAH86701.1	92
GOF124.ab1	similar to ENSANGP00000027643	*Apis mellifera*	XP_623876.1	38
GOF127.ab1	methionine adenosyltransferase	*Haliotis rufenscens*	AAZ30689.1	98
GOF128.ab1	GA18268-PA	*Drosophila pseudoobscura*	EAL30077.1	58
GOF129.ab1	MGC68601 protein	*Xenopus laevis*	AAH60466.1	30
GOF130.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	28
GOF131.ab1	similar to transmembrane protein 14C	*Strongylocentrotus purpuratus*	XP_795741.1	74
GOF132-2.ab1	hypothetical protein Tb11.01.6940	*Trypanosoma brucei TREU927*	XP_829587.1	34
GOF133.ab1	similar to kinesin family member 18A	*Strongylocentrotus purpuratus*	XP_786465.1	40
GOF134.ab1	-		_	0
GOF136.ab1	similar to CDA02 protein	*Gallus gallus*	NP_001026494.1	44
GOF138.ab1	guanine nucleotide-binding protein	*Petromyzon marinus*	AAM88904.1	83
GOF139.ab1	similar to chaperonin containing TCP1, subunit 5 (epsilon) isoform 1	*Strongylocentrotus purpuratus*	XP_780016.1	84
GOF140.ab1	similar to alpha 2 type IV collagen preproprotein; canstatin	*Gallus gallus*	XP_416952.1	32
GOF141.ab1	similar to transmembrane protein 2	*Bos taurus*	XP_593521.2	32
GOF142.ab1	67kD laminin receptor precursor	*Xenopus laevis*	AAW62261.1	79
GOF146-2.ab1	-		_	0
GOF146.ab1	hypothetical protein	*Gallus gallus*	CAH65290.1	58
GOF147.ab1	RPLP0 protein	*Homo sapiens*	AAH70194.1	80
GOF151.ab1	-		_	0
GOF152.ab1	NADH dehydrogenase subunit 2	*Pygopus orientalis*	AAO06338.1	29
GOF154.ab1	ubiquitin	*Biomphalaria glabrata*	AAG49540.1	99
GOF155.ab1	hypothetical protein LOC424533	*Gallus gallus*	NP_001026447.1	45
GOF156.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	29
GOF157.ab1	putative cell adhesion protein Sym32	*Anthopleura elegantissima*	AAF65308.1	48
GOF158.ab1	ENSANGP00000022045	*Anopheles gambiae str. PEST*	EAA05138.2	70
GOF160.ab1	keratin associated protein 5-8	*Homo sapiens*	NP_066384.1	23
GOF161.ab1	Hypothetical protein CBG12915	*Caenorhabditis briggsae*	CAE67426.1	50
GOF162.ab1	similar to nuclear receptor binding protein	*Danio rerio*	XP_691098.1	36
GOF163.ab1	apolipoprotein D	*Branchiostoma belcheri tsingtaunese*	AAP30079.1	47
GOF164.ab1	polyadenylate binding protein	*Petromyzon marinus*	AAB88449.1	89
GOF165.ab1	isocitrate dehydrogenase	*Crassostrea gigas*	AAT44354.1	74
GOF168.ab1	unknown protein	*Arabidopsis thaliana*	NP_197560.2	34
GOF170.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	31
GOF171.ab1	Transcription termination factor Rho	*Gluconobacter oxydans 621H*	AAW60839.1	28
GOF172.ab1	vitelline coat protein 41	*Tegula pfeifferi*	BAB15843.1	40
GOF175.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF90415.1	31

Table 9. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
GOF176.ab1	Hypothetical protein CBG16767	*Caenorhabditis briggsae*	CAE70263.1	22
GOF177.ab1	vitelline coat protein 42	*Tegula pfeifferi*	BAB15930.1	42
GOF179.ab1	ubiquitin ligase	*Giardia lamblia ATCC 50803*	XP_778668.1	25
GOF180.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	29
GOF181.ab1	60S acidic ribosomal protein P0	*Ixodes scapularis*	AAY66850.1	76
GOF182.ab1	-		_	0
GOF183.ab1	COP1-like protein	*Ostreococcus tauri*	AAU14277.1	37
GOF184.ab1	LOC400590 protein	*Homo sapiens*	AAH62632.1	69
GOF185.ab1	beta-tubulin	*Sycon sp. AR-2003*	AAP49559.1	100
GOF186.ab1	Acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	*Danio rerio*	AAH95591.1	80
GOF187.ab1	ribosomal protein P1	*Biomphalaria glabrata*	AAZ39530.1	56
GOF188.ab1	SJCHGC02697 protein	*Schistosoma japonicum*	AAW26877.1	82
GOF189.ab1	unknown protein	*Arabidopsis thaliana*	AAG51466.1	36
GOF190.ab1	-		_	0
GOF191.ab1	ENSANGP00000000511	*Anopheles gambiae str. PEST*	EAA07049.3	48
GOF192.ab1	P2RF1	*Human herpesvirus 6*	AAA16738.1	30

[a] Identity(%)

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Chapter III.

Bulk isolation of stress-related genes by expressed sequence tags of abalone, *Haliotis discus hannai*

Chapter Ⅲ. Bulk isolation of stress-related genes by expressed sequence tags of abalone, *Haliotis discus hannai*

ABSTRACT

Temperature and salinity are the primary physical factors affecting the life of molluscs. In order to analyze expressed genes under temperature and salinity change, suppression subtractive hybridization (SSH) method was used and constructed three cDNA libraries from abalone exposed to heat-shock, cold-shock or hyposalinity stress. Putative function could be assigned to 75.8% of the 1,536 sequenced cDNAs. Based on sequence similarities, candidate genes was identified that may be involved in stress response or immune function. Among them, several stress- and immune-related genes HSC, HSP, major histocompatibility complex (MHC) class IIa chain, CD45 homolog, and IRF7 were identified from three subtracted cDNA libraries. The expressions of these genes were investigated in abalone exposed to stress. They were induced in response to stress, supporting their involvement in abalone immunity.

These EST libraries could be the rich source of stress or immune-related genes, and supplements for other publicly available libraries for comprehensive analysis of the invertebrate stress-response transcriptome.

Key words : abalone, *Haliotis discus hannai*, expressed sequence tag (EST), suppression subtractive hybridization (SSH), environmental stress

INTRODUCTION

Invertebrates are increasingly being brought into mariculture, where it is important to monitor immune function and to minimise stresses that could suppress immunity. The understanding of immune systems and stress responses in abalone is based on limited work on abalone, variable amounts of work on other molluscs (mostly focused on a few gastropods and bivalves), work on other invertebrates and a huge body of work on vertebrates [1].

The stress response has a complex relationship with disease and has been implicated in disease outbreaks in many animals, including abalone. Especially, temperature and salinity are the primary physical factors affecting the life of molluscs. Limited research has been undertaken to date on stress and/or immune responses in abalone. Recently, a link has been established in abalone between increased stress and decreased immune functional capacity [2-8], leading to increased rates of bacterial infections and increased mortality [4-8]. This link is based on immune function tests carried out after applying stressors such as altered salinity, shaking, decreased dissolved oxygen, increased concentrations of ammonia and nitrate and increased temperature. In abalone and other molluscs, both the stress response and the immune response appear to be centered on the hemocyte, as these blood cells produce mediators of stress and the main immune responses [9-11].

Partial cDNA sequencing to generate expressed sequence tags (ESTs) has been used for the fast and efficient gathering of a detailed profile of genes expressed in various tissues, cell types, or developmental stages [12]. Recently, Munasinghe and coworkers have identified expressed genes in digestive gland of Disk abalone, *Haliotis duscus discus* by expressed sequence tags [13]. However, this previous EST study showed limited number of stress or immune response genes. This is due to the abundant transcripts such as housekeeping genes masked the identification of rare genes, i.e, stress inducible genes which are specifically expressed and degraded during short periods of time in stress response cells.

In this study, three subtracted cDNA libraries were constructed from heat-shock, cold shock or hyposalinity treated abalone for the screening of stress or immune response related genes from abalone, *Haliotis discus hannai*.

MATERIALS AND METHODS

Animals

Abalones, *Haliotis discus hannai* were suplied by Jeju Fisheries Research Institute (Jeju, Republic of Korea) and maintained in 6 tons flow-through tank at 18-20°C under a natural photoperiod. Abalone was exposed heat-shock or cold-shock from ambient sea water (18°C) to 28°C or 4°C, respectively. Hyopsalinity was derived from ambient sea water (35‰) to 20‰. The treated abalones (3 individuals per each time point) were sampled at 0, 0.5, 1, 2, 3, 4 and 5h in heat-shock treatment, or at 0, 3, 6, 9 and 24h in cold-shock treatment, or at 0, 1, 3, 6, 9 and 24h in hyosalinity treatment. The collected samples were ground immediately under liquid nitrogen for RNA preparation.

Total RNA isolation

Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Collected samples were pooled and ground into liquid nitrogen. Three volumes Trizol of sample was added and mixed with 0.2 volumes chloroform. The suspension was then centrifuged at 12,000 × *g* for 15 min. The clear upper phase was aspirated and placed in a clean tube. The same volumes of isopropanol was then added, and samples were centrifuged at 12,000 × *g* for 10 min. The RNA pellet was washed with 75% ethanol, dissolved in diethylpyrocarbonate-treated water and stored at −80°C.

mRNA isolation

mRNAs were isolated using a PolyA Tract mRNA isolation kit (Promega). After total RNA of each samples were pooled and adjusted to 0.5 mg, then, placed into 15 ml tube and RNase-free water was added to make 500 ul of final volume, and heated for 10 min at 65 °C. Mixture of 3㎕ biotinylated-Oligo (dT) probe and 13㎕ 20X SSC was added to the RNA tube. The annealed mixture was hybridized to Streptavidin-Paramagnetic Particles (SM-PMP), then mRNA-bound SM-PMP was captured with magnetic. Hybrid mRNA was dissolved with 250㎕ DEPC-water. After addition of 0.1 volume of 3M sodium acetate (NaOAc) and 1 volume of isopropanol the mRNA was, stored at -20°C for cDNA synthesis.

Suppression subtractive hybridization

The suppression subtractive hybridization technique (SSH) [14] was used to characterize new genes involved in stress and immune response. Briefly, cDNA was synthesized from 1㎍ of each mRNA (stress treated and control) using the SMART PCR cDNA Synthesis Kit (Clontech), which allowed the full-length amplification of cDNA from mRNA transcripts. A SSH assay was then performed using the PCR-Select cDNA Subtraction Kit (Clontech). The cDNA from the tester and from the driver were digested with *Rsa* I, and the tester cDNA was then ligated to either two different cDNA adaptors. During a first hybridization, excess driver was added to tester cDNA samples, which were then denatured and allowed to anneal. In the second hybridization, the two primary hybridization samples were mixed without denaturation. For further selection of differentially expressed sequences, denatured driver cDNA was again added to these hybrid samples. As a result, the remaining subtracted, equalized single-stranded tester cDNA reassociated to form hybrids with a different adaptor on each end. This forward-subtracted sample (genes present or up-regulated in stress treated compared with controls) was then used in PCR to amplify the differentially expressed sequences. PCR mixture was ligated using pGEM-T Easy system (Promega) and transformed into *E. coli* competent cells. A reverse subtracted library was also performed following the same protocol identify genes present or up-regulated in controls compared with infected tissues.

Selected colonies were amplified by PCR using Nested PCR primer 1 and 2R from PCR-Select cDNA Subtraction Kit. Agarose gel electrophoresis was performed to visualize the amplified fragments and to select by size the samples to be sequenced and arrayed. The PCR profile consisted of: initial denaturation for 5 min at 94°C; 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 65°C and 1.5 min elongation at 72°C; final extension for 7 min at 72°C. Excess of primers and nucleotides was removed by enzymatic digestion using 10 and 1 U of ExoI and SAP, respectively (Amersham Biosciences) at 37°C for 1 h followed by inactivation of the enzymes at 80°C for 15 min. DNA sequencing was performed using the ABI 3730 automatic DNA sequencer (PE Applied Biosystems) and the ABI Prism Big Dye Terminatior Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

Bioinformatic analysis

Bioinformatic analysis was conducted to determine gene identities using GeneMaster 3.0 software (Ensoltek). Briefly, vector sequence was removed and database search was limited to ESTs >100bp in length. ESTs were then assembled in clusters of contigous sequences (contig) using ICAtools program [15]. Gene annotation procedures and homology searches of the sequenced ESTs was locally done by BLASTX for amino acid similarity comparisons [16]. Matches were considered to be significant only when the probability (P) was less than 1×10^{-3} using BLASTX with all parameters at the defaults. All ESTs that were not identified as orthologues of known genes were designated as unknown EST clones and hypothetical proteins were considered as known EST clones.

RT-PCR verification

Verification of expression levels of candidate genes was carried out by RT-PCR. Total RNA samples were extracted from the collected samples according to each stress condition using TRIzol reagent (Invitrogen). Subsequently, first-strand cDNA synthesis was carried out using the Advantage RT-for-PCR Kit (BD Biosciences). The expression levels of selected genes were detected by RT-PCR using specific primers, which were based on the nucleotide sequence of EST clones. As an internal control, β-actin was amplified using the appropriate primers (Table 1). The PCR conditions were as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final step of 72°C for 7 min. The amplified PCR products were analyzed on an 1.5% agarose gel containing ethidium bromide (100 ng/ml).

Identification of microsatellite containing cDNAs

During the complication of EST sequences, genes that contained microsatellites were identified and their microsatellites were characterized in terms of complexity and repeat number. Clones containing microsatellites were identified by determination of a minimal number of repeats in the microsatellite sequences: dinucleotide, eight repeats; trinucleotide, five repeats; tetranucleotide, three repeats. Single nucleotide repeats were not included since they are not very useful for polymorphic markers. Some cDNA clones contains more than one type of repeat, in which case these clones were categorized according to the longest repeats.

Table 1. Primers used in RT-PCR

β-actin-RT-F	5'-GCCGCTTGACTCTTGTGTGC-3'
β-actin-RT-R	5'-CTCCTCTGGTGCAACGCGG-3'
HSC-RT-F	5'-CTGTGCGCTGACCTGTTTCG-3'
HSC-RT-R	5'-CGAGGTACGCAACTGCTTC-3'
HSC70-RT-F	5'-GTCGGCCTTGCTTAGACGAC-3'
HSC70-RT-R	5'-CTTGGCAAGTTTGAGCTCAC-3'
HSP84-RT-F	5'-GGTTTCCAATCGTCTGGTTAC-3'
HSP84-RT-R	5'-GTCTTCCAGGGAGAAGCC-3'
HSP26-RT-F	5'-CAGAGTCCTTGGGCGATCTAAC-3'
HSP26-RT-R	5'-GCAGGAGCTTTCTCATCGGCTTC-3'
cHSP70-RT-F	5'-GGACTTGCCAGTGCTCTTGTC-3'
cHSP70-RT-R	5'-GGACACGAGCGACGCCATC-3'
HSP71-RT-F	5'-GCCACGTGGTGTGCCTCAG-3'
HSP71-RT-R	5'-CGGCTTGTCTCACGCTGAAG-3'

RESULTS AND DISCUSSION

Summary of EST clones in heat-shock, cold-shock or hyposalinity subtracted cDNA libraries

A total of 1,536 random clones from the heat-shock, cold-shock or hyposalinity subtracted cDNA libraries were partially sequenced; 384 clones from the heat-shock library, 672 clones from the cold-shock library and 480 clones from hyposalinity library. A summary of the identified genes from three subtracted cDNA libraries is shown in Table 2.

The initial ESTs were grouped into 1,074 consensus sequences, comprised of 124 clusters (heat-shock: 42, cold-shock: 52 and hyposalinity: 21) and 950 singletons (heat-shock: 217, cold-shock: 491 and hyposalinity: 278). This suggests that the library had an overall redundany of 18.3%. BLASTX comparisons established that 1,150 (87.4%) of the clones were orthologues of known genes (82 (7.1%) of known genes were identified as hypothetical protein), and the remaining 166 (12.6%) clones were unidentifiable via similarity comparisons ($E \pm 1 \times e^{-3}$). Among the 1,074 EST clones, only 4 unique genes were identified as homologues of previously reported abalone ESTs and 1,070 (99.6%) genes were identified as orthologues of known genes from other organisms. These results suggest that EST analysis constitues a powerful technique for the rapid discovery of large numbers of useful genes in shellfish.

Table 2. General characteristics of the three subtracted cDNA libraries from abalone.

	Stress condition			Sum
	Heat-shock	Cold-shock	Hyposalinity	
Number of clones sequenced	384	672	480	1,536
Number of clones analysed	346	639	331	1316
EST clusters	42	52	21	124
Unigene	259	543	299	1074
Redundancy (%)	25.1	15	9.7	18.3
ESTs with E value $< 1\times e^{-3}$ (matched)(%)	303 (87.6)	403(63.1)	292 (88.2)	1150 (87.4)
ESTs with E value $\geq 1\times e^{-3}$ (unknown)	43 (12.4)	236 (36.9)	39 (11.8)	166 (12.6)

Expression profile in the three subtracted cDNA libraries

The expression profiles of the known genes identified in the three subtracted cDNA libraries are provided in Fig. 1. Among 1101 identified genes, 986 genes (89.6%) were sequenced only once; 91 genes (8.3%) 2-4 times; 24 genes (2.2%) 5 times or more. The percentage of singletons may be acceptable, although redundancy will tend to increase with increasing numbers of sequenced clones. The most abundantly expressed gene in the heat-shock subtracted cDNA library were as follows: small heat shock protein p26 (3.2%), heat shock 27kDa protein 2 (2.9%), erythrocyte membrane protein 1 (2.6%), heat shock protein 70 (2.0%) and cytoplasmic heat shock protein 70 (1.7%). The most abundantly expressed genes in the cold-shock subtracted cDNA library were as follows: trypsinogen 2 (1.4%), chymotrypsinogen 2 (1.1%), trypsinogen 1(1.1%), carboxypeptidase A1 (0.9%) and hemoglobin beta-A chain (0.6%). The most abundantly expressed genes in the hyposalinity subtracted cDNA library were as follows: actin (1.8%), cytoplasmic heat shock protein 70 (1.5%) and calcium binding protein 1(0.6%). The expression profile in the heat-shock or cold-shock subtracted cDNA library then, was more polarized than in its hyposalinity subtracted cDNA libraries.

Distribution of the identified clones in the three subtracted cDNA libraries

Based on the major functions of their encoded proteins, the identified clones were classified into sixteen broad categories; biogenesis of cellular components, cell cycle/ DNA processing, cell fate, cell rescue/ defense/ virulence, cell type differentiation, cellular communication/signal transduction mechanism, cellular transport/ transport facilitation/ transport routes, development (Systemic), energy, interaction with the cullular environment, metabolism, protein activity regulation, protein fate (folding, modification, destination), protein synthesis, protein with binding function/ cofactor requirement (structural or catalytic) and transcription. The distribution of identified clones from the three subtracted cDNA libraries is shown in Fig 2. No notable differences were detected between the cold-shock and hyposalinity subtracted cDNA libraries. However, the distribution of identified clones from the heat-shock subtracted library showed some differences, compared to others. Especially, cell rescue/ defense/ virulence, cellular transport/ transport facilitation/ transport routes, protein fate (folding, modification, destination) and protein with binding function/ cofactor requirement (structural or catalytic) were more abundant than the others.

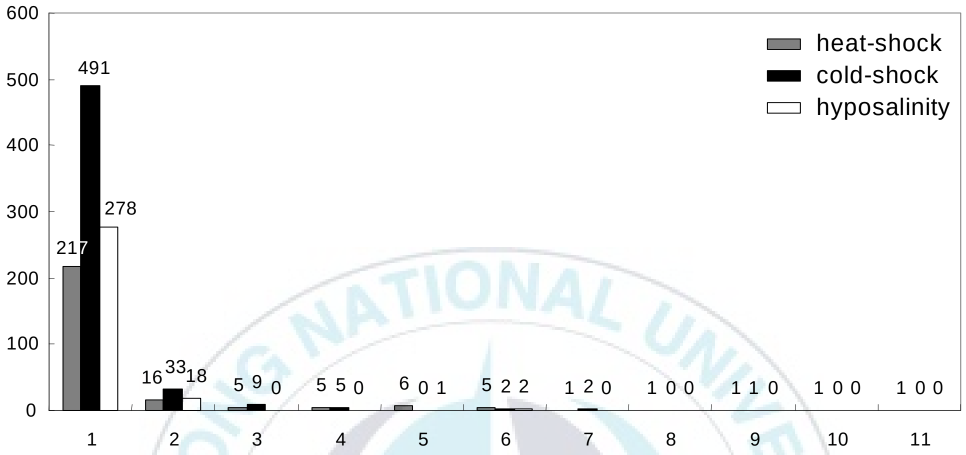

Fig. 1. Expression profiles and sequencing redundancy in the analysis of ESTs from the the three subtracted cDNA libraries of abalone. (redundancy, X axis; hits per gene, Y axis)

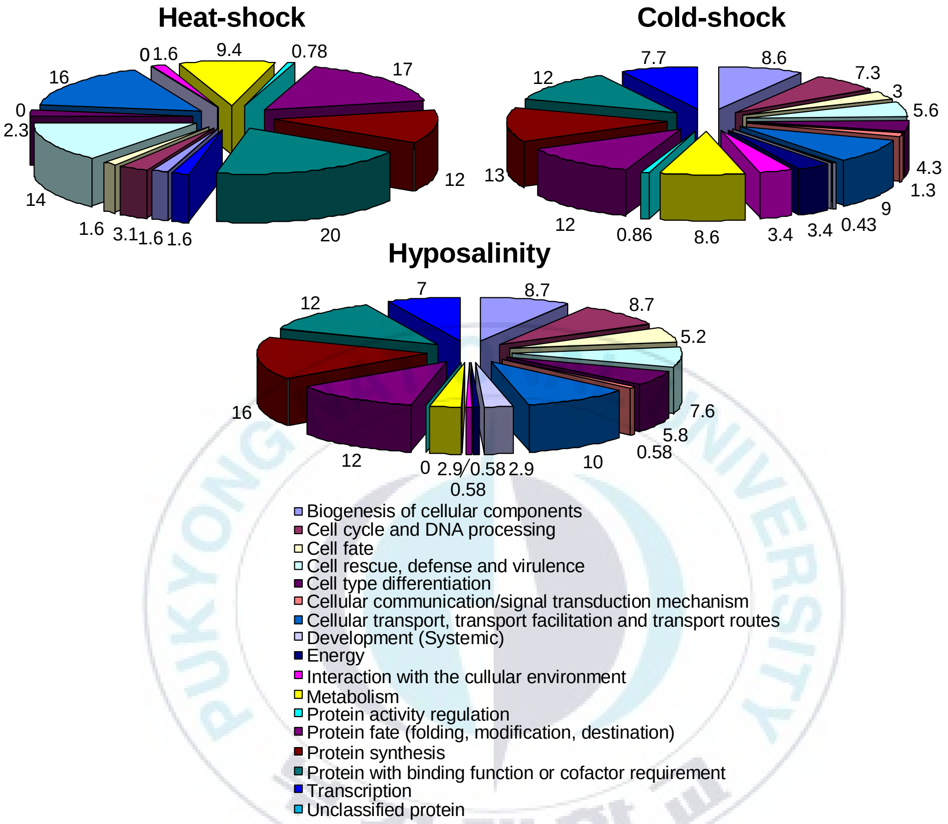

Fig. 2. Functional categorization of the three subtracted cDNA libraries taken from abalone.

Isolation of stress or immune response-related genes from the three subtracted cDNA libraries

Database searches identified putative stress response-related genes that were expressed in the three subtracted cDNA libraries (Table 3). The putative amino acid sequence deduced from several cDNA clone were identified as the heat shock protein. Heat shock proteins (HSPs) are well known as stress proteins because various forms of stress enhance their transcriptional activation and biosynthesis in organisms, ranging from bacteria to humans [17]. According to their average apparent molecular masses they are designed as HSP100, HSP90, HSP70, HSP60, and small-size HSPs [18, 19]. In this study, HSP molecules of various size were identified and RT-PCR was performed using some HSP genes (Table 3) to confirm the results of our study. Several HSPs was gradually expressed in thermal stress conditions. Interestingly, the expression sHSP26 was not induced by cold shock even that of HSP70 was strongly induced (Fig. 3). These results indicate that the abalone sHSP26 induced only by cold shock.

Various stress- or immune-related genes were identified (Table 4). The identified clones included a major component of immune system and revealed significant homology to other known immune-related genes. This work initiates a complementary approach to EST mass sequencing by identification of highly up-regulated genes in response to thermal and salinity stress. Most of the genes identified in this work have not yet been characterized in molluscs. Data of this work will complement those already obtained on the same species and will provide more information in future studies. In addition, the genes and ESTs identified in this study can be mapped onto genetic linkage maps and potentially serve as candidates for the mapping of stress-resistant genes/Quantitative Traits of Loci(QTLs).

Table 3. Putative stress-related genes of abalone SSH cDNA libraries

Clone no.	**Putative identification**	**Closest species**	**Identity**
ABHSL-4-H11-T3.ab1	heat shock cognate protein		82
ABHSL-3_F06_46_12.ab1	heat shock cognate 70	*Tetranychus urticae*	79
ABHSL-3_G04_31_14.ab1	84kDa heat shock protein	*Haliotis tuberculata*	91
AHSL-1_E04_29_10.ab1	heat shock protein hsp40	*homo sapiens*	98
AHSL-2_D11_84_07.ab1	small heat shock protein p26	*Artemia persimilis*	23
ABHSL-4-A9-T3.ab1	heat shock protein 71	*Haliotis tuberculata*	96
ABHSL-4-H9-T3.ab1	cytoplasmic heat shock protein 70	*strea edulis*	79
ABHSL-4-D2-T3.ab1	heat shock 70kD protein 1B	*Strongylocentrotus purpuratus*	91
ABHSL-3_A06_41_02.ab1	heat shock protein 70	*Bombyx mori*	52
ABHSL-3_H08_64_16.ab1	70kDa heat shock protein	*Crassostrea gigas*	61
AHSL-2_G05_39_13.ab1	heat shock protein 70	*Wuchereria bancrofti*	66

Table 4. Putative immune-related genes of abalone SSH cDNA libraries

Clone no.	**Putative identification**	**Closest species**	**Identity**
ABLSL-1-A10-T7.ab1	cathepsin L-like cysteine proteinase B	*Rhipicephalus haemaphysaloides haemaphysaloides*	76
ABLSL-1-B8-T7.ab1	MHC class II A antigen	*Paralichthys olivaceus*	70
ABLSL-1-C6-T7.ab1	IRF7	*Siniperca chuatsi*	71
ABLSL-1-C8-T7.ab1	leukocyte elastase inhibitor	*Oncorhynchus mykiss*	72
ABLSL-1-F6-T7.ab1	CD45	*Takifugu rubripes*	70
ABLSL-1-H9-T7.ab1	B-cell receptor CD22-A isoform	*Danio rerio*	28
ABLSL-2-F3-T7.ab1	CD36 antigen	*Bos taurus*	55
ABLSL-3-B4-T7.ab1	pancreatic protein with two somatomedin B domains	*Paralichthys olivaceus*	97
ABLSL-3-C11-T7.ab1	suppressor of cytokine signaling 3	*Takifugu rubripes*	77
ABLSL-3-H11-T7.ab1	Contains similarity to NBS/LRR disease resistance protein		38
ABLSL-3-H3-T7.ab1	CD3 epsilon precursor	*Gallus gallus*	33
ABLSL-4-B9-T7.ab1	ubiquitin fusion degradation 1-like	*Danio rerio*	90
ABLSL-4-C10-T7.ab1	hemoglobin beta-A chain	*Siniperca chuatsi*	88
ABLSL-4-F2-T7.ab1	MHC II invariant chain	*Siniperca chuatsi*	73

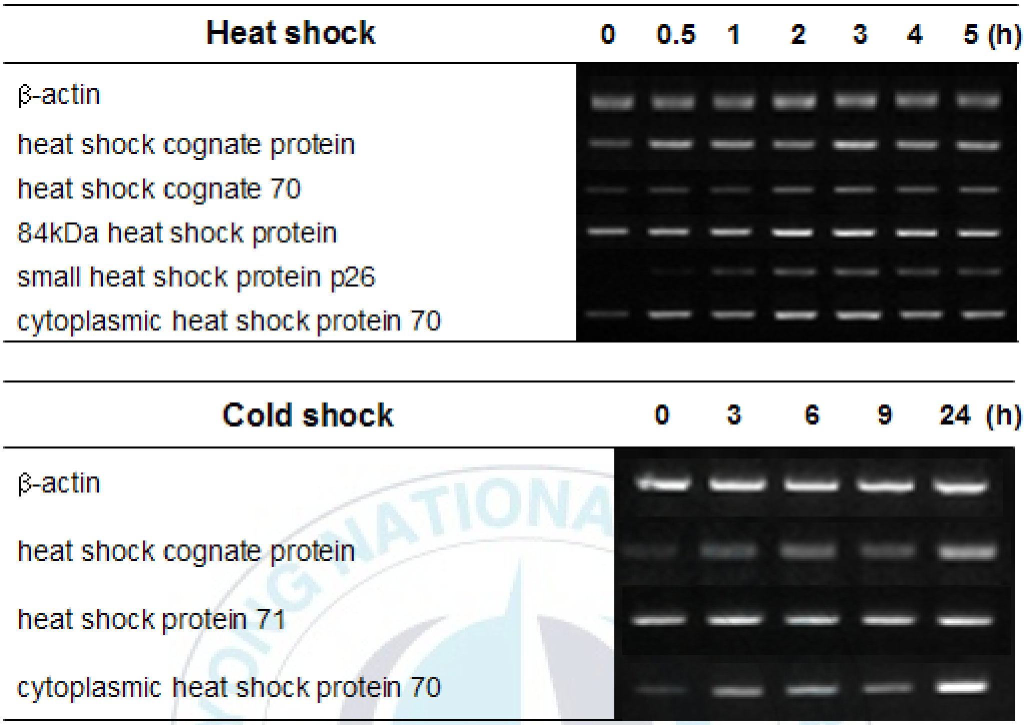

Fig. 3. Expression level of putative stress-related genes. HSP familes were analysed by RT-PCR.

Isolation of EST clones containing microsatellite

Among 1,536 sequenced cDNA clones, 18 unique EST clones harbor microsatellite sequences (Table 5). These microsatellites are potentially useful for genomic mapping if they are polymorphic. This study have been found that targeting microsatellite regions within cDNAs is an efficient way to develop type I molecular markers representing genes of known functions [20]. Because of the evolutionary conservation, mutation rates within gene-coding sequences are lower than those in non-coding genomic sequences. As a result, type I polymorphic markers are often more difficult to identify. By tagging the highly polymorphic microsatellites to known genes, the efficiency for the development of type I markers can be dramatically enhanced. The major objective of this study was to develop EST resources, polymorphism of these microsatellite clones was not attempted to characterize.

EST analysis may be one of the most efficient ways for the development of polymorphic type I markers as well as through tagging of microsatellites existing within cDNAs. ESTs will also be valuable molecular reagents for the production of microarrays. In particular, the application of cDNA microarrays may facilitate research attempting to answer questions concerning stress responses and other protective responses of abalone exposured by environmental stress.

Table 5. EST-containing MS cDNA clones from the abalone SSH cDNA libraries

Clone Name	Nucleotide repeat unit	Microsatellite repeats	Gene name
LSASL-2_C4_06.ab1	Dinucleotide	(GT)6	inhibitor of DNA binding 4
LSASL-2_B11_03.ab1	Tetranucleotide	(TCAC)4	unknown
AHSL-2_G10_79_14.ab1	Trinucleotide	(TCT)4	ribosomal protein S15
ABLSL7-H6-T7.ab1	Dinucleotide	(CA)5	type 1 collagen alpha 1
ABHSL-3_C06_43_06.ab1	Dinucleotide	(AT)7	Twin-arginine translocation pathway signal
ABLSL7-D6-T7.ab1	Dinucleotide	(AT)6	unknown
ABLSL6-D2-T7.ab1	Dinucleotide	(GA)6	unknown
ABLSL7-E6-T7.ab1	Trinucleotide	(TTG)4	unnamed protein product
ABLSL7-A11-T7.ab1	Trinucleotide	(CCT)4	beta actin
ABLSL7-A10-T7.ab1	Trinucleotide	(GGA)4	unknown
ABLSL5-E7-T7.ab1	Dinucleotide	(GT)8	unknown
ABLSL-4-E12-T7.ab1	Trinucleotide	(GGA)4	unknown
ABLSL-3-C11-T7.ab1	Dinucleotide	(AC)6	suppressor of cytokine signaling 3
ABLSL-4-B2-T7.ab1	Trinucleotide	(AGG)4	unnamed protein product
ABLSL-3-F12-T7.ab1	Trinucleotide ,/Tetranucleotide	(TTA)4 /(TCAA)4	unknown
ABLSL-3-D1-T7.ab1	Trinucleotide	(TCC)4	beta actin
ABLSL-1-E6-T7.ab1	Dinucleotide	(AC)5	unknown
ABLSL-1-G3-T7.ab1	Trinucleotide	(CTT)4	ribosomal protein S24

Table 6. List of identified ESTs from the heat-shock abalone subtracted cDNA library

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
ABHSL-3_A02_9_02.ab1	CG3696-PA, isoform A	*Drosophila melanogaster*	NP_523441.1	24
ABHSL-3_A04_25_02.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
ABHSL-3_A06_41_02.ab1	heat shock protein 70	*Bombyx mori*	ABD36134.1	52
ABHSL-3_A07_49_01.ab1	-		_	0
ABHSL-3_A08_57_02.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
ABHSL-3_A09_65_01.ab1	PREDICTED: similar to alanyl-tRNA synthetase	*Strongylocentrotus purpuratus*	XP_794517.1	30
ABHSL-3_A10_73_02.ab1	cellulase EGX	*Ampullaria crossean*	AAP31839.1	50
ABHSL-3_A11_81_01.ab1	ribosomal protein L18	*Crassostrea gigas*	CAD91422.1	77
ABHSL-3_B02_10_04.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
ABHSL-3_B03_18_03.ab1	GM03761p	*Drosophila melanogaster*	AAL47998.1	47
ABHSL-3_B04_26_04.ab1	-		_	0
ABHSL-3_B05_34_03.ab1	BAG-family molecular chaperone regulator-3; BAG-3	*Homo sapiens*	AAD16122.2	40
ABHSL-3_B06_42_04.ab1	hypothetical protein LOC548934	*Xenopus tropicalis*	NP_001016180.1	70
ABHSL-3_B07_50_03.ab1	proline rich protein MP4	*Mus musculus*	NP_444481.1	30
ABHSL-3_B08_58_04.ab1	nitric oxide synthase 1, neuronal	*Mus musculus*	NP_032738.1	42
ABHSL-3_B09_66_03.ab1	-		_	0
ABHSL-3_B10_74_04.ab1	heat shock protein 70	*Mytilus galloprovincialis*	BAD99026.1	53
ABHSL-3_B11_82_03.ab1	BAGS_BOMMO BAG domain-containing protein Samui		Q9BLJ6	38
ABHSL-3_B12_90_04.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	80
ABHSL-3_C01_3_05.ab1	similar to Homeodomain-interacting protein kinase 2	*Gallus gallus*	XP_416335.1	31
ABHSL-3_C03_19_05.ab1	similar to mitochondrial ATP synthase lipid binding protein precursor	*Apis mellifera*	XP_623661.1	42
ABHSL-3_C04_27_06.ab1	similar to 40S ribosomal protein S16	*Rattus norvegicus*	XP_341816.2	74
ABHSL-3_C05_35_05.ab1	heat shock protein 90	*Chlamys farreri*	AAR11781.1	64
ABHSL-3_C06_43_06.ab1	Twin-arginine translocation pathway signal	*Rhodobacter sphaeroides ATCC 17025*	ZP_00915527.1	27
ABHSL-3_C07_51_05.ab1	formin-related protein, possible	*Cryptosporidium parvum*	CAD98456.1	52
ABHSL-3_C08_59_06.ab1	actine	*Elaeis guineensis*	AAT45848.1	95
ABHSL-3_C09_67_05.ab1	similar to TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	*Mus musculus*	XP_122081.2	57
ABHSL-3_C10_75_06.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	73
ABHSL-3_C11_83_05.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
ABHSL-3_D01_4_07.ab1	hypothetical protein BC1906	*Bacteriophage phBC6A51*	NP_852540.1	24
ABHSL-3_D02_12_08.ab1	similar to CG5290-PA	*Bos taurus*	XP_614813.2	25
ABHSL-3_D03_20_07.ab1	-		_	0
ABHSL-3_D04_28_08.ab1	similar to notch1 preproprotein	*Strongylocentrotus purpuratus*	XP_789629.1	41

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABHSL-3_D05_36_07.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG08894.1	30
ABHSL-3_D06_44_08.ab1	hypothetical protein UM04162.1	*Ustilago maydis 521*	XP_760309.1	33
ABHSL-3_D08_60_08.ab1	hypothetical protein LOC541384	*Danio rerio*	NP_001013529.1	46
ABHSL-3_D09_68_07.ab1	-		_	0
ABHSL-3_D10_76_08.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
ABHSL-3_D12_92_08.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99499.1	57
ABHSL-3_E01_5_09.ab1	hypothetical protein Gmet_0599	*Geobacter metallireducens GS-15*	ABB30842.1	31
ABHSL-3_E02_13_10.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
ABHSL-3_E03_21_09.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
ABHSL-3_E04_29_10.ab1	similar to PTPL1-associated RhoGAP 1	*Pan troglodytes*	XP_513574.1	48
ABHSL-3_E06_45_10.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
ABHSL-3_E07_53_09.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
ABHSL-3_E08_61_10.ab1	cytoplasmic heat shock protein 70	*Ostrea edulis*	AAM46634.1	73
ABHSL-3_E09_69_09.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
ABHSL-3_E10_77_10.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	80
ABHSL-3_E11_85_09.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	92
ABHSL-3_E12_93_10.ab1	Mfd	*Pasteurella multocida subsp. multocida str. Pm70*	AAK03125.1	43
ABHSL-3_F02_14_12.ab1	-		_	0
ABHSL-3_F03_22_11.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	33
ABHSL-3_F04_30_12.ab1	malate-H+/Na+-lactate antiporter	*Bacillus subtilis subsp. subtilis str. 168*	CAB14288.1	35
ABHSL-3_F05_38_11.ab1	Hypothetical protein ZK380.2	*Caenorhabditis elegans*	AAC17011.1	43
ABHSL-3_F06_46_12.ab1	heat shock cognate 70	*Tetranychus urticae*	ABC33921.1	79
ABHSL-3_F07_54_11.ab1	similar to ENSANGP00000018891	*Apis mellifera*	XP_393575.2	43
ABHSL-3_F09_70_11.ab1	cytoplasmic heat shock protein 70	*Ostrea edulis*	AAM46634.1	83
ABHSL-3_F10_78_12.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
ABHSL-3_F11_86_11.ab1	ribosomal protein S1 (rpsA)	*Treponema pallidum subsp. pallidum str. Nichols*	AAC65266.1	39
ABHSL-3_F12_94_12.ab1	vdg3	*Mytilus edulis*	ABB76764.1	41
ABHSL-3_G01_7_13.ab1	-		_	0
ABHSL-3_G03_23_13.ab1	SBCFR-1 protein	*Paralabrax nebulifer*	CAA67355.1	42
ABHSL-3_G04_31_14.ab1	heat shock protein 90	*Chlamys farreri*	AAR11781.1	66
ABHSL-3_G07_55_13.ab1	hypothetical protein	*Oryza sativa (japonica cultivar-group)*	BAD82383.1	36
ABHSL-3_G08_63_14.ab1	heat shock protein 70	*Haliotis discus hannai*	ABC54952.1	98
ABHSL-3_G10_79_14.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF93683.1	41
ABHSL-3_G11_87_13.ab1	similar to PTPL1-associated RhoGAP 1	*Pan troglodytes*	XP_513574.1	39
ABHSL-3_H01_8_15.ab1	similar to COP9 signalosome subunit 6; H_NH0506M12.12; COP9 subunit 6; MOV34 homolog, 34 kD	*Pan troglodytes*	XP_519247.1	68
ABHSL-3_H02_16_16.ab1	BAGS_BOMMO BAG domain-containing protein Samui		Q9BLJ6	30

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABHSL-3_H03_24_15.ab1	RE15268p	*Drosophila melanogaster*	AAS93748.1	32
ABHSL-3_H07_56_15.ab1	Cell division transporter substrate-binding protein FtsY	*Pseudoalteromonas tunicata D2*	ZP_01136122.1	36
ABHSL-3_H08_64_16.ab1	70kDa heat shock protein	*Crassostrea gigas*	BAD15286.1	61
ABHSL-3_H10_80_16.ab1	PREDICTED: similar to baculoviral IAP repeat-containing 2, partial	*Strongylocentrotus purpuratus*	XP_789042.1	27
ABHSL-3_H11_88_15.ab1	-		_	0
ABHSL-3_H12_96_16.ab1	ribosomal protein S15A	*Lysiphlebus testaceipes*	AAX62449.1	85
ABHSL-4-A10-T3.ab1	-		_	0
ABHSL-4-A11-T3.ab1	-		_	0
ABHSL-4-A12-T3.ab1	similar to Paxillin	*Pan troglodytes*	XP_509424.1	36
ABHSL-4-A2-T3.ab1	SJCHGC02336 protein	*Schistosoma japonicum*	AAW25825.1	34
ABHSL-4-A3-T3.ab1	unnamed protein product	*Kluyveromyces lactis*	XP_454560.1	47
ABHSL-4-A4-T3.ab1	similar to guanine nucleotide exchange factor p532	*Strongylocentrotus purpuratus*	XP_781527.1	41
ABHSL-4-A5-T3.ab1	hypothetical protein 3	*Microplitis demolitor bracovirus*	YP_239367.1	42
ABHSL-4-A7-T3.ab1	-		_	0
ABHSL-4-A8-T3.ab1	ribosomal protein S5	*Crassostrea gigas*	CAD91445.1	95
ABHSL-4-A9-T3.ab1	heat shock protein 70	*Haliotis discus hannai*	ABC54952.1	96
ABHSL-4-B10-T3.ab1	ribosomal protein L24e	*Hister sp. APV-2005*	CAJ17316.1	59
ABHSL-4-B11-T3.ab1	vdg3	*Mytilus edulis*	ABB76764.1	40
ABHSL-4-B12-T3.ab1	hypothetical protein Pfl_2663	*Pseudomonas fluorescens PfO-1*	ABA74404.1	26
ABHSL-4-B2-T3.ab1	vdg3	*Mytilus edulis*	ABB76764.1	38
ABHSL-4-B3-T3.ab1	similar to Protein transport protein Sec24D (SEC24-related protein D)	*Bos taurus*	XP_580541.2	41
ABHSL-4-B5-T3.ab1	hypothetical protein Noc_0748	*Nitrosococcus oceani ATCC 19707*	ABA57261.1	42
ABHSL-4-B6-T3.ab1	ENSANGP00000014375	*Anopheles gambiae str. PEST*	EAA14477.2	30
ABHSL-4-B7-T3.ab1	HYPOTHETICAL PROTEIN	*Lactococcus lactis subsp. lactis Il1403*	AAK05469.1	26
ABHSL-4-B8-T3.ab1	hypothetical protein PTD2_08194	*Pseudoalteromonas tunicata D2*	ZP_01133610.1	28
ABHSL-4-B9-T3.ab1	-		_	0
ABHSL-4-C1-T3.ab1	developmentally-regulated vdg3	*Haliotis asinina*	AAX11341.1	63
ABHSL-4-C10-T3.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_473185.2	30
ABHSL-4-C11-T3.ab1	-		_	0
ABHSL-4-C3-T3.ab1	erythrocyte membrane protein 1	*Plasmodium falciparum*	AAL11649.1	25
ABHSL-4-C4-T3.ab1	hypothetical protein	*Yarrowia lipolytica*	XP_503304.1	38
ABHSL-4-C6-T3.ab1	-		_	0
ABHSL-4-C9-T3.ab1	unknown	*Crassostrea gigas*	AAU93882.1	33
ABHSL-4-D1-T3.ab1	novel protein similar to vertebrate gliacolin (C1Q)	*Danio rerio*	CAE17630.1	37
ABHSL-4-D10-T3.ab1	-		_	0
ABHSL-4-D11-T3.ab1	-		_	0

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
ABHSL-4-D2-T3.ab1	PREDICTED: similar to heat shock 70kD protein 1B	*Strongylocentrotus purpuratus*	XP_780151.1	91
ABHSL-4-D4-T3.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10208.1	26
ABHSL-4-D5-T3.ab1	-		_	0
ABHSL-4-D6-T3.ab1	Hypothetical protein F41G3.12	*Caenorhabditis elegans*	AAP13779.1	48
ABHSL-4-D8-T3.ab1	-		_	0
ABHSL-4-E1-T3.ab1	78kDa glucose regulated protein	*Crassostrea gigas*	BAD15288.1	69
ABHSL-4-E10-T3.ab1	ribosomal protein S2	*Chlamys farreri*	AAM94271.1	90
ABHSL-4-E12-T3.ab1	similar to sushi domain containing 1	*Mus musculus*	XP_620037.2	34
ABHSL-4-E2-T3.ab1	upregulated in colorectal cancer gene 1 protein precursor	*Homo sapiens*	NP_060019.1	27
ABHSL-4-E3-T3.ab1	heat shock protein 70	*Mytilus galloprovincialis*	CAH04108.1	69
ABHSL-4-E5-T3.ab1	-		_	0
ABHSL-4-E6-T3.ab1	General substrate transporter:Major facilitator superfamily	*Burkholderia ambifaria AMMD*	ZP_00686283.1	34
ABHSL-4-E7-T3.ab1	similar to Protein transport protein Sec61 beta subunit	*Strongylocentrotus purpuratus*	XP_786999.1	70
ABHSL-4-E8-T3.ab1	heat-shock protein 70	*Biomphalaria glabrata*	AAB99911.1	86
ABHSL-4-E9-T3.ab1	yhaP	*Bacillus subtilis subsp. subtilis str. 168*	CAB12830.1	45
ABHSL-4-F10-T3.ab1	-		_	0
ABHSL-4-F11-T3.ab1	hypothetical protein	*Plasmodium chabaudi chabaudi*	XP_743395.1	29
ABHSL-4-F12-T3.ab1	-		_	0
ABHSL-4-F2-T3.ab1	NADH dehydrogenase subunit 4	*Haliotis rubra*	YP_026074.1	91
ABHSL-4-F3-T3.ab1	-		_	0
ABHSL-4-F4-T3.ab1	heat shock protein protein	*Paracentrotus lividus*	CAA43653.1	57
ABHSL-4-F6-T3.ab1	hypothetical protein	*Yarrowia lipolytica*	XP_501418.1	31
ABHSL-4-F8-T3.ab1	PREDICTED: similar to ENSANGP00000018891	*Apis mellifera*	XP_393575.2	32
ABHSL-4-F9-T3.ab1	small heat shock protein p26	*Artemia sinica*	ABC41137.1	31
ABHSL-4-G11-T3.ab1	4Fe-4S ferredoxin, iron-sulfur binding protein	*Anaeromyxobacter dehalogenans 2CP-C*	YP_467283.1	39
ABHSL-4-G12-T3.ab1	hypothetical protein 151.t00004	*Entamoeba histolytica HM-1:IMSS*	XP_651700.1	40
ABHSL-4-G2-T3.ab1	unnamed protein product	*Mus musculus*	BAE27774.1	26
ABHSL-4-G3-T3.ab1	PREDICTED: similar to hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1	*Danio rerio*	XP_698091.1	45
ABHSL-4-G5-T3.ab1	GA21098-PA	*Drosophila pseudoobscura*	EAL32786.1	30
ABHSL-4-G6-T3.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_704192.1	44
ABHSL-4-G8-T3.ab1	similar to mucin 19, partial	*Strongylocentrotus purpuratus*	XP_793965.1	35
ABHSL-4-G9-T3.ab1	-		_	0
ABHSL-4-H10-T3.ab1	LIM-homeobox domain protein		AAB08752.1	42
ABHSL-4-H3-T3.ab1	Col3a1 protein	*Mus musculus*	AAH28248.1	40
ABHSL-4-H5-T3.ab1	blastula protease-10	*Paracentrotus lividus*	CAA39673.1	32

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABHSL-4-H2-T3.ab1	similar to hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 1	*Danio rerio*	XP_698091.1	45
ABHSL-4-H6-T3.ab1	heat shock protein 70	*Manduca sexta*	AAO65964.1	57
ABHSL-4-H8-T3.ab1	fatty acid binding protein	*Clonorchis sinensis*	AAN04089.1	29
AHSL-1_A01_1_01.ab1	ENSANGP00000009540	*Anopheles gambiae str. PEST*	EAA04007.2	35
AHSL-1_A02_9_02.ab1	developmentally-regulated vdg3	*Haliotis asinina*	AAX11341.1	73
AHSL-1_A07_49_01.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-1_A09_65_01.ab1	similar to pim (mammalian oncogene) Related Kinase (prk-1), partial	*Danio rerio*	XP_699228.1	49
AHSL-1_A11_81_01.ab1	-		_	0
AHSL-1_A12_89_02.ab1	conserved hypothetical protein	*Chlamydophila abortus S26/3*	CAH64374.1	43
AHSL-1_B04_26_04.ab1	156D suface antigen	*Paramecium primaurelia*	CAA65436.1	39
AHSL-1_B06_42_04.ab1	ribosomal protein S14	*Bombyx mori*	AAU11819.1	36
AHSL-1_B09_66_03.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	83
AHSL-1_B11_82_03.ab1	polyketide synthase	*Mycosphaerella zeae-maydis*	AAR85531.1	35
AHSL-1_B12_90_04.ab1	midasin	*Aspergillus fumigatus Af293*	XP_755547.1	35
AHSL-1_C01_3_05.ab1	ependymin-related protein precursor; EpenHg precursor	*Holothuria glaberrima*	AAR89380.1	46
AHSL-1_C02_11_06.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-1_C03_19_05.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-1_C05_35_05.ab1	fasciclin domain protein	*Cyanobacteria bacterium Yellowstone A-Prime*	YP_473783.1	51
AHSL-1_C08_59_06.ab1	hypothetical protein, conserved	*Leishmania major*	CAJ03405.1	34
AHSL-1_C09_67_05.ab1	hypothetical protein	*Oryza sativa (japonica cultivar-group)*	XP_462982.1	46
AHSL-1_C11_83_05.ab1	hypothetical protein XP_686536	*Danio rerio*	XP_691628.1	41
AHSL-1_C12_91_06.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	80
AHSL-1_D01_4_07.ab1	ribosomal protein L24	*Crassostrea gigas*	CAD91424.1	87
AHSL-1_D02_12_08.ab1	-		_	0
AHSL-1_D04_28_08.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99140.1	38
AHSL-1_D05_36_07.ab1	heat shock protein 70	*Bombyx mori*	ABD36134.1	57
AHSL-1_D06_44_08.ab1	brain-specific fatty acid binding protein	*Oryzias latipes*	BAE72542.1	35
AHSL-1_D07_52_07.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-1_D08_60_08.ab1	alginate lyase	*Haliotis discus hannai*	BAC87758.1	100
AHSL-1_D09_68_07.ab1	-		_	0
AHSL-1_D10_76_08.ab1	-		_	0
AHSL-1_D11_84_07.ab1	surface antigen S	*Paramecium primaurelia*	AAS59562.1	48
AHSL-1_E01_5_09.ab1	expressed protein	*Cryptococcus neoformans var. neoformans JEC21*	AAW42212.1	30
AHSL-1_E04_29_10.ab1	heat shock protein hsp40 homolog - human	*human*	G02272	68
AHSL-1_E06_45_10.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	50
AHSL-1_E08_61_10.ab1	asparaginyl-tRNA synthetase	*Pan troglodytes*	XP_512149.1	74

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
AHSL-1_E10_77_10.ab1	matrilin 1, cartilage matrix protein	*Rattus norvegicus*	NP_001006980.1	30
AHSL-1_E12_93_10.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-1_F01_6_11.ab1	ribosomal protein L35	*Mus musculus*	NP_079868.1	79
AHSL-1_F02_14_12.ab1	ATP-dependent RNA helicase, DEAD/DEAH box family protein	*Rhodobacterales bacterium HTCC2654*	ZP_01012424.1	48
AHSL-1_F03_22_11.ab1	2-hydroxymuconic semialdehyde dehydrogenase	*Burkholderia cepacia*	AAP32788.1	32
AHSL-1_F04_30_12.ab1	heat shock protein 70	*Bombyx mori*	ABD36134.1	55
AHSL-1_F07_54_11.ab1	Dienelactone hydrolase	*Synechococcus sp. WH 7805*	ZP_01122724.1	31
AHSL-1_F08_62_12.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	82
AHSL-1_F09_70_11.ab1	SJCHGC01954 protein	*Schistosoma japonicum*	AAX26850.2	53
AHSL-1_F10_78_12.ab1	hypothetical protein, unknown function	*Leishmania major*	CAJ08568.1	51
AHSL-1_F11_86_11.ab1	temptin	*Aplysia brasiliana*	AAS92605.1	56
AHSL-1_F12_94_12.ab1	similar to Lactase-phlorizin hydrolase precursor (Lactase-glycosylceramidase)	*Strongylocentrotus purpuratus*	XP_783049.1	58
AHSL-1_G01_7_13.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	84
AHSL-1_G02_15_14.ab1	-		_	0
AHSL-1_G04_31_14.ab1	similar to angiomotin isoform 2	*Bos taurus*	XP_879786.1	33
AHSL-1_G05_39_13.ab1	heat shock protein 70	*Bombyx mori*	ABD36134.1	57
AHSL-1_G06_47_14.ab1	MGC80644 protein	*Xenopus laevis*	AAH73276.1	34
AHSL-1_G07_55_13.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-1_H01_8_15.ab1	F1F0-type ATP synthase subunit g	*Ixodes scapularis*	AAY66986.1	46
AHSL-1_H02_16_16.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	62
AHSL-1_H03_24_15.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
AHSL-1_H05_40_15.ab1	OSJNBa0035I04.9	*Oryza sativa (japonica cultivar-group)*	CAE05421.2	45
AHSL-1_H07_56_15.ab1	-		_	0
AHSL-1_H08_64_16.ab1	heat shock protein Hsp70b	*Drosophila lummei*	AAR17097.2	48
AHSL-1_H10_80_16.ab1	-		_	0
AHSL-1_H11_88_15.ab1	alpha-amylase	*Corbicula fluminea*	AAO17927.2	53
AHSL-1_H12_96_16.ab1	tetraspanin-CD63 receptor	*Geodia cydonium*	CAA77025.1	33
AHSL-2_A01_1_01.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
AHSL-2_A02_9_02.ab1	CONSERVED HYPOTHETICAL PROTEIN	*Mycobacterium bovis AF2122/97*	CAD94260.1	29
AHSL-2_A04_25_02.ab1	DRIM-related protein, putative	*Paramecium tetraurelia*	YP_054339.1	46
AHSL-2_A06_41_02.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-2_A08_57_02.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	92
AHSL-2_A09_65_01.ab1	similar to Sulfatase modifying factor 2 precursor (C-alpha-formyglycine-generating enzyme 2), partial	*Strongylocentrotus purpuratus*	XP_789318.1	58
AHSL-2_A10_73_02.ab1	RH19795p	*Drosophila melanogaster*	AAM29578.1	36
AHSL-2_B01_2_03.ab1	-		_	0
AHSL-2_B02_10_04.ab1	-		_	0
AHSL-2_B03_18_03.ab1	actin	*Pinus taeda*	AAD47902.1	77
AHSL-2_B05_34_03.ab1	similar to hypothetical protein MGC67567	*Rattus norvegicus*	XP_577950.1	31

Table 6. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
AHSL-2_B06_42_04.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	100
AHSL-2_B07_50_03.ab1	proline rich protein 2 (predicted)	*Rattus norvegicus*	NP_001013229.1	40
AHSL-2_B08_58_04.ab1	hypothetical protein PF14_0644	*Plasmodium falciparum 3D7*	AAN37257.1	35
AHSL-2_B09_66_03.ab1	nonmuscle myosin essential light chain	*Ixodes scapularis*	AAY66877.1	68
AHSL-2_C02_11_06.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	92
AHSL-2_C03_19_05.ab1	MGC80065 protein	*Xenopus laevis*	AAH72146.1	80
AHSL-2_C04_27_06.ab1	myosin:SUBUNIT=regulatory light chain		1803425D	62
AHSL-2_C05_35_05.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
AHSL-2_C07_51_05.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	46
AHSL-2_C08_59_06.ab1	ENSANGP00000025203	*Anopheles gambiae str. PEST*	EAA45629.2	35
AHSL-2_C09_67_05.ab1	-		_	0
AHSL-2_C11_83_05.ab1	-		_	0
AHSL-2_D03_20_07.ab1	Ac2-246	*Rattus norvegicus*	AAP86271.1	93
AHSL-2_D05_36_07.ab1	-		_	0
AHSL-2_D06_44_08.ab1	Heat shock protein Hsp20	*Solibacter usitatus Ellin6076*	ZP_00527347.1	28
AHSL-2_D08_60_08.ab1	ribosomal protein S18	*Branchiostoma belcheri*	AAN52390.1	87
AHSL-2_E03_21_09.ab1	RL4_URECA 60S ribosomal protein L4 (L1)		P49165	51
AHSL-2_E04_29_10.ab1	-		_	0
AHSL-2_E05_37_09.ab1	NADH dehydrogenase subunit 1	*Haliotis rubra*	YP_026078.1	78
AHSL-2_E06_45_10.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	92
AHSL-2_E07_53_09.ab1	hypothetical protein Pfl_2663	*Pseudomonas fluorescens PfO-1*	ABA74404.1	26
AHSL-2_E08_61_10.ab1	Flagellar biosynthetic protein FlhB	*Nitrosococcus oceani ATCC 19707*	ABA58619.1	34
AHSL-2_E09_69_09.ab1	small heat shock protein p26	*Artemia urmiana*	ABC41141.1	24
AHSL-2_E12_93_10.ab1	small heat shock protein p26	*Artemia franciscana*	ABC41136.1	32
AHSL-2_F01_6_11.ab1	hypothetical protein DDB0169123	*Dictyostelium discoideum*	XP_642739.1	28
AHSL-2_F02_14_12.ab1	-		_	0
AHSL-2_F03_22_11.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
AHSL-2_F05_38_11.ab1	-		_	0
AHSL-2_F07_54_11.ab1	GA20963-PA	*Drosophila pseudoobscura*	EAL26527.1	47
AHSL-2_F09_70_11.ab1	-		_	0
AHSL-2_F12_94_12.ab1	Hypothetical protein CBG21955	*Caenorhabditis briggsae*	CAE74264.1	43
AHSL-2_G01_7_13.ab1	heat shock protein 70	*Mytilus galloprovincialis*	BAD99026.1	54
AHSL-2_G02_15_14.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	100
AHSL-2_G03_23_13.ab1	vdg3	*Mytilus edulis*	ABB76764.1	31
AHSL-2_G05_39_13.ab1	heat shock protein 70	*Wuchereria bancrofti*	AAF66987.1	66
AHSL-2_G09_71_13.ab1	hypothetical protein	*Arabidopsis thaliana*	AAD25572.1	30
AHSL-2_G10_79_14.ab1	ribosomal protein S15	*Mytilus edulis*	ABB73035.1	62
AHSL-2_G12_95_14.ab1	MK2 non-allergic IgE heavy chain IGHV2-5	*Homo sapiens*	AAW29114.1	92
AHSL-2_H01_8_15.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	85
AHSL-2_H03_24_15.ab1	glyceraldehyde phosphate dehydrogenase	*Vibrio fischeri*	AAC80272.2	78

Table 6. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
AHSL-2_H05_40_15.ab1	-		_	0
AHSL-2_H07_56_15.ab1	Hypothetical protein CBG22012	*Caenorhabditis briggsae*	CAE74305.1	36
AHSL-2_H10_80_16.ab1	-		_	0
AHSL-2_H11_88_15.ab1	-		_	0
AHSL-2_H12_96_16.ab1	-		_	0

[a] Identity(%)

Table 7. List of identified ESTs from the cold-shock abalone subtracted cDNA library

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
ABLSL-1-A1-T7.ab1	-		_	0
ABLSL-1-A10-T7.ab1	cathepsin L-like cysteine proteinase B	*Rhipicephalus haemaphysaloides haemaphysaloides*	AAQ16118.1	76
ABLSL-1-A11-T7.ab1	-		_	0
ABLSL-1-A2-T7.ab1	-		_	0
ABLSL-1-A3-T7.ab1	heat shock protein 90 beta	*Paralichthys olivaceus*	AAO92751.1	90
ABLSL-1-A4-T7.ab1	Ruvbl1 protein	*Xenopus laevis*	AAH71105.1	92
ABLSL-1-A5-T7.ab1	-		_	0
ABLSL-1-A6-T7.ab1	-		_	0
ABLSL-1-A7-T7.ab1	-		_	0
ABLSL-1-A8-T7.ab1	similar to DNA-damage-inducible transcript 4-like	*Danio rerio*	XP_699459.1	43
ABLSL-1-B1-T7.ab1	-		_	0
ABLSL-1-B10-T7.ab1	similar to Hypothetical protein MGC76218	*Gallus gallus*	XP_415059.1	76
ABLSL-1-B11-T7.ab1	sorting nexin 3	*Takifugu rubripes*	AAM61764.1	88
ABLSL-1-B12-T7.ab1	-		_	0
ABLSL-1-B2-T7.ab1	-		_	0
ABLSL-1-B3-T7.ab1	aminopeptidase N	*Pseudopleuronectes americanus*	AAC32754.1	81
ABLSL-1-B4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF91569.1	74
ABLSL-1-B5-T7.ab1	ARP3_FUGRU Actin-like protein 3		100O73723	
ABLSL-1-B6-T7.ab1	chitinase	*Oncorhynchus mykiss*	CAD59687.1	62
ABLSL-1-B7-T7.ab1	ribosomal protein P1	*Biomphalaria glabrata*	AAZ39530.1	55
ABLSL-1-B8-T7.ab1	MHC class II A antigen	*Paralichthys olivaceus*	AAY18782.1	70
ABLSL-1-B9-T7.ab1	-		_	0
ABLSL-1-C1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF93821.1	84
ABLSL-1-C10-T7.ab1	-		_	0
ABLSL-1-C12-T7.ab1	elongation factor 1 alpha	*Paralichthys olivaceus*	BAE48211.1	97
ABLSL-1-C2-T7.ab1	-		_	0
ABLSL-1-C3-T7.ab1	-		_	0
ABLSL-1-C4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF98499.1	97
ABLSL-1-C6-T7.ab1	IRF7	*Siniperca chuatsi*	AAV65045.1	71
ABLSL-1-C7-T7.ab1	-		_	0
ABLSL-1-C8-T7.ab1	leukocyte elastase inhibitor	*Oncorhynchus mykiss*	AAU14875.1	72
ABLSL-1-C9-T7.ab1	-		_	0
ABLSL-1-D1-T7.ab1	-		_	0
ABLSL-1-D10-T7.ab1	Hypothetical protein LOC449806	*Danio rerio*	AAH83431.1	94
ABLSL-1-D11-T7.ab1	Hypothetical protein MGC66080	*Danio rerio*	AAH56561.1	93
ABLSL-1-D12-T7.ab1	hsc71	*Paralichthys olivaceus*	BAD05136.1	99
ABLSL-1-D2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG12999.1	83
ABLSL-1-D3-T7.ab1	-		_	0
ABLSL-1-D5-T7.ab1	ribosomal protein L28	*Haliotis asinina*	AAX11340.1	74

Table 7. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
ABLSL-1-D6-T7.ab1	-		_	0
ABLSL-1-D7-T7.ab1	-		_	0
ABLSL-1-D9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG04978.1	66
ABLSL-1-E10-T7.ab1	-		_	0
ABLSL-1-E12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF91411.1	87
ABLSL-1-E3-T7.ab1	-		_	0
ABLSL-1-E5-T7.ab1	-		_	0
ABLSL-1-E6-T7.ab1	-		_	0
ABLSL-1-E7-T7.ab1	-		_	0
ABLSL-1-E8-T7.ab1	N-myc downstream regulated gene 1 protein, ndrg1	*Danio rerio*	CAE18169.1	75
ABLSL-1-E9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF94246.1	73
ABLSL-1-F1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02536.1	57
ABLSL-1-F12-T7.ab1	-		_	0
ABLSL-1-F3-T7.ab1	-		_	0
ABLSL-1-F4-T7.ab1	hsc71	*Paralichthys olivaceus*	BAD05136.1	98
ABLSL-1-F5-T7.ab1	receptor for activated protein kinase C	*Paralichthys olivaceus*	AAT35603.1	98
ABLSL-1-F6-T7.ab1	CD45	*Takifugu rubripes*	CAB96212.1	70
ABLSL-1-F7-T7.ab1	-		_	0
ABLSL-1-F8-T7.ab1	similar to transitional epithelia response protein	*Danio rerio*	XP_686705.1	96
ABLSL-1-F9-T7.ab1	similar to Thbs2, partial	*Danio rerio*	XP_694690.1	51
ABLSL-1-G1-T7.ab1	-		_	0
ABLSL-1-G10-T7.ab1	RhAG-1 protein	*Takifugu rubripes*	NP_001027816.1	61
ABLSL-1-G11-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG05898.1	80
ABLSL-1-G12-T7.ab1	-		_	0
ABLSL-1-G2-T7.ab1	-		_	0
ABLSL-1-G3-T7.ab1	ribosomal protein S24	*Ixodes scapularis*	AAY66904.1	63
ABLSL-1-G4-T7.ab1	-		_	0
ABLSL-1-G5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03728.1	74
ABLSL-1-G8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09558.1	69
ABLSL-1-H1-T7.ab1	-		_	0
ABLSL-1-H10-T7.ab1	-		_	0
ABLSL-1-H11-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99350.1	48
ABLSL-1-H12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09775.1	74
ABLSL-1-H2-T7.ab1	elastase A precursor	*Gadus morhua*	AAB58351.1	77
ABLSL-1-H3-T7.ab1	-		_	0
ABLSL-1-H4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG05778.1	65
ABLSL-1-H5-T7.ab1	similar to ZDHHC7 protein	*Gallus gallus*	XP_414183.1	72
ABLSL-1-H6-T7.ab1	-		_	0
ABLSL-1-H8-T7.ab1	-		_	0
ABLSL-1-H9-T7.ab1	similar to B-cell receptor CD22-A isoform	*Danio rerio*	XP_699631.1	28
ABLSL-2-A1-T7.ab1	triglyceride lipase	*Anguilla japonica*	BAB85636.1	47
ABLSL-2-A10-T7.ab1	phospholipase A2	*Pagrus major*	BAB47142.1	75

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABLSL-2-A11-T7.ab1	-		_	0
ABLSL-2-A12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06284.1	33
ABLSL-2-A2-T7.ab1	-		_	0
ABLSL-2-A3-T7.ab1	40S ribosomal protein S7	*Oncorhynchus mykiss*	CAJ43764.1	98
ABLSL-2-A4-T7.ab1	-		_	0
ABLSL-2-A5-T7.ab1	activin A receptor type II-like 1	*Danio rerio*	NP_705929.1	71
ABLSL-2-A7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF95307.1	80
ABLSL-2-A8-T7.ab1	-		_	0
ABLSL-2-A9-T7.ab1	NS1-associated protein 1	*Danio rerio*	AAQ97822.1	96
ABLSL-2-B1-T7.ab1	-		_	0
ABLSL-2-B10-T7.ab1	S26	*Suberites domuncula*	AAX48902.1	69
ABLSL-2-B12-T7.ab1	similar to Actin, cytoplasmic 2 (Gamma-actin)	*Rattus norvegicus*	XP_215761.3	71
ABLSL-2-B2-T7.ab1	-		_	0
ABLSL-2-B3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06211.1	67
ABLSL-2-B4-T7.ab1	-		_	0
ABLSL-2-B5-T7.ab1	-		_	0
ABLSL-2-B6-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG04058.1	91
ABLSL-2-B7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03524.1	96
ABLSL-2-B8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06540.1	62
ABLSL-2-B9-T7.ab1	-		_	0
ABLSL-2-C11-T7.ab1	triglyceride lipase	*Anguilla japonica*	BAB85636.1	42
ABLSL-2-C12-T7.ab1	-		_	0
ABLSL-2-C2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06540.1	95
ABLSL-2-C3-T7.ab1	Hypothetical protein LOC550513	*Danio rerio*	AAH92803.1	98
ABLSL-2-C4-T7.ab1	similar to notch1 preproprotein	*Strongylocentrotus purpuratus*	XP_789629.1	43
ABLSL-2-C5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF98815.1	81
ABLSL-2-C7-T7.ab1	stk19	*Danio rerio*	XP_691236.1	75
ABLSL-2-C9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06540.1	60
ABLSL-2-D10-T7.ab1	ribosomal protein L19	*Argopecten irradians*	AAN05588.1	67
ABLSL-2-D12-T7.ab1	similar to MGC97608 protein	*Apis mellifera*	XP_395079.2	46
ABLSL-2-D2-T7.ab1	-		_	0
ABLSL-2-D3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF95090.1	82
ABLSL-2-D4-T7.ab1	-		_	0
ABLSL-2-D5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG07447.1	92
ABLSL-2-D7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG04213.1	83
ABLSL-2-D9-T7.ab1	-		_	0
ABLSL-2-E1-T7.ab1	-		_	0
ABLSL-2-E11-T7.ab1	-		_	0
ABLSL-2-E12-T7.ab1	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	*Mus musculus*	NP_033005.1	31
ABLSL-2-E2-T7.ab1	heat shock cognate 70 kDa protein	*Pimephales promelas*	AAS46619.1	100
ABLSL-2-E3-T7.ab1	-		_	0
ABLSL-2-E4-T7.ab1	TCP1-beta	*Notothenia coriiceps*	AAX19463.1	89

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
ABLSL-2-E5-T7.ab1	-		_	0
ABLSL-2-E6-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG14264.1	86
ABLSL-2-E7-T7.ab1	similar to alpha 2 type VI collagen isoform 2C2a precursor	*Danio rerio*	XP_696164.1	36
ABLSL-2-E9-T7.ab1	HBAB_SERQU Hemoglobin alpha-B subunit (Hemoglobin alpha-B chain) (Alpha-B-globin)		68Q9PVM3	
ABLSL-2-F1-T7.ab1	-		_	0
ABLSL-2-F10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF96772.1	77
ABLSL-2-F11-T7.ab1	-		_	0
ABLSL-2-F2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG08351.1	59
ABLSL-2-F3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02411.1	58
ABLSL-2-F4-T7.ab1	-		_	0
ABLSL-2-F5-T7.ab1	-		_	0
ABLSL-2-F6-T7.ab1	GA18441-PA	*Drosophila pseudoobscura*	EAL29084.1	29
ABLSL-2-F7-T7.ab1	-		_	0
ABLSL-2-F9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG12186.1	59
ABLSL-2-G1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97838.1	70
ABLSL-2-G10-T7.ab1	hypothetical protein LOC550527	*Danio rerio*	NP_001017829.1	81
ABLSL-2-G12-T7.ab1	heat shock cognate 70 kDa protein	*Pimephales promelas*	AAS46619.1	98
ABLSL-2-G2-T7.ab1	hemoglobin beta-A chain	*Siniperca chuatsi*	AAY79276.1	87
ABLSL-2-G3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97070.1	50
ABLSL-2-G4-T7.ab1	similar to MGC86483 protein	*Danio rerio*	XP_689383.1	63
ABLSL-2-G5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97552.1	81
ABLSL-2-G6-T7.ab1	PDZ/coiled-coil domain binding partner for the rho-family GTPase TC10	*Danio rerio*	AAH56565.1	91
ABLSL-2-G7-T7.ab1	ribosomal protein L18	*Crassostrea gigas*	CAD91422.1	75
ABLSL-2-G8-T7.ab1	-		_	0
ABLSL-2-H1-T7.ab1	-		_	0
ABLSL-2-H10-T7.ab1	-		_	0
ABLSL-2-H11-T7.ab1	HBAB_SERQU Hemoglobin alpha-B subunit		66Q9PVM3	
ABLSL-2-H12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97070.1	50
ABLSL-2-H2-T7.ab1	-		_	0
ABLSL-2-H3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09847.1	63
ABLSL-2-H5-T7.ab1	cytochrome c oxidase subunit III	*Haliotis rubra*	YP_026066.1	83
ABLSL-2-H8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06475.1	57
ABLSL-2-H9-T7.ab1	Zgc:56053	*Danio rerio*	AAH45887.1	62
ABLSL-3-A10-T7.ab1	elastase 2-like protein	*Sparus aurata*	AAT45251.1	89
ABLSL-3-A11-T7.ab1	trypsinogen 1 precursor	*Pseudopleuronectes americanus*	AAC32751.1	43
ABLSL-3-A12-T7.ab1	-		_	0
ABLSL-3-A2-T7.ab1	40S ribosomal protein S5	*Pagrus major*	AAP20199.1	98
ABLSL-3-A3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10857.1	95

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABLSL-3-A4-T7.ab1	MEP1A protein	*Danio rerio*	NP_001025452.1	78
ABLSL-3-A5-T7.ab1	sp2 protein	*Ciona intestinalis*	NP_001027740.1	35
ABLSL-3-A7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09292.1	85
ABLSL-3-A8-T7.ab1	Surf4 protein	*Danio rerio*	AAH66509.1	81
ABLSL-3-A9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97758.1	81
ABLSL-3-B10-T7.ab1	GDP dissociation inhibitor 2	*Danio rerio*	AAQ91240.1	87
ABLSL-3-B11-T7.ab1	-		_	0
ABLSL-3-B12-T7.ab1	-		_	0
ABLSL-3-B2-T7.ab1	-		_	0
ABLSL-3-B3-T7.ab1	similar to Histone H3.3 isoform 5	*Bos taurus*	XP_888206.1	95
ABLSL-3-B4-T7.ab1	pancreatic protein with two somatomedin B domains	*Paralichthys olivaceus*	BAA88246.1	97
ABLSL-3-B5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06065.1	64
ABLSL-3-B6-T7.ab1	-		_	0
ABLSL-3-B7-T7.ab1	-		_	0
ABLSL-3-B8-T7.ab1	hypothetical protein LOC550606	*Danio rerio*	NP_001017907.1	70
ABLSL-3-B9-T7.ab1	-		_	0
ABLSL-3-C10-T7.ab1	-		_	0
ABLSL-3-C11-T7.ab1	suppressor of cytokine signaling 3	*Takifugu rubripes*	ABC73078.1	77
ABLSL-3-C12-T7.ab1	-		_	0
ABLSL-3-C2-T7.ab1	RPL27 protein	*Homo sapiens*	AAH21886.1	73
ABLSL-3-C4-T7.ab1	Degenerative spermatocyte homolog, lipid desaturase	*Danio rerio*	AAH53183.1	87
ABLSL-3-C7-T7.ab1	trypsinogen 1	*Paralichthys olivaceus*	BAA82362.1	96
ABLSL-3-D10-T7.ab1	-		_	0
ABLSL-3-D11-T7.ab1	-		_	0
ABLSL-3-D12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF94990.1	81
ABLSL-3-D2-T7.ab1	-		_	0
ABLSL-3-D3-T7.ab1	-		_	0
ABLSL-3-D4-T7.ab1	HBAA_SERQU Hemoglobin alpha-A subunit		73Q9PVM4	
ABLSL-3-D5-T7.ab1	-		_	0
ABLSL-3-D6-T7.ab1	similar to ribosomal protein L23	*Gallus gallus*	XP_418122.1	90
ABLSL-3-D7-T7.ab1	-		_	0
ABLSL-3-D9-T7.ab1	-		_	0
ABLSL-3-E1-T7.ab1	RPL18A protein	*Xenopus laevis*	AAH42256.1	73
ABLSL-3-E10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF96615.1	56
ABLSL-3-E11-T7.ab1	-		_	0
ABLSL-3-E12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG05204.1	73
ABLSL-3-E2-T7.ab1	phosphoinositide-3-kinase, regulatory subunit 5, p101	*Gallus gallus*	NP_001025868.1	55
ABLSL-3-E3-T7.ab1	cytochrome c oxidase subunit I	*Haliotis discus discus*	AAQ84300.1	80
ABLSL-3-E5-T7.ab1	procollagen C-proteinase enhancer protein	*Takifugu rubripes*	AAB94049.1	78

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABLSL-3-E6-T7.ab1	-		_	0
ABLSL-3-E7-T7.ab1	-		_	0
ABLSL-3-E9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03300.1	78
ABLSL-3-F1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF93955.1	58
ABLSL-3-F11-T7.ab1	type 1 collagen alpha 1	*Paralichthys olivaceus*	BAD77968.1	93
ABLSL-3-F12-T7.ab1	-		_	0
ABLSL-3-F2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF91199.1	66
ABLSL-3-F3-T7.ab1	-		_	0
ABLSL-3-F4-T7.ab1	-		_	0
ABLSL-3-F6-T7.ab1	bile salt-activated lipase	*Pseudopleuronectes americanus*	AAN63868.1	84
ABLSL-3-G10-T7.ab1	carboxypeptidase B	*Paralichthys olivaceus*	BAC53789.1	97
ABLSL-3-G11-T7.ab1	Unknown (protein for MGC:66050)	*Danio rerio*	AAH59804.1	74
ABLSL-3-G2-T7.ab1	-		_	0
ABLSL-3-G3-T7.ab1	-		_	0
ABLSL-3-G6-T7.ab1	-		_	0
ABLSL-3-G8-T7.ab1	-		_	0
ABLSL-3-H1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02842.1	83
ABLSL-3-H10-T7.ab1	similar to PYRIN-containing APAF1-like protein 7	*Danio rerio*	XP_689864.1	66
ABLSL-3-H11-T7.ab1	-		_	0
ABLSL-3-H12-T7.ab1	developmentally-regulated vdg3	*Haliotis asinina*	AAX11341.1	38
ABLSL-3-H2-T7.ab1	ribosomal protein L27	*Fundulus heteroclitus*	AAU50549.1	88
ABLSL-3-H3-T7.ab1	-		_	0
ABLSL-3-H4-T7.ab1	-		_	0
ABLSL-3-H5-T7.ab1	-		_	0
ABLSL-3-H6-T7.ab1	triglyceride lipase	*Anguilla japonica*	BAB85636.1	59
ABLSL-3-H7-T7.ab1	similar to CCCH zinc finger protein C3H-1	*Danio rerio*	XP_692984.1	32
ABLSL-3-H9-T7.ab1	Actin-related protein 10 homolog	*Danio rerio*	AAH45412.1	92
ABLSL-4-A11-T7.ab1	-		_	0
ABLSL-4-A12-T7.ab1	Thioredoxin interacting protein	*Danio rerio*	AAH49493.1	73
ABLSL-4-A2-T7.ab1	Hypothetical protein LOC450016	*Danio rerio*	AAH83378.1	92
ABLSL-4-A4-T7.ab1	cytochrome c oxidase subunit III	*Paralichthys olivaceus*	NP_037588.1	67
ABLSL-4-A5-T7.ab1	-		_	0
ABLSL-4-A6-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99511.1	97
ABLSL-4-A7-T7.ab1	-		_	0
ABLSL-4-B1-T7.ab1	-		_	0
ABLSL-4-B10-T7.ab1	-		_	0
ABLSL-4-B11-T7.ab1	Nuclear protein 1 (Protein p8) (Candidate of metastasis 1) isoform 1	*Bos taurus*	XP_872550.1	61
ABLSL-4-B12-T7.ab1	LOC495127 protein	*Xenopus laevis*	AAH92115.1	79
ABLSL-4-B4-T7.ab1	ferritin-like protein	*Pinctada fucata*	AAQ12076.1	78
ABLSL-4-B6-T7.ab1	ribosomal protein S24	*Ixodes scapularis*	AAY66904.1	62
ABLSL-4-B7-T7.ab1	unnamed protein product	*Haliotis rufescens*	CAA50572.1	92
ABLSL-4-B8-T7.ab1	-		_	0

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I[a]**
ABLSL-4-B9-T7.ab1	ubiquitin fusion degradation 1-like	*Danio rerio*	NP_001002451.1	90
ABLSL-4-C1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09552.1	81
ABLSL-4-C10-T7.ab1	hemoglobin beta-A chain	*Siniperca chuatsi*	AAY79276.1	88
ABLSL-4-C12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10891.1	90
ABLSL-4-C3-T7.ab1	thymosin beta 12	*perch, liver, Peptide, 43 aa*	AAB21004.1	65
ABLSL-4-C4-T7.ab1	-		_	0
ABLSL-4-C7-T7.ab1	MGC86316 protein	*Xenopus laevis*	AAH77634.1	84
ABLSL-4-C8-T7.ab1	-		_	0
ABLSL-4-C9-T7.ab1	40S ribosomal protein S3	*Pseudopleuronectes americanus*	AAT01919.1	95
ABLSL-4-D1-T7.ab1	-		_	0
ABLSL-4-D10-T7.ab1	tropomyoshin1-1	*Takifugu rubripes*	BAC57564.1	78
ABLSL-4-D12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09508.1	95
ABLSL-4-D2-T7.ab1	-		_	0
ABLSL-4-D4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09508.1	87
ABLSL-4-D5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG04219.1	92
ABLSL-4-D6-T7.ab1	-		_	0
ABLSL-4-D7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG08374.1	80
ABLSL-4-D8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02645.1	81
ABLSL-4-D9-T7.ab1	carboxypeptidase A1 (pancreatic)	*Gallus gallus*	NP_989915.1	65
ABLSL-4-E1-T7.ab1	-		_	0
ABLSL-4-E11-T7.ab1	bile salt-activated lipase	*Pseudopleuronectes americanus*	AAN63868.1	89
ABLSL-4-E2-T7.ab1	splicing factor 3b, subunit 1 isoform 1	*Canis familiaris*	XP_857064.1	90
ABLSL-4-E3-T7.ab1	-		_	0
ABLSL-4-E4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG07447.1	92
ABLSL-4-E5-T7.ab1	ribosomal protein L1	*Lonomia obliqua*	AAV91386.1	63
ABLSL-4-E7-T7.ab1	PAS1	*Takifugu rubripes*	AAC34392.1	72
ABLSL-4-E9-T7.ab1	KRP-A	*Aplysia californica*	CAA48558.1	83
ABLSL-4-F10-T7.ab1	-		_	0
ABLSL-4-F11-T7.ab1	-		_	0
ABLSL-4-F2-T7.ab1	MHC II invariant chain	*Siniperca chuatsi*	AAS77256.1	73
ABLSL-4-F3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF93208.1	60
ABLSL-4-F5-T7.ab1	serum lectin isoform 2	*Verasper variegatus*	BAE44114.1	59
ABLSL-4-F8-T7.ab1	-		_	0
ABLSL-4-F9-T7.ab1	-		_	0
ABLSL-4-G1-T7.ab1	-		_	0
ABLSL-4-G11-T7.ab1	-		_	0
ABLSL-4-G12-T7.ab1	-		_	0
ABLSL-4-G3-T7.ab1	-		_	0
ABLSL-4-G4-T7.ab1	elastase A precursor	*Gadus morhua*	AAB58351.1	77
ABLSL-4-G6-T7.ab1	-		_	0
ABLSL-4-G7-T7.ab1	-		_	0

Table 7. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I^a
ABLSL-4-G8-T7.ab1	-		_	0
ABLSL-4-G9-T7.ab1	Unknown (protein for MGC:112201)	*Danio rerio*	AAH93263.1	44
ABLSL-4-H12-T7.ab1	-		_	0
ABLSL-4-H2-T7.ab1	S26	*Suberites domuncula*	AAX48902.1	51
ABLSL-4-H3-T7.ab1	ribosomal protein L27	*Homo sapiens*	NP_000979.1	75
ABLSL-4-H4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99822.1	76
ABLSL-4-H7-T7.ab1	serum lectin isoform 2	*Verasper variegatus*	BAE44114.1	67
ABLSL-4-H8-T7.ab1	-		_	0
ABLSL5-A1-T7.ab1	-		_	0
ABLSL5-A11-T7.ab1	triglyceride lipase	*Anguilla japonica*	BAB85636.1	46
ABLSL5-A12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG12999.1	84
ABLSL5-A2-T7.ab1	chymotrypsinogen 2	*Paralichthys olivaceus*	BAA82366.1	93
ABLSL5-A3-T7.ab1	sodium potassium ATPase alpha subunit	*Rhabdosargus sarba*	AAT48993.1	84
ABLSL5-A4-T7.ab1	-		_	0
ABLSL5-A5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06274.1	86
ABLSL5-A6-T7.ab1	unnamed protein product	*Mus musculus*	BAE33678.1	95
ABLSL5-A7-T7.ab1	-		_	0
ABLSL5-A8-T7.ab1	perforin	*Fundulus heteroclitus*	AAU50521.1	67
ABLSL5-A9-T7.ab1	L-threonine dehydrogenase	*Danio rerio*	AAH63962.1	93
ABLSL5-B1-T7.ab1	Calrl protein	*Danio rerio*	AAH46906.1	69
ABLSL5-B10-T7.ab1	mediator of RNA polymerase II transcription, subunit 31 homolog	*Danio rerio*	NP_001002417.1	61
ABLSL5-B11-T7.ab1	perforin	*Fundulus heteroclitus*	AAU50521.1	74
ABLSL5-B12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02712.1	85
ABLSL5-B2-T7.ab1	trypsinogen 1	*Paralichthys olivaceus*	BAA82362.1	98
ABLSL5-B3-T7.ab1	-		_	0
ABLSL5-B4-T7.ab1	-		_	0
ABLSL5-B5-T7.ab1	-		_	0
ABLSL5-B6-T7.ab1	-		_	0
ABLSL5-B7-T7.ab1	similar to 40S ribosomal protein S29	*Mus musculus*	XP_910773.1	97
ABLSL5-B8-T7.ab1	60S ribosomal protein L13A	*Platichthys flesus*	CAH57701.1	66
ABLSL5-B9-T7.ab1	-		_	0
ABLSL5-C1-T7.ab1	-		_	0
ABLSL5-C10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99350.1	42
ABLSL5-C11-T7.ab1	cytochrome c oxidase subunit I	*Haliotis discus discus*	AAQ84300.1	63
ABLSL5-C12-T7.ab1	Hypothetical protein CBG01620	*Caenorhabditis briggsae*	CAE58478.1	31
ABLSL5-C2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF91666.1	76
ABLSL5-C3-T7.ab1	-		_	0
ABLSL5-C4-T7.ab1	-		_	0
ABLSL5-C5-T7.ab1	-		_	0
ABLSL5-C6-T7.ab1	unnamed protein product	*Mus musculus*	BAC31241.1	47
ABLSL5-C7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06274.1	88
ABLSL5-C8-T7.ab1	RNA helicase	*Takifugu rubripes*	AAB96360.1	96
ABLSL5-C9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99350.1	48
ABLSL5-D1-T7.ab1	-		_	0

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABLSL5-D10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06274.1	87
ABLSL5-D11-T7.ab1	similar to Lysyl oxidase homolog 2 precursor (Lysyl oxidase-like protein 2) (Lysyl oxidase related protein 2) (Lysyl oxidase-related protein WS9-14)	*Danio rerio*	XP_693880.1	72
ABLSL5-D2-T7.ab1	-		_	0
ABLSL5-D3-T7.ab1	-		_	0
ABLSL5-D4-T7.ab1	hypothetical protein	*Magnetospirillum gryphiswaldense*	CAJ30042.1	57
ABLSL5-D5-T7.ab1	-		_	0
ABLSL5-D6-T7.ab1	-		_	0
ABLSL5-D7-T7.ab1	cytochrome P450 1C2	*Stenotomus chrysops*	AAL78299.1	79
ABLSL5-D8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG01916.1	44
ABLSL5-D9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06540.1	74
ABLSL5-E1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF94413.1	65
ABLSL5-E10-T7.ab1	apolipoprotein E1	*Takifugu rubripes*	BAD83859.1	58
ABLSL5-E11-T7.ab1	H2A histone family, member V	*Gallus gallus*	NP_001026545.1	98
ABLSL5-E12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF95107.1	75
ABLSL5-E2-T7.ab1	-		_	0
ABLSL5-E4-T7.ab1	-		_	0
ABLSL5-E6-T7.ab1	-		_	0
ABLSL5-E7-T7.ab1	-		_	0
ABLSL5-E8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03215.1	93
ABLSL5-E9-T7.ab1	unknown	*Sparus aurata*	AAT45247.1	75
ABLSL5-F1-T7.ab1	hypothetical protein XP_697578	*Danio rerio*	XP_702670.1	73
ABLSL5-F10-T7.ab1	-		_	0
ABLSL5-F11-T7.ab1	Casein kinase 2 alpha 1	*Danio rerio*	AAH44403.1	80
ABLSL5-F12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG12450.1	76
ABLSL5-F2-T7.ab1	-		_	0
ABLSL5-F3-T7.ab1	-		_	0
ABLSL5-F4-T7.ab1	-		_	0
ABLSL5-F5-T7.ab1	-		_	0
ABLSL5-F6-T7.ab1	novel protein	*Mus musculus*	CAI24017.1	55
ABLSL5-F7-T7.ab1	developmentally-regulated vdg3	*Haliotis asinina*	AAX11341.1	68
ABLSL5-F8-T7.ab1	-		_	0
ABLSL5-F9-T7.ab1	Unknown (protein for MGC:83116)	*Xenopus laevis*	AAH68911.1	75
ABLSL5-G1-T7.ab1	-		_	0
ABLSL5-G10-T7.ab1	-		_	0
ABLSL5-G11-T7.ab1	-		_	0
ABLSL5-G12-T7.ab1	trypsinogen 2	*Paralichthys olivaceus*	BAA82363.1	93
ABLSL5-G2-T7.ab1	tubulin alpha 6	*Danio rerio*	AAQ94598.1	93
ABLSL5-G4-T7.ab1	unknown	*Sparus aurata*	AAT45249.1	46
ABLSL5-G5-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03362.1	73
ABLSL5-G6-T7.ab1	Hypothetical protein LOC406819	*Danio rerio*	AAH65893.1	66

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABLSL5-G7-T7.ab1	-		_	0
ABLSL5-G9-T7.ab1	-		_	0
ABLSL5-H10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF92165.1	48
ABLSL5-H11-T7.ab1	perforin	*Fundulus heteroclitus*	AAU50521.1	71
ABLSL5-H12-T7.ab1	NADH dehydrogenase subunit 4	*Paralichthys olivaceus*	NP_037591.1	86
ABLSL5-H2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02185.1	77
ABLSL5-H3-T7.ab1	-		_	0
ABLSL5-H4-T7.ab1	-		_	0
ABLSL5-H5-T7.ab1	-		_	0
ABLSL5-H6-T7.ab1	-		_	0
ABLSL5-H7-T7.ab1	-		_	0
ABLSL5-H8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF95281.1	100
ABLSL5-H9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10432.1	76
ABLSL6-A1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG12092.1	75
ABLSL6-A10-T7.ab1	-		_	0
ABLSL6-A11-T7.ab1	-		_	0
ABLSL6-A12-T7.ab1	trypsinogen 2	*Paralichthys olivaceus*	BAA82363.1	99
ABLSL6-A2-T7.ab1	complement component C4	*Takifugu rubripes*	CAD45003.1	65
ABLSL6-A3-T7.ab1	receptor for activated protein kinase C	*Paralichthys olivaceus*	AAT35603.1	84
ABLSL6-A4-T7.ab1	NADH dehydrogenase subunit 2	*Paralichthys olivaceus*	NP_037583.1	69
ABLSL6-A5-T7.ab1	LOC565117 protein	*Danio rerio*	AAH76049.1	64
ABLSL6-A6-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG11361.1	89
ABLSL6-A7-T7.ab1	ribosomal protein L17	*Paralichthys olivaceus*	AAF61071.1	98
ABLSL6-A8-T7.ab1	-		_	0
ABLSL6-A9-T7.ab1	-		_	0
ABLSL6-B1-T7.ab1	kelch-like ECH-associated protein 1	*Danio rerio*	NP_878284.1	73
ABLSL6-B10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG13267.1	96
ABLSL6-B11-T7.ab1	-		_	0
ABLSL6-B12-T7.ab1	-		_	0
ABLSL6-B2-T7.ab1	-		_	0
ABLSL6-B5-T7.ab1	trypsinogen 3	*Paralichthys olivaceus*	BAA82364.2	100
ABLSL6-B6-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF95073.1	79
ABLSL6-B8-T7.ab1	-		_	0
ABLSL6-B9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG01716.1	33
ABLSL6-C1-T7.ab1	-		_	0
ABLSL6-C11-T7.ab1	similar to RIKEN cDNA 2810457I06	*Danio rerio*	XP_686867.1	91
ABLSL6-C12-T7.ab1	ubiquitously transcribed tetratricopeptide repeat, X chromosome	*Homo sapiens*	CAI41479.1	77
ABLSL6-C2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF96616.1	84
ABLSL6-C3-T7.ab1	-		_	0
ABLSL6-C4-T7.ab1	-		_	0
ABLSL6-C6-T7.ab1	-		_	0
ABLSL6-C7-T7.ab1	-		_	0
ABLSL6-C8-T7.ab1	Eukaryotic translation elongation factor 2, like	*Danio rerio*	AAH45488.1	81
ABLSL6-C9-T7.ab1	-		_	0
ABLSL6-D10-T7.ab1	-		_	0

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
ABLSL6-D12-T7.ab1	Similar to tubulin, gamma 1	*Danio rerio*	AAH45486.1	96
ABLSL6-D2-T7.ab1	-		_	0
ABLSL6-D4-T7.ab1	-		_	0
ABLSL6-D6-T7.ab1	-		_	0
ABLSL6-D7-T7.ab1	-		_	0
ABLSL6-D8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06445.1	84
ABLSL6-D9-T7.ab1	antifreeze protein type IV	*Paralichthys olivaceus*	AAM46175.1	95
ABLSL6-E1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF90221.1	61
ABLSL6-E10-T7.ab1	-		_	0
ABLSL6-E11-T7.ab1	Hypothetical protein FLJ11749-like	*Danio rerio*	AAH95171.1	75
ABLSL6-E12-T7.ab1	-		_	0
ABLSL6-E2-T7.ab1	-		_	0
ABLSL6-E3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG06274.1	93
ABLSL6-E4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF95324.1	75
ABLSL6-E5-T7.ab1	-		_	0
ABLSL6-E6-T7.ab1	-		_	0
ABLSL6-E7-T7.ab1	antifreeze protein type IV	*Paralichthys olivaceus*	AAM46175.1	98
ABLSL6-E8-T7.ab1	chaperonin containing TCP-1 delta	*Takifugu rubripes*	NP_001027851.1	92
ABLSL6-E9-T7.ab1	-		_	0
ABLSL6-F10-T7.ab1	elastase 2 precursor	*Paralichthys olivaceus*	BAA82368.1	98
ABLSL6-F11-T7.ab1	beta actin	*Acanthopagrus schlegelii*	AAR84618.1	100
ABLSL6-F12-T7.ab1	-		_	0
ABLSL6-F3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG12384.1	54
ABLSL6-F4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10851.1	51
ABLSL6-F5-T7.ab1	-		_	0
ABLSL6-F6-T7.ab1	-		_	0
ABLSL6-F7-T7.ab1	-		_	0
ABLSL6-F8-T7.ab1	triglyceride lipase	*Anguilla japonica*	BAB85636.1	45
ABLSL6-F9-T7.ab1	apolipoprotein E1	*Takifugu rubripes*	BAD83859.1	71
ABLSL6-G1-T7.ab1	-		_	0
ABLSL6-G10-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG13267.1	84
ABLSL6-G11-T7.ab1	actin-related protein 2/3 complex	*Pagrus major*	AAP20158.1	83
ABLSL6-G12-T7.ab1	unnamed protein product	*Mus musculus*	BAC37136.1	97
ABLSL6-G3-T7.ab1	hypothetical protein XP_678391	*Danio rerio*	XP_683483.1	44
ABLSL6-G4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF89942.1	40
ABLSL6-G5-T7.ab1	-		_	0
ABLSL6-G8-T7.ab1	-		_	0
ABLSL6-H10-T7.ab1	-		_	0
ABLSL6-H11-T7.ab1	-		_	0
ABLSL6-H12-T7.ab1	-		_	0
ABLSL6-H2-T7.ab1	-		_	0
ABLSL6-H3-T7.ab1	trypsinogen 1	*Paralichthys olivaceus*	BAA82362.1	90
ABLSL6-H5-T7.ab1	-		_	0
ABLSL6-H6-T7.ab1	-		_	0

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
ABLSL6-H7-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF90567.1	72
ABLSL7-A1-T7.ab1	-		_	0
ABLSL7-A10-T7.ab1	-		_	0
ABLSL7-A11-T7.ab1	beta actin	*Acanthopagrus schlegelii*	AAR84618.1	100
ABLSL7-A12-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03728.1	77
ABLSL7-A2-T7.ab1	glutathione S-transferase alpha	*Pseudopleuronectes americanus*	AAO13011.1	56
ABLSL7-A3-T7.ab1	-		_	0
ABLSL7-A5-T7.ab1	-		_	0
ABLSL7-A6-T7.ab1	similar to cofactor required for Sp1 transcriptional activation, subunit 6, 77kDa, partial	*Danio rerio*	XP_695952.1	89
ABLSL7-A7-T7.ab1	-		_	0
ABLSL7-A8-T7.ab1	Hypothetical protein LOC406819	*Danio rerio*	AAH65893.1	77
ABLSL7-A9-T7.ab1	transglutaminase 1	*Danio rerio*	XP_694950.1	87
ABLSL7-B1-T7.ab1	similar to H3 histone, family 3B	*Bos taurus*	XP_869677.1	71
ABLSL7-B10-T7.ab1	-		_	0
ABLSL7-B11-T7.ab1	ornithine decarboxylase antizyme large isoform	*Paralichthys olivaceus*	AAP82035.1	98
ABLSL7-B3-T7.ab1	ENSANGP00000023518	*Anopheles gambiae str. PEST*	EAA45498.1	60
ABLSL7-B4-T7.ab1	-		_	0
ABLSL7-B5-T7.ab1	claudin 5	*Danio rerio*	NP_998439.1	64
ABLSL7-B6-T7.ab1	Beta-Ig-H3/fasciclin	*Polaromonas sp. JS666*	ZP_00505509.1	32
ABLSL7-B8-T7.ab1	similar to type 1 tumor necrosis factor receptor shedding aminopeptidase regulator, partial	*Bos taurus*	XP_592495.2	70
ABLSL7-B9-T7.ab1	-		_	0
ABLSL7-C10-T7.ab1	-		_	0
ABLSL7-C11-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG04362.1	53
ABLSL7-C12-T7.ab1	-		_	0
ABLSL7-C2-T7.ab1	-		_	0
ABLSL7-C3-T7.ab1	LOC553228 protein	*Danio rerio*	AAH93328.1	51
ABLSL7-C4-T7.ab1	Hypothetical protein FLJ11749-like	*Danio rerio*	AAH95171.1	75
ABLSL7-C5-T7.ab1	-		_	0
ABLSL7-C6-T7.ab1	-		_	0
ABLSL7-C7-T7.ab1	-		_	0
ABLSL7-C8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG02279.1	39
ABLSL7-C9-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF92165.1	90
ABLSL7-D1-T7.ab1	-		_	0
ABLSL7-D10-T7.ab1	-		_	0
ABLSL7-D11-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF89831.1	71
ABLSL7-D12-T7.ab1	-		_	0
ABLSL7-D2-T7.ab1	carboxypeptidase B	*Paralichthys olivaceus*	BAC53789.1	99

Table 7. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
ABLSL7-D3-T7.ab1	-		_	0
ABLSL7-D4-T7.ab1	-		_	0
ABLSL7-D5-T7.ab1	-		_	0
ABLSL7-D6-T7.ab1	-		_	0
ABLSL7-E1-T7.ab1	-		_	0
ABLSL7-E10-T7.ab1	-		_	0
ABLSL7-E11-T7.ab1	elastase 4 precursor	*Paralichthys olivaceus*	BAA82370.1	99
ABLSL7-E12-T7.ab1	-		_	0
ABLSL7-E2-T7.ab1	-		_	0
ABLSL7-E3-T7.ab1	unknown protein	*Arabidopsis thaliana*	BAC42245.1	63
ABLSL7-E4-T7.ab1	-		_	0
ABLSL7-E5-T7.ab1	similar to procollagen, type XVI, alpha 1	*Danio rerio*	XP_684157.1	75
ABLSL7-E6-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10864.1	89
ABLSL7-E7-T7.ab1	apolipoprotein B	*Herpestes javanicus*	AAY18256.1	34
ABLSL7-E8-T7.ab1	-		_	0
ABLSL7-E9-T7.ab1	-		_	0
ABLSL7-F1-T7.ab1	heat shock protein 90 beta	*Paralichthys olivaceus*	AAO92751.1	90
ABLSL7-F10-T7.ab1	-		_	0
ABLSL7-F11-T7.ab1	-		_	0
ABLSL7-F12-T7.ab1	similar to protein phosphatase 1 (formerly 2C)-like	*Danio rerio*	XP_685040.1	65
ABLSL7-F2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG00997.1	67
ABLSL7-F3-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF94246.1	71
ABLSL7-F4-T7.ab1	-		_	0
ABLSL7-F6-T7.ab1	-		_	0
ABLSL7-F7-T7.ab1	-		_	0
ABLSL7-F8-T7.ab1	-		_	0
ABLSL7-F9-T7.ab1	-		_	0
ABLSL7-G1-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG01849.1	70
ABLSL7-G11-T7.ab1	similar to Protein UNQ6350/PRO21055 homolog precursor	*Canis familiaris*	XP_546575.1	51
ABLSL7-G12-T7.ab1	-		_	0
ABLSL7-G2-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF99203.1	84
ABLSL7-G3-T7.ab1	-		_	0
ABLSL7-G4-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF90729.1	53
ABLSL7-G5-T7.ab1	Zgc:56053	*Danio rerio*	AAH45887.1	54
ABLSL7-G6-T7.ab1	Zgc:56053	*Danio rerio*	AAH45887.1	50
ABLSL7-G7-T7.ab1	intestinal fatty acid-binding protein	*Danio rerio*	AAO16213.1	65
ABLSL7-G8-T7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG08259.1	89
ABLSL7-G9-T7.ab1	MGC80804 protein	*Xenopus laevis*	AAH73375.1	97
ABLSL7-H1-T7.ab1	-		_	0
ABLSL7-H10-T7.ab1	carboxypeptidase A1	*Paralichthys olivaceus*	BAC53788.1	100
ABLSL7-H12-T7.ab1	-		_	0
ABLSL7-H2-T7.ab1	mitochondrial citrate synthase precursor	*Katsuwonus pelamis*	AAR98860.1	93
ABLSL7-H3-T7.ab1	-		_	0

Table 7. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**^{a}
ABLSL7-H4-T7.ab1	EF1A_ORYLA Elongation factor 1-alpha aa(EF-1-alpha)		90Q9YIC0	
ABLSL7-H5-T7.ab1	-		_	0
ABLSL7-H6-T7.ab1	type 1 collagen alpha 1	*Paralichthys olivaceus*	BAD77968.1	93
ABLSL7-H7-T7.ab1	peroxisome proliferator-activated receptor beta	*Pleuronectes platessa*	CAD62448.1	96
ABLSL7-H8-T7.ab1	-		_	0
ABLSL7-H9-T7.ab1	chymotrypsinogen 2	*Paralichthys olivaceus*	BAA82366.1	92

[a] Identity(%)

Table 8. List of identified ESTs from the hyposalinity abalone subtracted cDNA library

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
LSASL-2_A02_02.ab1	-		_	0
LSASL-2_A03_01.ab1	calcium binding protein 1	*Biomphalaria glabrata*	AAV91525.1	26
LSASL-2_A04_02.ab1	actin	*Onychoteuthis compacta*	AAF81170.1	97
LSASL-2_A05_01.ab1	G-protein-coupled receptor induced protein GIG2	*Rattus norvegicus*	AAG35664.1	67
LSASL-2_A06_02.ab1	RL44_PICJA 60S ribosomal protein L44 (60S ribosomal protein L41)		85P52809	
LSASL-2_A07_01.ab1	ribosomal protein L10a isoform B	*Lysiphlebus testaceipes*	AAX62471.1	62
LSASL-2_A09_01.ab1	putative ATPase	*Monkeypox virus*	AAY97545.1	31
LSASL-2_A10_02.ab1	2 (zwei) ig-domain protein protein 4	*Caenorhabditis elegans*	AAA68768.1	33
LSASL-2_A11_01.ab1	similar to WD repeat domain 36	*Strongylocentrotus purpuratus*	XP_780489.1	48
LSASL-2_A12_02.ab1	BAGS_BOMMO BAG domain-containing protein Samui		35Q9BLJ6	
LSASL-2_B02_04.ab1	ribosomal protein L10	*Branchiostoma belcheri tsingtaunese*	AAO31769.1	90
LSASL-2_B04_04.ab1	cI repressor	*Bacteriophage EJ-1*	NP_945243.1	31
LSASL-2_B05_03.ab1	envelope glycoprotein	*Human immunodeficiency virus 1*	AAP60103.1	41
LSASL-2_B06_04.ab1	similar to Coagulation factor VII (Serum prothrombin conversion accelerator)	*Pan troglodytes*	XP_509745.1	48
LSASL-2_B07_03.ab1	ribosomal protein P2, putative	*Cryptococcus neoformans var. neoformans JEC21*	AAW44232.1	34
LSASL-2_B08_04.ab1	variant-specific surface protein TSA417	*Giardia intestinalis*	AAD05040.1	25
LSASL-2_B10_04.ab1	-		_	0
LSASL-2_B11_03.ab1	-		_	0
LSASL-2_B12_04.ab1	-		_	0
LSASL-2_C01_05.ab1	ETS-family transcription factor	*Chlamys farreri*	AAU11487.2	55
LSASL-2_C02_06.ab1	CCAAT/enhancer binding protein	*Aplysia kurodai*	AAG61258.1	44
LSASL-2_C03_05.ab1	ribosomal protein L5	*Argopecten irradians*	AAN05603.1	79
LSASL-2_C04_06.ab1	inhibitor of DNA binding 4	*Rattus norvegicus*	NP_783172.1	46
LSASL-2_C06_06.ab1	a-agglutinin anchorage subunit	*Giardia lamblia ATCC 50803*	XP_767315.1	42
LSASL-2_C07_05.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG03416.1	38
LSASL-2_C08_06.ab1	LD46584p	*Drosophila melanogaster*	AAL68241.1	77
LSASL-2_C10_06.ab1	similar to golgi membrane protein SB140 isoform 1	*Strongylocentrotus purpuratus*	XP_780638.1	87

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**^{a}
LSASL-2_C12_06.ab1	baseplate wedge subunit	*Enterobacteria phage RB49*	NP_891710.1	36
LSASL-2_D02_08.ab1	similar to Calcium-binding protein 2 (CaBP2)	*Canis familiaris*	XP_854627.1	34
LSASL-2_D03_07.ab1	dipeptidase, putative	*Chlamydophila pneumoniae AR39*	AAF38320.1	32
LSASL-2_D04_08.ab1	similar to amylase 1, salivary	*Strongylocentrotus purpuratus*	XP_782094.1	28
LSASL-2_D05_07.ab1	complement component 2	*Homo sapiens*	CAI17453.1	39
LSASL-2_D06_08.ab1	polymerase	*Lymphocytic choriomeningitis virus*	AAR15899.1	40
LSASL-2_D07_07.ab1	Chaperonin Containing TCP-1 family member (cct-5)	*Caenorhabditis elegans*	NP_497915.3	44
LSASL-2_D08_08.ab1	actin	*Ommastrephes bartramii*	AAF81166.1	93
LSASL-2_D10_d10_08.ab1	similar to RIKEN cDNA A330021E22	*Gallus gallus*	XP_418644.1	42
LSASL-2_D11__07.ab1	type X collagen		AAA48736.1	65
LSASL-2_D12_08.ab1	polymerase (RNA) II (DNA directed) polypeptide F	*Gallus gallus*	NP_990065.1	77
LSASL-2_E02_10.ab1	novel hemicentin protein	*Danio rerio*	CAI11663.1	48
LSASL-2_E03_09.ab1	Elongation FacTor family member (eft-2)	*Caenorhabditis elegans*	NP_492457.1	76
LSASL-2_E04_10.ab1	regulatory protein, LuxR:Response regulator receiver	*Alkaliphilus metalliredigenes QYMF*	ZP_00799383.1	34
LSASL-2_E05_09.ab1	hypothetical protein	*Wolinella succinogenes*	CAE10266.1	33
LSASL-2_E06_10.ab1	RPL15 protein	*Homo sapiens*	AAH81565.1	91
LSASL-2_E09_09.ab1	actin	*Scyliorhinus torazame*	AAG22088.1	73
LSASL-2_E10_10.ab1	Glycosyl transferase, family 2:Glycosyl transferase, group 1	*Trichodesmium erythraeum IMS101*	ZP_00675068.1	40
LSASL-2_E11_09.ab1	-		_	0
LSASL-2_E12_10.ab1	similar to cytosolic sialic acid 9-O-acetylesterase homolog, partial	*Strongylocentrotus purpuratus*	XP_789419.1	30
LSASL-2_F01_11.ab1	-		_	0
LSASL-2_F03_11.ab1	similar to 40S ribosomal protein S28	*Mus musculus*	XP_914732.1	68
LSASL-2_F04_12.ab1	similar to Lysosomal alpha-mannosidase precursor (Mannosidase, alpha B) (Lysosomal acid alpha-mannosidase) (Laman) (Mannosidase alpha class 2B member 1)	*Strongylocentrotus purpuratus*	XP_795985.1	40
LSASL-2_F05_11.ab1	similar to CG5742-PA	*Strongylocentrotus purpuratus*	XP_791617.1	24
LSASL-2_F06_12.ab1	ATP synthase beta subunit	*Pinctada fucata*	ABC86835.1	28
LSASL-2_F07_11.ab1	conserved Theileria-specific sub-telomeric protein, SVSP family, putative	*Theileria annulata*	CAI74978.1	31

Table 8. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I^a
LSASL-2_F09_11.ab1	hypothetical protein Tb10.100.0140	*Trypanosoma brucei TREU927*	XP_822285.1	37
LSASL-2_F10_12.ab1	ribosomal protein S8	*Argopecten irradians*	AAN05595.1	76
LSASL-2_F12_12.ab1	similar to deleted in malignant brain tumors 1 isoform c precursor	*Pan troglodytes*	XP_521642.1	31
LSASL-2_G01_13.ab1	adaptive-response sensory histidine kinase SasA	*Cyanobacteria bacterium Yellowstone A-Prime*	YP_474113.1	37
LSASL-2_G03_13.ab1	beta-tubulin	*Halichondria sp. AR-2003*	AAP49554.1	97
LSASL-2_G05_13.ab1	similar to CG4785-PA	*Strongylocentrotus purpuratus*	XP_795990.1	51
LSASL-2_G06_14.ab1	Eukaryotic translation initiation factor 3, subunit 8	*Danio rerio*	AAH57465.1	72
LSASL-2_G07_13.ab1	-		_	0
LSASL-2_G08_14.ab1	ENSANGP00000025543	*Anopheles gambiae str. PEST*	EAL41105.1	44
LSASL-2_G09_13.ab1	transcriptional regulator, ArsR family	*Rhodobacter sphaeroides 2.4.1*	ABA80224.1	34
LSASL-2_G11_13.ab1	similar to Ubiquitin carboxyl-terminal hydrolase 7 (Ubiquitin thiolesterase 7) (Ubiquitin-specific processing protease 7) (Deubiquitinating enzyme 7) (Herpesvirus associated ubiquitin-specific protease)	*Apis mellifera*	XP_392848.2	56
LSASL-2_G12_14.ab1	serine/threonine-specific protein kinase -like	*Oryza sativa (japonica cultivar-group)*	XP_550053.1	35
LSASL-2_H02_16.ab1	similar to sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	*Canis familiaris*	XP_532030.2	43
LSASL-2_H03_15.ab1	odorant receptor	*Danio rerio*	ABC43377.1	31
LSASL-2_H04_16.ab1	MGC68600 protein	*Xenopus laevis*	AAH56661.1	43
LSASL-2_H06_16.ab1	similar to Sulfatase modifying factor 2 precursor (C-alpha-formyglycine-generating enzyme 2), partial	*Strongylocentrotus purpuratus*	XP_789318.1	58
LSASL-2_H08_16.ab1	-		_	0
LSASL-2_H10_16.ab1	-		_	0
LSASL-2_H11_15.ab1	cellulase	*Haliotis discus*	BAC67186.1	55
LSASL-1_A01_01.ab1	vitellogenin	*Poecilia reticulata*	AAN05434.1	35
LSASL-1_A02_02.ab1	polymerase (RNA) II (DNA directed) polypeptide F	*Gallus gallus*	NP_990065.1	76
LSASL-1_A03_01.ab1	thioester-containing protein	*Euphaedusa tau*	BAE44110.1	43
LSASL-1_A05_01.ab1	homeobox protein	*Homo sapiens*	CAB86198.1	39
LSASL-1_A06_02.ab1	ribosomal protein L19	*Argopecten irradians*	AAN05588.1	97
LSASL-1_A07_01.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF97630.1	78

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
LSASL-1_A08_02.ab1	unknown protein	*Arabidopsis thaliana*	NP_187126.1	32
LSASL-1_A10_02.ab1	alginate lyase	*Haliotis discus hannai*	BAC87758.1	80
LSASL-1_A11_01.ab1	RPL18A protein	*Xenopus laevis*	AAH42256.1	73
LSASL-1_A12_02.ab1	PTPL1-associated RhoGAP 1	*Pan troglodytes*	XP_513574.1	37
LSASL-1_B01_03.ab1	-		_	0
LSASL-1_B02_04.ab1	hypothetical protein Noc_0748	*Nitrosococcus oceani ATCC 19707*	ABA57261.1	41
LSASL-1_B03_03.ab1	fructose-biphosphate aldolase	*Biomphalaria glabrata*	AAZ39527.1	77
LSASL-1_B04_04.ab1	6-phospho-alpha-glucosidase (maltose-6'-phosphate hydrolase)	*Lactobacillus johnsonii NCC 533*	NP_965620.1	29
LSASL-1_B05_03.ab1	RC159	*Ruegeria sp. PR1b*	AAN05232.1	35
LSASL-1abalone1-050716_B06_b6_04.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG09202.1	48
LSASL-1_B07_03.ab1	bifunctional dihydrofolate reductase-thymidylate synthase	*Plasmodium falciparum 3D7*	NP_702821.1	30
LSASL-1_B08_04.ab1	hypothetical protein Bcep18194_A5494	*Burkholderia sp. 383*	ABB09088.1	65
LSASL-1_B09_03.ab1	fertilization protein		AAC37229.1	46
LSASL-1_B10_04.ab1	RE74713p	*Drosophila melanogaster*	AAV36859.1	36
LSASL-1_B11_03.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG10456.1	74
LSASL-1_B12_04.ab1	similar to cubilin, partial	*Strongylocentrotus purpuratus*	XP_784754.1	31
LSASL-1_C01_05.ab1	hypothetical protein	*Homo sapiens*	CAB63768.1	37
LSASL-1_C02_06.ab1	-		_	0
LSASL-1_C03_05.ab1	similar to Protein C20orf29	*Rattus norvegicus*	XP_575239.1	53
LSASL-1_C05_05.ab1	-		_	0
LSASL-1_C06_06.ab1	Pancreatic lipase-related protein 2	*Mus musculus*	AAH94923.1	53
LSASL-1_C07_05.ab1	-		_	0
LSASL-1_C08_06.ab1	ferritin	*Branchiostoma belcheri tsingtaunese*	AAQ21039.1	67
LSASL-1_C10_06.ab1	ribosomal protein S10	*Branchiostoma belcheri*	AAN52385.1	73
LSASL-1_C11_05.ab1	similar to ribosomal protein L22	*Apis mellifera*	XP_625009.1	70
LSASL-1_D01_07.ab1	-		_	0
LSASL-1_D02_08.ab1	hypothetical protein MGG_ch7g424	*Magnaporthe grisea 70-15*	EAQ71017.1	44
LSASL-1_D03_07.ab1	ENSANGP00000011058	*Anopheles gambiae str. PEST*	EAA07595.2	71
LSASL-1_D04_08.ab1	-		_	0
LSASL-1_D055_07.ab1	-		_	0
LSASL-1_D06_08.ab1	Hypothetical protein CBG19221	*Caenorhabditis briggsae*	CAE72125.1	28
LSASL-1_D07_07.ab1	agmatinase, putative	*Pseudomonas fluorescens Pf-5*	AAY90871.1	60
LSASL-1_D08_08.ab1	unnamed protein product	*Homo sapiens*	BAC87052.1	33

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I^a**
LSASL-1_D09_07.ab1	Y48C3A.4	*Caenorhabditis elegans*	NP_496811.1	32
LSASL-1_D10_08.ab1	40S ribosomal protein S9	*Ictalurus punctatus*	AAK95191.1	87
LSASL-1_D11_07.ab1	-		_	0
LSASL-1_D12_08.ab1	-		_	0
LSASL-1_E01_09.ab1	Myc homolog	*Crassostrea virginica*	AAB34577.1	40
LSASL-1_E02_10.ab1	-		_	0
LSASL-1_E03_09.ab1	similar to methyltransferase Cyt19	*Gallus gallus*	XP_421735.1	57
LSASL-1_E04_10.ab1	MyoD	*Branchiostoma belcheri tsingtaunese*	AAR12640.1	60
LSASL-1_E05_09.ab1	Citrate synthase	*Nitrococcus mobilis Nb-231*	ZP_01126607.1	29
LSASL-1_E06_10.ab1	Hypothetical protein CBG01620	*Caenorhabditis briggsae*	CAE58478.1	32
LSASL-1_E08_10.ab1	alcohol dehydrogenase class 3	*Branchiostoma lanceolatum*	AAF73255.1	86
LSASL-1_E10_10.ab1	similar to casein kinase 2, beta subunit	*Canis familiaris*	XP_532075.2	33
LSASL-1_E11_09.ab1	-		_	0
LSASL-1_E12_10.ab1	similar to Thyrotropin-releasing hormone degrading ectoenzyme (TRH-degrading ectoenzyme) (TRH-DE) (TRH-specific aminopeptidase) (Thyroliberinase) (Pyroglutamyl-peptidase II) (PAP-II)	*Danio rerio*	XP_691936.1	37
LSASL-1_F01_11.ab1	-		_	0
LSASL-1_F04_12.ab1	MGC64389 protein	*Xenopus laevis*	AAH53792.1	30
LSASL-1_F05_11.ab1	similar to Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit precursor (Ribophorin I) (RPN-I)	*Gallus gallus*	XP_414360.1	79
LSASL-1_F06_12.ab1	CG32644-PB	*Drosophila melanogaster*	NP_727652.1	38
LSASL-1_F07_11.ab1	-		_	0
LSASL-1_F08_12.ab1	-		_	0
LSASL-1_F09_11.ab1	actin	*Haliotis discus hannai*	AAQ92368.1	100
LSASL-1_F11_11.ab1	hypothetical protein	*Plasmodium berghei strain ANKA*	XP_679773.1	35
LSASL-1_F12_12.ab1	NADH dehydrogenase subunit F	*Symphoricarpos orbiculatus*	AAM28656.1	36
LSASL-1_G01_13.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAG01270.1	64
LSASL-1_G02_14.ab1	inhibitor of apoptosis protein	*Trichoplusia ni*	AAF19819.1	40
LSASL-1_G03_13.ab1	similar to	*Danio rerio*	XP_684696.1	37
LSASL-1_G04_14.ab1	ribosomal protein S9	*Rattus norvegicus*	NP_112370.1	88
LSASL-1_G05_13.ab1	developmentally-regulated vdg3	*Haliotis asinina*	AAX11341.1	66

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
LSASL-1_G06_14.ab1	CYT_CYPCA Cystatin precursor (Ovarian cystatin) (P12)		41P35481	
LSASL-1_G07_13.ab1	heat shock protein 70	*Culex pipiens*	AAX84696.1	76
LSASL-1_G08_14.ab1	metalloprotease-disintegrin	*Gallus gallus*	XP_418639.1	37
LSASL-1_G10_14.ab1	unnamed protein product	*Mus musculus*	BAE40001.1	73
LSASL-1_G11_13.ab1	-		_	0
LSASL-1_G12_14.ab1	-		_	0
LSASL-1_H02_16.ab1	phytocyanin protein, PUP2	*Arabidopsis thaliana*	CAD66637.1	35
LSASL-1_H04_16.ab1	heat shock protein 70	*Manduca sexta*	AAO65964.1	57
LSASL-1_H05_15.ab1	actin	*Stylophora pistillata*	AAR13014.1	94
LSASL-1_H0616.ab1	UDP-N-acetylglucosamine pyrophosphorylase	*Shewanella sp. PV-4*	ZP_00837958.1	25
LSASL-1_H08_16.ab1	thioester-containing protein	*Euphaedusa tau*	BAE44110.1	60
LSASL-1_H09_15.ab1	unnamed protein product	*Xenopus laevis*	CAA62212.1	45
LSASL-1_H11_15.ab1	cytochrome b	*Haliotis tuberculata*	AAX35438.1	86
LSASL-5-A1-T3.ab1	HYPOTHETICAL TRANSMEMBRANE PROTEIN	*Ralstonia solanacearum*	CAD17006.1	33
LSASL-5-A10-T3.ab1	Cytochrome oxidase assembly	*Chloroflexus aurantiacus J-10-fl*	ZP_00767116.1	35
LSASL-5-A11-T3.ab1	hypothetical protein XP_778835	*Strongylocentrotus purpuratus*	XP_783928.1	32
LSASL-5-A12-T3.ab1	ferritin	*Branchiostoma belcheri tsingtaunese*	AAQ21039.1	71
LSASL-5-A3-T3.ab1	hypothetical protein PTD2_08194	*Pseudoalteromonas tunicata D2*	ZP_01133610.1	35
LSASL-5-A4-T3.ab1	disulfide isomerase	*Ostertagia ostertagi*	CAD11865.1	76
LSASL-5-A5-T3.ab1	vitelline envelope sperm lysin receptor	*Haliotis rufescens*	AAL50827.1	46
LSASL-5-A8-T3.ab1	eosinophil chemotactic cytokine	*Gallus gallus*	NP_989760.1	42
LSASL-5-B1-T3.ab1	Tubulin, alpha 1	*Mus musculus*	AAH83344.1	92
LSASL-5-B10-T3.ab1	fructose-biphosphate aldolase	*Biomphalaria glabrata*	AAZ39527.1	83
LSASL-5-B11-T3.ab1	hypothetical protein Chro.30218	*Cryptosporidium hominis TU502*	XP_668663.1	27
LSASL-5-B3-T3.ab1	chitinase	*Aedes aegypti*	AAZ39947.1	33
LSASL-5-B4-T3.ab1	microneme protein 4	*Eimeria tenella*	CAC34726.1	27
LSASL-5-B5-T3.ab1	-		_	0
LSASL-5-B7-T3.ab1	Hdlbp-prov protein	*Xenopus laevis*	AAH44314.1	43
LSASL-5-B9-T3.ab1	actin	*Ommastrephes bartramii*	AAF81166.1	98
LSASL-5-C10-T3.ab1	-		_	0
LSASL-5-C11-T3.ab1	ubiquitin	*Biomphalaria glabrata*	AAG49540.1	98
LSASL-5-C12-T3.ab1	CoA enzyme activase	*Desulfitobacterium hafniense DCB-2*	ZP_00557506.1	31
LSASL-5-C4-T3.ab1	ENSANGP00000016526, partial	*Apis mellifera*	XP_623859.1	33
LSASL-5-C5-T3.ab1	tropomyosin	*Crassostrea gigas*	AAK96889.1	90
LSASL-5-C9-T3.ab1	similar to ENSANGP00000007148	*Apis mellifera*	XP_392871.2	54

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
LSASL-5-D10-T3.ab1	proteasome subunit Y	*Lethenteron japonicum*	BAA19761.1	72
LSASL-5-D11-T3.ab1	hypothetical protein XP_868675	*Bos taurus*	XP_873768.1	34
LSASL-5-D12-T3.ab1	Rps9 protein	*Mus musculus*	AAH12491.1	90
LSASL-5-D2-T3.ab1	similar to BTG2 protein (NGF-inducible protein TIS21)	*Bos taurus*	XP_586687.2	58
LSASL-5-D3-T3.ab1	hypothetical protein Afu5g06460	*Aspergillus fumigatus Af293*	XP_753987.1	21
LSASL-5-D8-T3.ab1	smooth muscle alpha actin	*Xenopus laevis*	AAX85448.1	83
LSASL-5-D9-T3.ab1	hypothetical protein, unknown function	*Leishmania major*	CAJ02124.1	27
LSASL-5-E10-T3.ab1	paramyosin protein	*Crassostrea gigas*	CAD79338.1	70
LSASL-5-E11-T3.ab1	MANA_MYTED Mannan endo-1,4-beta-mannosidase precursor (Beta-mannanase) (Endo-beta-1,4-mannanase) (ManA)		51Q8WPJ2	
LSASL-5-E3-T3.ab1	-		_	0
LSASL-5-E4-T3.ab1	similar to cGMP-dependent protein kinase 2 (CGK 2) (cGKII) (Type II cGMP-dependent protein kinase)	*Danio rerio*	XP_686609.1	33
LSASL-5-E5-T3.ab1	ferritin GF2	*Crassostrea gigas*	AAP83794.1	81
LSASL-5-E6-T3.ab1	conserved hypothetical protein	*Symbiobacterium thermophilum IAM 14863*	BAD39566.1	36
LSASL-5-E7-T3.ab1	Tubulin, alpha 1	*Mus musculus*	AAH83344.1	97
LSASL-5-F1-T3.ab1	-		_	0
LSASL-5-F11-T3.ab1	similar to heat shock 70kD protein 1B	*Strongylocentrotus purpuratus*	XP_780151.1	84
LSASL-5-F4-T3.ab1	ENSANGP00000001678	*Anopheles gambiae str. PEST*	EAA06914.2	42
LSASL-5-F8-T3.ab1	beta-1,4-endoglucanase	*Biomphalaria glabrata*	AAT76428.1	60
LSASL-5-F9-T3.ab1	dissimilatory (bi-)sulfite reductase alpha subunit	*uncultured sulfate-reducing bacterium*	AAK61938.1	41
LSASL-5-G10-T3.ab1	ACT_CALFI Actin		Q92192	96
LSASL-5-G11-T3.ab1	KARG_NORMA Arginine kinase (AK)		93P51544	
LSASL-5-G12-T3.ab1	662aa long hypothetical acetyl-CoA synthetase	*Sulfolobus tokodaii str. 7*	BAB65737.1	38
LSASL-5-G3-T3.ab1	alginate lyase	*Haliotis discus hannai*	BAC87758.1	100
LSASL-5-G4-T3.ab1	cytoplasmic heat shock protein 70	*Ostrea edulis*	AAM46635.1	78
LSASL-5-G5-T3.ab1	ribosomal protein L31	*Crassostrea gigas*	CAD91431.1	85
LSASL-5-G6-T3.ab1	-		_	0
LSASL-5-G8-T3.ab1	GTP-binding nuclear protein RAN1	*Chironomus tentans*	CAE55862.1	70
LSASL-5-G9-T3.ab1	hypothetical protein	*Plasmodium falciparum 3D7*	NP_704653.1	33

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**^{a}
LSASL-5-H12-T3.ab1	hillarin	*Hirudo medicinalis*	AAK49949.1	37
LSASL-5-H2-T3.ab1	ribosomal protein L3 variant 1	*Lysiphlebus testaceipes*	AAX62422.1	70
LSASL-5-H3-T3.ab1	retinitis pigmentosa GTPase regulator	*Homo sapiens*	AAG00551.1	36
LSASL-5-H4-T3.ab1	similar to CG10687-PA	*Apis mellifera*	XP_623490.1	91
LSASL-5-H5-T3.ab1	70kDa heat shock protein	*Crassostrea gigas*	BAD15286.1	88
LSASL-5-H6-T3.ab1	actin	*Pyrocystis lunula*	AAO14682.1	85
LSASL-5-H7-T3.ab1	-		_	0
LSASL-5-H9-T3.ab1	cytochrome c oxidase subunit III	*Haliotis rubra*	YP_026066.1	83
LSASL3-A-1.ab1	-		_	0
LSASL3-A-10.ab1	hypothetical protein XP_775808	*Strongylocentrotus purpuratus*	XP_780901.1	65
LSASL3-A-11.ab1	ribosomal protein L19	*Argopecten irradians*	AAN05588.1	51
LSASL3-A-12.ab1	-		_	0
LSASL3-A-2.ab1	RH08259p	*Drosophila melanogaster*	AAM29564.1	37
LSASL3-A-3.ab1	similar to predicted CDS, reverse transcriptase family member (1O840)	*Strongylocentrotus purpuratus*	XP_795356.1	40
LSASL3-A-4.ab1	Myc homolog	*Crassostrea virginica*	AAB34577.1	59
LSASL3-A-5.ab1	vitelline coat protein 42	*Tegula pfeifferi*	BAB15930.1	44
LSASL3-A-6.ab1	F54D5.3	*Caenorhabditis elegans*	NP_496473.1	35
LSASL3-A-7.ab1	tubulin	*Crassostrea gigas*	BAD88768.1	98
LSASL3-B-1.ab1	Hypothetical protein LOC554120	*Danio rerio*	AAH95631.1	64
LSASL3-B-10.ab1	OSJNBa0093F16.20	*Oryza sativa (japonica cultivar-group)*	NP_914878.1	33
LSASL3-B-2.ab1	ribosomal protein S24	*Ixodes scapularis*	AAY66904.1	62
LSASL3-B-3.ab1	hypothetical protein CHGG_01091	*Chaetomium globosum CBS 148.51*	EAQ92856.1	44
LSASL3-B-6.ab1	similar to ENSANGP00000003616	*Apis mellifera*	XP_624843.1	45
LSASL3-B-7.ab1	similar to hemicentin 1, partial	*Strongylocentrotus purpuratus*	XP_789045.1	43
LSASL3-C-1.ab1	probable phage resistance protein	*Hahella chejuensis KCTC 2396*	YP_434638.1	32
LSASL3-C-2.ab1	hypothetical protein DDB0185347	*Dictyostelium discoideum*	XP_639223.1	43
LSASL3-C-3.ab1	bicoid	*Drosophila affinis*	AAX13021.1	27
LSASL3-C-4.ab1	-		_	0
LSASL3-C-6.ab1	paramyosin protein	*Crassostrea gigas*	CAD79338.1	83
LSASL3-C-8.ab1	ribosomal protein L21	*Argopecten irradians*	AAN05604.1	72
LSASL3-D-1.ab1	similar to ENSANGP00000020389	*Apis mellifera*	XP_392114.1	54
LSASL3-D-3.ab1	60s ribosomal protein L10	*Ixodes scapularis*	AAY66849.1	52
LSASL3-D-4.ab1	ENSANGP00000014375	*Anopheles gambiae str. PEST*	EAA14477.2	30
LSASL3-D-5.ab1	RE15268p	*Drosophila melanogaster*	AAS93748.1	35

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**[a]
LSASL3-D-6.ab1	similar to CG9285-PA, isoform A, partial	*Strongylocentrotus purpuratus*	XP_795581.1	71
LSASL3-D-7.ab1	unnamed protein product	*Tetraodon nigroviridis*	CAF96213.1	54
LSASL3-D-8.ab1	ENSANGP00000027602	*Anopheles gambiae str. PEST*	EAL41007.1	29
LSASL3-D-9.ab1	Hypothetical protein B0546.1	*Caenorhabditis elegans*	AAB92010.1	52
LSASL3-E-1.ab1	similar to Thbs2, partial	*Danio rerio*	XP_694690.1	51
LSASL3-E-6.ab1	CG9476-PA	*Drosophila melanogaster*	NP_524297.1	95
LSASL3-E-7.ab1	hypothetical protein UM00449.1	*Ustilago maydis 521*	XP_756596.1	32
LSASL3-E-8.ab1	COG1198: Primosomal protein N' (replication factor Y) - superfamily II helicase	*Vibrio sp. Ex25*	ZP_00762026.1	52
LSASL3-E-9.ab1	Hypothetical protein LOC406855	*Danio rerio*	AAH65319.1	49
LSASL3-F-4.ab1	baculoviral IAP repeat-containing 3	*Homo sapiens*	AAU88144.1	56
LSASL3-F-5.ab1	diaminopimelate epimerase	*Prochlorococcus marinus str. NATL2A*	AAZ57814.1	44
LSASL3-F-6.ab1	hypothetical protein LOC_Os12g28910	*Oryza sativa (japonica cultivar-group)*	ABA98481.1	38
LSASL3-G-6.ab1	Vanin 3	*Xenopus tropicalis*	AAH87969.1	41
LSASL3-G-7.ab1	putative calcium dependent protein kinase	*Silene latifolia*	CAF74837.1	35
LSASL3-H-1.ab1	unnamed protein product	*Homo sapiens*	BAC87532.1	43
LSASL3-H-12.ab1	ribosomal protein L19	*Argopecten irradians*	AAN05588.1	67
LSASL3-H-2.ab1	YadA-like, C-terminal:Haemagluttinin motif:Hep_Hag	*Burkholderia vietnamiensis G4*	ZP_00423121.1	23
LSASL3-H-3.ab1	ENSANGP00000016786	*Anopheles gambiae str. PEST*	EAA14690.3	94
LSASL3-H-4.ab1	putative alkaline phosphatase	*Idiomarina baltica OS145*	ZP_01042468.1	36
LSASL3-H-5.ab1	similar to hypothetical protein A930013K19	*Pan troglodytes*	XP_526806.1	41
LSASL3-H-6.ab1	CG5394-PA, isoform A	*Drosophila melanogaster*	NP_524471.2	28
LSASL3-H-7.ab1	-		_	0
LSASL3-H-8.ab1	transmembrane receptor Unc5B	*Danio rerio*	AAU94928.1	37
LSASL4-A-11.ab1	repressible high-affinity phosphate permease		47AAA74899.1	
LSASL4-A-12.ab1	-		_	0
LSASL4-A-2.ab1	thioester-containing protein	*Euphaedusa tau*	BAE44110.1	59
LSASL4-A-3.ab1	odz, odd Oz\/ten-m homolog 1(Drosophila)	*Homo sapiens*	CAI42721.1	31
LSASL4-A-4.ab1	cathepsin L	*Mytilus galloprovincialis*	AAT39505.2	80
LSASL4-A-5.ab1	ARHE	*Homo sapiens*	CAG46835.1	37
LSASL4-B-2.ab1	hypothetical protein Afu5g08450	*Aspergillus fumigatus Af293*	XP_753792.1	41
LSASL4-B-3.ab1	GA20963-PA	*Drosophila pseudoobscura*	EAL26527.1	47

Table 8. (*continued*)

Clone no.	**Putative identification**	**Closest species**	**Accession no.**	**I**^{a}
LSASL4-B-4.ab1	MGC108090 protein	*Xenopus tropicalis*	AAH90371.1	36
LSASL4-B-5.ab1	GA22016-PA	*Drosophila pseudoobscura*	EAL30854.1	40
LSASL4-B-6.ab1	hypothetical protein DSM3645_09727	*Blastopirellula marina DSM 3645*	ZP_01088750.1	37
LSASL4-B-7.ab1	endo-1,4-beta-D-glucanase	*Mytilus edulis*	CAC59695.1	52
LSASL4-C-1.ab1	similar to Plasmodium falciparum. Hypothetical protein	*Dictyostelium discoideum*	AAO53175.1	29
LSASL4-C-2.ab1	branched-chain amino acid aminotransferase, putative	*Rhodobacter sphaeroides ATCC 17025*	ZP_00915995.1	31
LSASL4-C-3.ab1	HBA_TURTR Hemoglobin alpha subunit (Hemoglobin alpha chain) (Alpha-globin)		45P18978	
LSASL4-C-4.ab1	-		_	0
LSASL4-C-5.ab1	Hypothetical protein H34I24.2	*Caenorhabditis elegans*	AAK18967.1	26
LSASL4-C-6.ab1	PDZ/DHR/GLGF	*Shewanella sp. MR-7*	ZP_00856533.1	32
LSASL4-D-11.ab1	hypothetical protein AN0759.2	*Aspergillus nidulans FGSC A4*	XP_658363.1	42
LSASL4-D-12.ab1	calcium binding protein 2	*Biomphalaria glabrata*	AAV91522.1	41
LSASL4-D-2.ab1	fat tumor suppressor homolog 2	*Rattus norvegicus*	NP_075243.1	35
LSASL4-D-3.ab1	CEll Death abnormality family member (ced-1)	*Caenorhabditis elegans*	NP_001021773.1	43
LSASL4-D-4.ab1	acetyl-CoA synthetase beta subunit	*Syntrophus aciditrophicus SB*	YP_460502.1	34
LSASL4-D-5.ab1	integrin beta 5	*Bos taurus*	AAS93434.1	37
LSASL4-D-6.ab1	ribosomal protein L21	*Argopecten irradians*	AAN05604.1	73
LSASL4-D-7.ab1	similar to Peptidyl-prolyl cis-trans isomerase C (PPIase) (Rotamase) (Cyclophilin C)	*Gallus gallus*	XP_424412.1	38
LSASL4-D-8.ab1	-		_	0
LSASL4-E-12.ab1	hypothetical protein DEHA0B04213g	*Debaryomyces hansenii CBS767*	XP_457149.1	33
LSASL4-E-8.ab1	MYSP_MYTGA Paramyosin		O96064	61
LSASL4-F-10.ab1	chromodomain helicase DNA binding protein	*Giardia lamblia ATCC 50803*	XP_769479.1	25
LSASL4-F-5.ab1	hypothetical protein LOC549394	*Xenopus tropicalis*	NP_001016640.1	87
LSASL4-G-11.ab1	similar to AMP deaminase 3	*Danio rerio*	XP_684156.1	30
LSASL4-G-12.ab1	similar to ENSANGP00000018891	*Apis mellifera*	XP_393575.2	37
LSASL4-G-6.ab1	cell cycle dependent kinase B	*Ostreococcus tauri*	AAV68596.1	35
LSASL4-H-1.ab1	Unknown (protein for IMAGE:7977719)	*Xenopus laevis*	AAI06200.1	69
LSASL4-H-11.ab1	transcription factor Crx	*Gallus gallus*	AAQ14340.1	51
LSASL4-H-12.ab1	Exodeoxyribonuclease V, RecC subunit	*Geobacter uraniumreducens Rf4*	ZP_01140309.1	40

Table 8. (*continued*)

Clone no.	Putative identification	Closest species	Accession no.	I[a]
LSASL4-H-2.ab1	40S ribosomal protein S23	*Dermacentor variabilis*	AAP04351.1	94
LSASL4-H-3.ab1	similar to ENSANGP00000012845	*Apis mellifera*	XP_624775.1	38
LSASL4-H-5.ab1	-		_	0

[a] Identity(%)

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Chapter IV.

Expression profiling of environmental stress-related genes from abalone, *Haliotis discus hannai* using cDNA microarrays

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ABSTRACT

Changes in environmental conditions challenge an organism to maintain their homeostasis. Salinity and temperature are two factors that fluctuate greatly in marine environmental. These factors affect not only the physiological mechanisms but also mortality of aquatic organisms such as fish and shellfish. In the present study, to investigate the response of abalone to environmental stress by heat-shock, cold-shock or hyposalinity using a cDNA microarray consisting of over 2,993 different amplicons was done. Reverse transcription-RCR assays were used to verify the differential expression of candiate genes. The differentially expressed genes were previously revealed by suppression subtractive hybridization and EST surveys and were recognized to encode other organisms components of the stress or immune system. Some of genes identified in this study were not previously recognized as a stress-associated genes. In addition, a number of genes with no known homologs were uncovered. Determination of their specific roles during stress condition may lead to a better understanding of stress response system.

Key words : abalone, *Haliotis discus hannai*, expressed sequence tag (EST), stress, microarray.

INTRODUCTION

Host defense mechanisms in molluscan are comprised of various innate and nonadaptive mechanisms. The primary cell responsible for immunoregulation is the hemocyte, which participates in a variety of functions, such as digestion and nutrient transport, wound healing, shell repair, excretion, and internal defense [1]. Since there are some methological restrictions in studying the abalone immune system, information on stress- or immune-related molecules of abalone is still limited.

Recently, remarkable progress has been made in genetic technology. Rapid expressed sequence tag (EST) analysis allows acquisition of huge number of DNA sequence information in a short time for many organisms including several species of aquatic organisms. In chapter II & III, EST analysis was done to determine the stress-related genes of abalone using subtracted cDNA libraries from abalone exposed to environmental stresses (heat-shock, cold-shock or hyposalinity).

Among the 1,316 ESTs analyzed, many ESTs were identified as stress- or immune-related genes involving in cell division, signal transduction, defense, metabolism and proliferation. However, simple BLAST searches allowed identification of 12.6% ESTs which did not show any significant identity and homology to DNA sequences available in the GenBank. Some of these unknown genes may be involved in function of the stress system. One way to elucidate the functions of these genes is to analyze their expression patterns.

Microarray technology is suitable for analysis of expression patterns of a large number of genes [2]. The analysis of gene expressions of large sets of genes facilitates the discovery of function of genes and signal transduction pathways. Microarray is particularly useful for study of species with limited genetical information and for analysis of complicated system like stress response. In this study, microarray analysis was conducted in order to investigate shellfish stress response under thermal or salinity stress.

MESTERIALS AND METHODS

Preparation of abalone cDNA microarray

A cDNA chip was made using abalone ESTs selected from the cDNA libraries prepared from the seven tissues of abalone and the environmental stress induced abalone subtracted cDNA libraries. The cDNA inserts were amplified by PCR using the T3 primer (5'-AATTA ACCCTCACTAAAGGG-3') and T7 primer (5'- TAATACGACTCACTATA GGG-3') in 90㎕ reactions using 2㎕ of the appropriate plasmids as templates. The samples were preheated at 95°C for 5 min and subjected to 30 cycles of amplification each consisting of 30s at 95°C, 30s at 5 °C, 1min at 72°C, with a 5min extension at 72°C during the last step. PCR products were purified using a PCR 96 cleanup kit (Millipore). The presenced of products was confirmed by agarose gel electrohporesis, and the remainder of the DNA was resuspended in 50% DMSO. The 2,993 clones were spotted in dupicate to amino-silane coated CMT GAPS II slides (Corning) using an OmniGrid microarrayer (GeneMachines).

RNA preparation, labeling and hybridization for microarray analysis

Abalone was exposed to heat-shock or cold-shock from ambient sea water (18°C) to 28°C or 4°C, respectively. Hyopsalinity was derived from ambient sea water (35‰) to 20‰. The treated abalones (3 individuals per each time point) were sampled at 0, 0.5, 1, 2, 3, 4 and 5h in heat-shock treatment (HT), or at 0, 3, 6, 9 and 24h in cold-shock treatment (CT), or at 0, 1, 3, 6, 9 and 24h in hyposalinity treatment (HST). The collected samples were ground immediately under liquid nitrogen for RNA preparation. Corresponding non-treated abalone (HTcon, CTcon, HSTcon) were sampled at the same time points to use as controls. Total RNA was prepared from pooled sample of each stess condition and 100 µg of each RNA was labeled with Cy3 (HTcon, CTcon, HSTcon) or Cy5 (HT, CT, HST) monoreactive dyes (Amersham) using a Superscript cDNA system (Gibco BRL). The Cy3- and Cy5-labeled cDNA probes were cleaned using a Qiaquick nucleotide removal kit (Qiagen). The purified probes were dried and resuspended in 40㎕ of hybridization buffer consisting of 5×SSC, 0.1% SDS, 20㎍ of Cot-1 DNA (Gibco BRL), 20㎍ of poly (A) RNA (Promega) and 20㎍ of yeast tRNA (Gibco BRL). The probes were denatured at 95°C for 3min and then applied to the microarray slides. An indirect comparison method was used in this study; i.e. total RNA, HTcon was used as a reference for heat-shock treatment abalone (HT). Total RNA, CTcon was used as a reference for cold-shock treatment abalone (CT) and total RNA, HSTcon was used as a reference for hyposalinity treatment abalone (HST). Hybridization was performed at 42°C for 16 h, whereupon the microarray slides were washed once with 2×SSC, 0.1% SDS at 42°C for 4 min, once with 0.1×SSC, 0.1% SDS at room temperature for 10 min and three times with 0.1×SSC at room temperature for 1 min. Finally, the microarray slides were rinsed with distilled water and spin-dried.

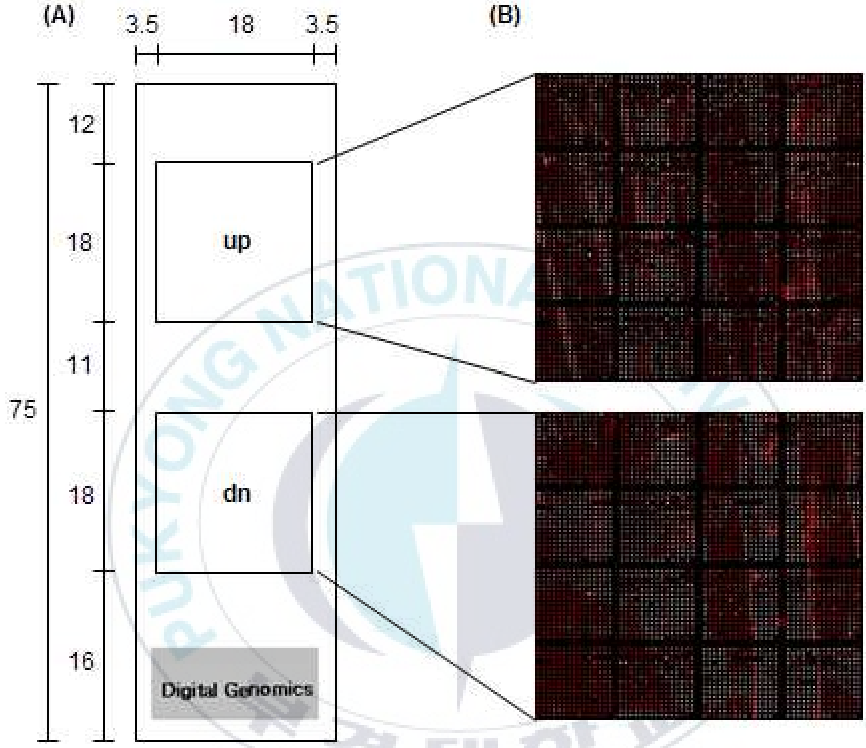


Fig. 1. The standard of constructed microarray , (A); Syto61 staining image of spotted microarrays, (B).

Microarray analysis

Microarray scanning and data normalizations were performed using a GenePix 4000B scanner and the GenePix Pro 6.0 software package (Axon Instruments). Poor-quality spots (sum of median <500) were filtered from the raw data prior to analysis. Background fluorescene was subtracted from the spot intensities and spots were flagged and removed from the data set if the signal to background ration was less than 1, or the signal intensity was less than 100. The filtered images were analyzed with GeneSpring GX 7.4 (Agilent technologies) to obtain gene expression ratios (treated vs. control). Logged gene expression ratios were normalized by the locally weighted non-linear regression (LOWESS) method [3].

RT-PCR verification

Verification of expression levels of candidate genes were carried out by RT-PCR. Total RNA samples were extracted from the collected samples according to each stress condition using TRIzol reagent (Invitrogen). Subsequently, first-strand cDNA synthesis was carried out using the Advantage RT-for-PCR Kit (BD Biosciences). The levels of selected genes expression were determined by RT-PCR using specific primers, which were based on the nucleotide sequence of EST clones. As an internal control, β-actin was amplified using the appropriate primers (Table 1). The PCR conditions were as follows: 95 C for 5 min, followed by 30 cycles of 95 C for 30 s, 55 C for 30 s, and 72 C for 30 s, with a final step of 72 C for 7 min. The amplified PCR products were analyzed on an 1.5% agarose gel containing ethidium bromide (100 ng/ml).

Table 1. Primers used in RT-PCR

ABHSL4G09-RT-F	5'-CGCCTCCTCCAAGACTT-3'
ABHSL4G09-RT-R	5'-GCCAAGCTATTTAGGTGACA-3'
DGT214-RT-F	5'-GCGGAAGTCTTGGAGGAG-3'
DGT214-RT-R	5'-GTGAGCTGGCTACCCGTTAC-3'
SKN070-RT-F	5'-GATAGAACGCTGAACAAATG-3'
SKN070-RT-R	5'-ACCTAGCCGTCTGTAGTCA-3'
β-actin-RT-F	5'-GCCGCTTGACTCTTGTGTGC-3'
β-actin-RT-R	5'-CTCCTCTGGTGCAACGCGG-3'

RESULTS AND DISCUSSION

Microarray analysis

With the cDNA clones that appeared to be involved in environmental stress response, Next study was expanded the target clones and used microarray analysis to examine their expression profiles under environmental stress by heat-shock, cold-shock or hyposalinity. In order to control against general stress-responsive genes, the differentially expressed genes were compared to those showing expression changes in stress non-treated control. Scatter-plot analysis of raw microarray data revealed that ~10% of the target genes showed more than a two-fold in signal intensity (Fig. 2). As expected, stress treatment resulted in marked, each stress condition changes in the gene expression profiles. Differentially expressed genes were defined as those showing a two-fold or greater change on at least one occasion over three stress conditions. A summary of the identified genes can be seen in Table 3-8.

Differentially expressed genes in three stress conditions

96, 144 and 139 clones were identified that differentially expressed in response to treatment with heat-shock, cold-shock or hyposalinity, respectively (Table 2). In heat-shock condition, the up- and down-regulated genes included stress-related genes such as HSP70 and HSP. In cold-shock condition, stress- or immune-related genes significantly up- or down- regulated. HSP70 was one of the strongest up-regulated genes in cold-shock (above 10- fold).

The up- or down- regulated genes include genes involved in cell structure and energy metabolism such as arginine kinase (AK), glyceraldehyde phosphate dehydrogenase and elongation factor. Clones encoding arginine kinase (AK) were strongly up-regulated in hyposalinity condition and slightly down-regulated in heat-shock condition. These date indicated that temperature or salinity stress has affected the expression of energy metabolism-related genes, which must have impaired the defense system of the abalone. It is interesting to note that the clones similar to arginine kinase (AK) were regulated significantly. Since AK is one of the allergens homologous to arginine kinase in invertebrate [4, 5], and was reported as a phosphagen-ATP phosphotransferas [6]. This result is consistent with the report of Astrofsky et al. [7] showed that AK was up-regulated after 30h of WSSV injection. However, AK was down-regulated in WSSV injected shrimp in Wang et al [8]. These results can be suggested that the expression pattern of AK may fluctuate at different stress condition. Because AK plays an important role in energy metabolisms [9], it is thought that the up- or down-regulation may reflect damage of energy metabolism.

An interesting result was the expression change of genes which participated in reproduction. Representative reproduction-related clones were identified as sperm lysin which was up-regulated in hyposalinity condition. In addition, clones encoding fertilization protein and vitelline coat protein were also strongly up-regulated in hyposalinity condition. However, a number of clones encoding vitelline coat protein was down-regulated in cold-shock condition.

In vertebrates, the primary response to stress is characterized by the release of neuroendocrine messengers such as catecholamines and glucocorticoids [10, 11]. These messengers act in turn to divert energy away from non-essential processes such as growth, reproduction and certain immune functions to particular bio-energetic processes, such as increased oxygen uptake and mobilization of energy substrates, which help the animal to adapt and overcome the threat [10, 12]. In invertebrates, there is still a need for information on the effects of stress and stress induced neuroendocrine changes on immunity and resistance to disease. Although it is not clear why reproduction-related gene expression in environmental stress condition was highly changed, we speculated that abalones are fatally influenced by environmental stress.

At least two genes were identified as unknown genes among strongly up-regulated genes (heat-shock ; ABHSL-4-G09, ABHSL-2-B01: cold shock; ABLSL-5-E02, GIL076: hyposalinity; LSASL-5-B05, ABLSL-7-B09, SKN164). Due to limited sequence information of these genes still remain to be identified and their role in stress response remains to be defined.

Confirmation of microarray results

To confirm the results of microarray study, RT-PCR was performed using three representative genes (Fig. 3). The gene expression of β-actin, which was used for normalization, was equivalent under control or stress treatment. The score of ration for each gene corresponds to the RT-PCR result. Gene expression pattern of these genes strongly support the results of microarray.

In conclusion, DNA microarray hybridization methodology has proved to be a powerful tool for the determination and quantitative analysis of the expressed genes in the abalone and has uncovered a wider and more detailed understanding of the molecular basis of stress response in the abalone. This study represents the first report of the production of cDNA libraries by subtractive suppressive hybridization and utilization of microarray techniques for the study of temperature or salinity stress in the abalone. This robust and powerful technique will allow the identification of a substantial number of genes that are potentially involved in stress response. Expanding the use of DNA microarrays to non-model species that have been crucial in the study of certain physiological fucntions will be valuable for the identification the genes associated with these processes [13]. The methodology has great advantages for the screening of genes and the devolopment of experimental hypotheses in many organisms when exposed to changes in their environmental conditions.

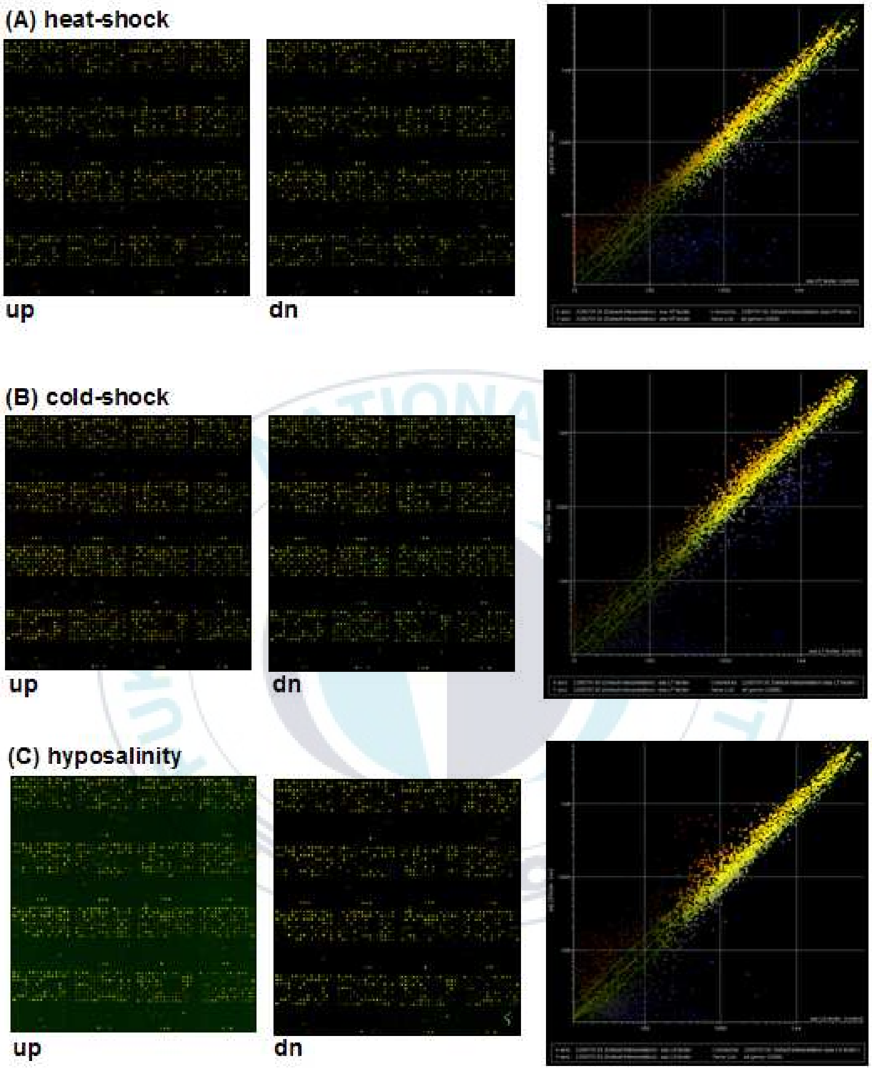
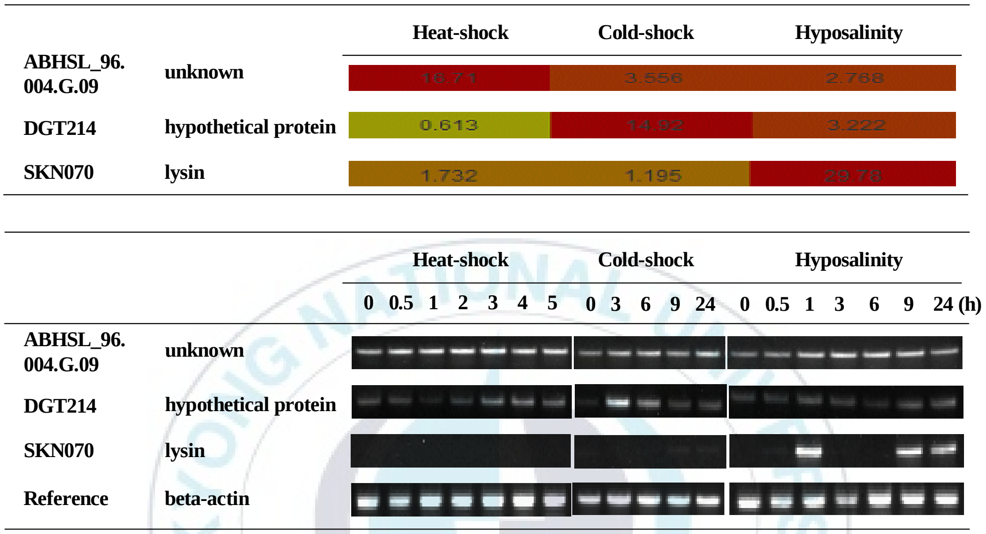


Fig. 2. Reliability of the hybridized microarrays. Technical replicates and scatter plot of signal values from replicates on a microarray.

Table 2. Gene expression changes of abalone exposed to three different environmental stresses

	Stress conditions		
	Heat-shock	Cold-shock	Hyposalinity
Up-regulated	56	70	116
Down-regulated	40	74	23
Total	96	144	139
Percent of 2993 genes	3.2%	4.8%	4.6%



		Heat-shock	Cold-shock	Hyposalinity
ABHSL_96.004.G.09	unknown	16.71	3.556	2.768
DGT214	hypothetical protein	0.613	14.92	3.222
SKN070	lysin	1.732	1.195	29.78

Fig. 3. Reverse transcription-RCR analysis of three representative genes identified as being differentially expressed in the microarray analysis.

Table 3. List of up-regulated genes by heat-shock

Systematic Name	Clone no.	Putative identification	Fold chage
ND_384.05.K.16	GIL-260	similar to ankyrin repeat domain protein 17 isoform b, partial	2.32
ND_384.04.I.07	GOM-052	fertilization protein	2.063
ND_384.04.C.17	GOM-016	70 kDa heat shock protein	2.04
ND_384.04.D.08	GIL-016	Asparagine synthetase	2.484
ND_384.04.K.06	GOM-159	elongation factor 1-alpha	3.038
ND_384.09.D.23	LSASL_96.004.B.12	unknown	2.746
ND_384.08.I.10	ABHSL_96.003.E.05	unknown	2.039
ND_384.08.E.03	ABHSL_96.002.C.02	MK2 non-allergic IgE heavy chain IGHV2-5	2.154
ND_384.08.M.15	ABHSL_96.002.G.08	unknown	2.845
ND_384.08.K.03	ABHSL_96.002.F.02	unknown	2.213
ND_384.08.I.09	ABHSL_96.002.E.05	NADH dehydrogenase subunit 1	2.305
ND_384.09.G.21	LSASL_96.002.D.11	type X collagen	2.607
ND_384.04.K.09	GOM-065	sperm lysin	2.1
ND_384.08.H.02	LSASL_96.001.D.01	unknown	2.424
ND_384.08.K.15	ABHSL_96.002.F.08	unknown	2.308
ND_384.08.N.24	LSASL_96.001.G.12	unknown	2.708
ND_384.08.E.17	ABHSL_96.002.C.09	unknown	3.846
ND_384.08.N.22	LSASL_96.001.G.11	unknown	3.946
ND_384.03.N.13	GOF-175	unnamed protein product	2.441
ND_384.06.J.01	ABLSL_96.003.E.01	RPL18A protein	2.158
ND_384.09.C.21	LSASL_96.002.B.11	unknown	5.755
ND_384.09.N.13	LSASL_96.004.G.07	unknown	3.763
ND_384.02.L.13	SKN-067	Mcm5-prov protein	4.201
ND_384.07.C.10	ABLSL_96.006.B.05	trypsinogen 3	3.812
ND_384.07.C.18	ABLSL_96.006.B.09	unnamed protein product	3.893
ND_384.01.P.17	DGT-285	unknown	2.485
ND_384.04.D.22	GIL-023	similar to Asparaginyl-tRNA synthetase, cytoplasmic (Asparagine--tRNA ligase) (AsnRS)	2.834
ND_384.09.C.13	LSASL_96.002.B.07	ribosomal protein P2, putative	2.106
ND_384.06.F.10	ABLSL_96.004.C.05	hypothetical protein 3	2.421
ND_384.06.F.23	ABLSL_96.003.C.12	unknown	2.266
ND_384.08.C.14	ABHSL_96.003.B.07	proline rich protein MP4	3.093
ND_384.07.P.10	ABHSL_96.001.H.05	OSJNBa0035I04.9	2.201
ND_384.07.L.15	ABLSL_96.007.F.08	unknown	2.455
ND_384.07.P.02	ABHSL_96.001.H.01	F1F0-type ATP synthase subunit g	2.568
ND_384.07.D.18	ABHSL_96.001.B.09	glyceraldehyde phosphate dehydrogenase	2.022
ND_384.02.B.17	SKN009	similar to CG16965-PA	2.436
ND_384.07.H.18	ABHSL_96.001.D.09	unknown	2.689
ND_384.01.M.23	DGT-084	transcriptional regulator, internal deletion	2.214
ND_384.02.H.21	SKN047	hypothetical protein	2.009
ND_384.02.F.06	SKN123	myosin regulatory light chain interacting protein	2.014
ND_384.02.D.04	SKN110	methionyl-tRNA synthetase	2.759
ND_384.02.F.03	SKN026	23S rRNA methyltransferase/RumA	2.109
ND_384.02.H.19	SKN046	caveolin-3	2.619

Table 3. (*Continued*)

Systematic Name	**Clone no.**	**Putative identification**	**Fold chage**
ND_384.01.J.05	DGT-243	Hypothetical protein LOC553722	2.889
ND_384.03.E.16	GOF-032	hypothetical protein cgd4_750	2.738
ND_384.09.P.21	LSASL_96.004.H.11	transcription factor Crx	2.31
ND_384.08.O.05	ABHSL_96.002.H.03	glyceraldehyde phosphate dehydrogenase	6.129
ND_384.08.N.17	ABHSL_96.004.G.09	unknown	16.71
ND_384.09.P.13	LSASL_96.004.H.07	unknown	2.034
ND_384.08.C.01	ABHSL_96.002.B.01	unknown	8.19
ND_384.08.G.16	ABHSL_96.003.D.08	hypothetical protein LOC541384	2.352
ND_384.06.N.10	ABLSL_96.004.G.05	GA21098-PA	4.534
ND_384.07.L.18	ABHSL_96.001.F.09	SJCHGC01954 protein	4.193
ND_384.08.I.07	ABHSL_96.002.E.04	unknown	2.249
ND_384.08.G.09	ABHSL_96.002.D.05	unknown	4.729
ND_384.08.K.17	ABHSL_96.002.F.09	unknown	3.957

Table 4. List of down-regulated genes by heat-shock

Systematic Name	Clone no.	Putative identification	Fold chage
ND_384.05.C.23	GIL120	inhibitor of apoptosis protein 3	0.229
ND_384.05.E.17	GIL129	unknown	0.29
ND_384.05.K.14	GIL259	Hypothetical protein CBG06905	0.089
ND_384.05.E.21	GIL131	similar to signal peptide, CUB domain, EGF-like 3	0.0805
ND_384.05.G.19	GIL142	COG3025: Uncharacterized conserved protein	0.434
ND_384.05.G.08	GIL232	putative ubiquitin-conjugating enzyme	0.492
ND_384.05.K.05	GIL159	Oxidoreductase, N-terminal:Oxidoreductase, C-terminal	0.478
ND_384.04.G.20	GOM142	Arginine kinase (AK)	0.405
ND_384.04.F.20	GIL034	inhibitor of apoptosis protein 3	0.17
ND_384.04.M.10	VHP257	D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase family protein	0.235
ND_384.04.M.22	VHP263	mature-parasite-infected erythrocyte surface antigen	0.338
ND_384.04.P.04	GIL086	polyubiquitin	0.314
ND_384.09.J.24	LSASL_96.005.E.12	unknown	0.288
ND_384.09.J.20	LSASL_96.005.E.10	paramyosin protein	0.0573
ND_384.04.L.20	GIL070	polyubiquitin	0.374
ND_384.04.L.18	GIL069	Hypothetical protein CBG21335	0.116
ND_384.10.A.08	VHP208	CPK27; ATP binding / calcium ion binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase	0.255
ND_384.08.M.03	ABHSL_96.002.G.02	glyceraldehyde phosphate dehydrogenase	0.117
ND_384.08.K.05	ABHSL_96.002.F.03	glyceraldehyde phosphate dehydrogenase	0.264
ND_384.08.N.16	LSASL_96.001.G.08	similar to metalloprotease-disintegrin	0.404
ND_384.08.M.07	ABHSL_96.002.G.04	40S ribosomal protein S26	0.392
ND_384.06.D.08	ABLSL_96.004.B.04	ferritin-like protein	0.445
ND_384.09.O.23	LSASL_96.002.H.12	alginase	0.355
ND_384.07.K.24	ABLSL_96.006.F.12	unknown	0.346
ND_384.06.K.12	ABLSL_96.002.F.06	GA18441-PA	0.45
ND_384.10.D.07	VHP226	P-553	0.361
ND_384.03.C.02	GOF013	vitelline coat protein 41	0.0694
ND_384.06.J.24	ABLSL_96.004.E.12	beta-actin	0.435
ND_384.07.I.03	ABLSL_96.005.E.02	unknown	0.44
ND_384.07.J.06	ABHSL_96.001.E.03	melanoma ubiquitous mutated protein	0.447
ND_384.10.A.12	VHP210	Myc homolog	0.472
ND_384.02.H.09	SKN041	X-box binding protein 1	0.373
ND_384.02.H.16	SKN140	unknown	0.371
ND_384.02.N.15	SKN080	hypothetical protein UM01098.1	0.491
ND_384.04.C.07	GOM1	ribosomal protein L24e	0.01
ND_384.02.J.17	SKN057	inhibitor of apoptosis 2 protein	0.207
ND_384.08.C.20	ABHSL_96.003.B.10	unknown	0.0193
ND_384.09.C.18	LSASL_96.003.B.09	unknown	0.13
ND_384.08.H.19	ABHSL_96.004.D.10	unknown	0.477
ND_384.07.B.22	ABHSL_96.001.A.11	unknown	0.0201
ND_384.08.E.18	ABHSL_96.003.C.09	similar to TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	0.0412

Table 5. List of up-regulated genes by cold-shock

Systematic Name	**Clone no.**	**Putative identification**	**Fold chage**
ND_384.05.D.11	VHP018	inhibitor of apoptosis protein	2.616
ND_384.05.C.23	GIL120	inhibitor of apoptosis protein 3	2.184
ND_384.05.E.12	GIL222	ZK829.7	2.206
ND_384.05.E.07	GIL124	MGC52542 protein	3.086
ND_384.05.K.17	GIL165	heat shock protein 70	2.873
ND_384.05.E.21	GIL131	PREDICTED: similar to signal peptide, CUB domain, EGF-like 3	6.837
ND_384.04.F.20	GIL034	inhibitor of apoptosis protein 3	5.483
ND_384.04.I.14	GOM151	unknown	2.469
ND_384.04.N.08	GIL076	unknown	4.197
ND_384.09.D.23	LSASL_96.004.B.12	unknown	3.365
ND_384.08.M.15	ABHSL_96.002.G.08	unknown	2.136
ND_384.09.G.21	LSASL_96.002.D.11	type X collagen	3.085
ND_384.10.A.08	VHP208	CPK27; ATP binding / calcium ion binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase	3.141
ND_384.04.K.09	GOM065	sperm lysin	2.537
ND_384.08.M.03	ABHSL_96.002.G.02	glyceraldehyde phosphate dehydrogenase	5.259
ND_384.08.L.19	ABHSL_96.004.F.10	unknown	3.406
ND_384.08.K.05	ABHSL_96.002.F.03	glyceraldehyde phosphate dehydrogenase	2.593
ND_384.08.E.17	ABHSL_96.002.C.09	unknown	2.191
ND_384.06.I.02	ABLSL_96.002.E.01	unknown	3.201
ND_384.04.G.24	GOM144	unknown	3.702
ND_384.07.C.10	ABLSL_96.006.B.05	trypsinogen 3	3.758
ND_384.08.C.14	ABHSL_96.003.B.07	proline rich protein MP4	3.188
ND_384.07.P.10	ABHSL_96.001.H.05	OSJNBa0035I04.9	2.221
ND_384.07.L.15	ABLSL_96.007.F.08	unknown	2.577
ND_384.07.L.04	ABHSL_96.001.F.02	ATP-dependent RNA helicase, DEAD/DEAH box family protein	5.532
ND_384.07.P.02	ABHSL_96.001.H.01	F1F0-type ATP synthase subunit g	2.367
ND_384.07.I.03	ABLSL_96.005.E.02	unknown	18.95
ND_384.07.D.18	ABHSL_96.001.B.09	unknown	2.284
ND_384.04.K.18	GOM165	sperm lysin	2.113
ND_384.07.F.11	ABLSL_96.007.C.06	unknown	2.093
ND_384.02.B.18	SKN105	PREDICTED: similar to RIKEN cDNA 1200009H11	2.478
ND_384.07.P.14	ABHSL_96.001.H.07	unknown	2.134
ND_384.02.B.09	SKN005	CG34043-PA	2.323
ND_384.02.B.14	SKN103	CG34043-PA	2.933
ND_384.07.H.18	ABHSL_96.001.D.09	unknown	2.215
ND_384.01.O.04	DGT182	LOC443666 protein	2.8
ND_384.02.D.10	SKN113	hypothetical protein Chro.60269	2.03
ND_384.02.D.11	SKN018	similar to guanylate cyclase OlGC-R2	5.336
ND_384.01.D.19	DGT214	hypothetical protein	14.92

Table 5. (*Continued*)

Systematic Name	**Clone no.**	**Putative identification**	**Fold chage**
ND_384.01.E.05	DGT027	unknown	2.2
ND_384.01.E.12	DGT126	hypothetical protein PB000759.00.0	2.132
ND_384.01.D.23	DGT216	Elongator protein 3/MiaB/NifB	2.047
ND_384.01.E.01	DGT025	ZK829.7	2.063
ND_384.01.F.11	DGT222	recombination activating protein 1	2.264
ND_384.01.F.17	DGT225	unnamed protein product	2.449
ND_384.01.E.22	DGT131	putative baseplate assembly protein	2.529
ND_384.01.C.23	DGT024	hypothetical protein, conserved	2.404
ND_384.01.J.11	DGT246	unknown	2.308
ND_384.01.L.07	DGT256	unknown	2.152
ND_384.02.N.07	SKN076	unknown	2.714
ND_384.02.N.23	SKN084	ENSANGP00000011567	2.296
ND_384.02.P.01	SKN085	sperm lysin	2.924
ND_384.04.C.07	GOM016	70 kDa heat shock protein	10.41
ND_384.02.J.17	SKN057	inhibitor of apoptosis 2 protein	7.043
ND_384.02.P.23	SKN096	immediate-early protein	3.145
ND_384.08.C.13	ABHSL_96.002.B.07	proline rich protein 2 (predicted)	2.741
ND_384.04.C.04	GOM110	lysin	2.596
ND_384.08.C.20	ABHSL_96.003.B.10	heat shock protein 70	4.2
ND_384.08.N.17	ABHSL_96.004.G.09	unknown	3.556
ND_384.08.C.01	ABHSL_96.002.B.01	unknown	2.886
ND_384.07.H.04	ABHSL_96.001.D.02	unknown	3.782
ND_384.08.G.16	ABHSL_96.003.D.08	hypothetical protein LOC541384	2.922
ND_384.08.K.11	ABHSL_96.002.F.06	unknown	3.094
ND_384.07.L.18	ABHSL_96.001.F.09	SJCHGC01954 protein	2.529
ND_384.08.I.07	ABHSL_96.002.E.04	unknown	2.362
ND_384.09.O.19	LSASL_96.002.H.10	unknown	2.244
ND_384.07.B.22	ABHSL_96.001.A.11	unknown	2.064
ND_384.02.B.12	SKN102	acidic ribosomal phosphoprotein P0	2.03
ND_384.08.K.17	ABHSL_96.002.F.09	unknown	3.339

Table 6. List of down-regulated gene by cold-shock

Systematic Name	Clone no.	Putative identification	Fold chage
ND_384.05.C.18	GIL213	cyclin B3	0.442
ND_384.04.P.17	VHP285	rhoptry protein homolog Bc60.2 - Babesia canis	0.411
ND_384.05.G.12	GIL234	unkown	0.395
ND_384.05.K.14	GIL259	Hypothetical protein CBG06905	0.495
ND_384.04.H.11	VHP243	V (tail component;256)	0.401
ND_384.04.M.22	VHP263	mature-parasite-infected erythrocyte surface antigen	0.364
ND_384.04.J.24	GIL060	repressor	0.284
ND_384.09.L.24	LSASL_96.005.F.12	alginase	0.444
ND_384.09.J.24	LSASL_96.005.E.12	alginase	0.438
ND_384.09.C.10	LSASL_96.003.B.05	unknown	0.292
ND_384.09.J.20	LSASL_96.005.E.10	paramyosin protein	0.387
ND_384.09.N.06	LSASL_96.005.G.03	alginate lyase	0.43
ND_384.09.B.02	LSASL_96.005.A.01	transmembrane protein	0.491
ND_384.08.L.02	LSASL_96.001.F.01	unkown	0.209
ND_384.09.N.21	LSASL_96.004.G.11	similar to AMP deaminase 3	0.379
ND_384.09.O.23	LSASL_96.002.H.12	alginase	0.438
ND_384.10.D.07	VHP226	P-553	0.281
ND_384.03.A.04	GOF002	vitelline coat protein 41	0.281
ND_384.03.C.02	GOF013	vitelline coat protein 41	0.0661
ND_384.04.L.22	GIL071	similar to bcl2-like, partial	0.282
ND_384.06.F.10	ABLSL_96.004.C.05	unknown	0.451
ND_384.07.N.02	ABHSL_96.001.G.01	glyceraldehyde phosphate dehydrogenase	0.422
ND_384.07.H.16	ABHSL_96.001.D.08	alginate lyase	0.35
ND_384.03.G.06	GOF039	vitelline coat protein 41	0.435
ND_384.03.H.05	GOF135	vitelline coat protein 41	0.388
ND_384.03.G.14	GOF043	vitelline coat protein 41	0.339
ND_384.03.N.21	GOF179	ubiquitin ligase	0.179
ND_384.03.F.09	GOF125	vitelline coat protein 41	0.26
ND_384.03.F.11	GOF126	vitelline coat protein 42	0.397
ND_384.03.D.15	GOF116	similar to contactin associated protein 1	0.241
ND_384.03.C.12	GOF018	vitelline coat protein 41	0.334
ND_384.03.E.20	GOF034	vitelline coat protein 41	0.253
ND_384.03.E.10	GOF029	vitelline coat protein 41	0.495
ND_384.03.K.16	GOF068	vitelline coat protein 41	0.24
ND_384.03.F.03	GOF122	vitelline coat protein 41	0.293
ND_384.03.G.20	GOF046	vitelline coat protein 41	0.169
ND_384.03.E.04	GOF026	vitelline coat protein 41	0.208
ND_384.03.K.04	GOF062	vitelline envelope sperm lysin receptor	0.176
ND_384.03.J.23	GOF156	vitelline coat protein 41	0.44
ND_384.03.L.09	GOF161	Hypothetical protein CBG12915	0.121
ND_384.03.M.10	GOF077	vitelline coat protein 41	0.367
ND_384.03.M.20	GOF082	vitelline coat protein 41	0.174
ND_384.03.K.02	GOF061	vitelline coat protein 41	0.395
ND_384.03.N.17	GOF177	vitelline coat protein 42	0.245
ND_384.03.I.06	GOF051	vitelline coat protein 42	0.1

Table 6. (*Continued*)

Systematic Name	Clone no.	Putative identification	Fold chage
ND_384.03.I.12	GOF054	vitelline coat protein 41	0.273
ND_384.03.H.09	GOF137	vitelline coat protein 42	0.319
ND_384.03.H.15	GOF140	similar to alpha 2 type IV collagen preproprotein; canstatin	0.38
ND_384.03.E.06	GOF027	vitelline coat protein 41	0.19
ND_384.03.G.18	GOF045	vitelline coat protein 41	0.459
ND_384.03.J.09	GOF149	vitelline coat protein 42	0.223
ND_384.03.N.09	GOF173	vitelline coat protein 42	0.189
ND_384.03.N.07	GOF172	vitelline coat protein 41	0.365
ND_384.03.H.21	GOF143	vitelline coat protein 42	0.203
ND_384.03.A.06	GOF003	CTP SYNTHASE	0.204
ND_384.03.B.06	GOF189	unknown	0.299
ND_384.03.B.03	GOF098	vitelline coat protein 42	0.216
ND_384.03.A.12	GOF006	similar to a disintegrin and metalloproteinase with thrombospondin motifs 9 preproprotein	0.283
ND_384.03.B.05	GOF099	vitelline coat protein 42	0.295
ND_384.03.C.10	GOF017	guanine nucleotide binding protein (G protein)	0.423
ND_384.03.I.22	GOF059	vitelline coat protein 41	0.297
ND_384.03.B.17	GOF105	vitelline coat protein 41	0.417
ND_384.03.A.20	GOF010	unkown	0.432
ND_384.03.C.18	GOF021	vitelline coat protein 41	0.468
ND_384.03.P.19	GOF190	unkown	0.332
ND_384.03.P.21	GOF191	ENSANGP00000000511	0.132
ND_384.03.C.08	GOF016	vitelline coat protein 41	0.181
ND_384.04.B.06	GIL003	similar to leucine rich repeat containing 49, partial	0.46
ND_384.04.B.10	GIL005	TonB dependent, hydroxamate-type ferrisiderophore, outer membrane receptor	0.5
ND_384.08.O.05	ABHSL_96.002.H.03	glyceraldehyde phosphate dehydrogenase	0.19
ND_384.06.N.10	ABLSL_96.004.G.05	40S ribosomal protein S3	0.481
ND_384.01.F.24	RM036	putative sugar transporter	0.322
ND_384.08.H.19	ABHSL_96.004.D.10	unkown	0.199

Table 7. List of up-regulated genes by hyposalinity

Systematic Name	Clone no.	Putative identification	Fold chage
ND_384.05.K.17	GIL165	heat shock protein 70	2.024
ND_384.05.E.21	GIL131	similar to CEGP1 protein	14.77
ND_384.04.G.20	GOM142	Arginine kinase (AK)	2.304
ND_384.04.F.20	GIL034	inhibitor of apoptosis protein 3	2.668
ND_384.04.I.07	GOM052	fertilization protein	18.6
ND_384.04.I.12	GOM150	fertilization protein	15.77
ND_384.04.I.04	GOM146	Arginine kinase (AK)	3.215
ND_384.04.I.10	GOM149	Arginine kinase (AK)	2.552
ND_384.04.C.15	GOM020	Arginine kinase (AK)	2.899
ND_384.04.D.02	GIL013	zonadhesin	2.166
ND_384.04.C.12	GOM114	PREDICTED: similar to eukaryotic translation elongation factor 1 beta 2; eukaryotic translation elongation factor 1 beta 1	2.908
ND_384.04.I.14	GOM151	unkown	5.152
ND_384.04.C.13	GOM019	unkown	2.282
ND_384.04.E.21	GOM035	unkown	2.353
ND_384.04.M.09	GOM077	unkown	2.492
ND_384.04.M.19	GOM082	unkown	2.241
ND_384.09.D.10	LSASL_96.005.B.05	unkown	20.38
ND_384.10.A.08	VHP208	CPK27; ATP binding / calcium ion binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase	2.022
ND_384.04.K.12	GOM162	Arginine kinase (AK)	3.451
ND_384.04.K.09	GOM065	sperm lysin	15.36
ND_384.08.M.03	ABHSL_96.002.G.02	unkown	4.245
ND_384.08.H.02	LSASL_96.001.D.01	unkown	3.285
ND_384.09.A.11	LSASL_96.002.A.06	RL44_PICJA 60S ribosomal protein L44	11.1
ND_384.08.M.07	ABHSL_96.002.G.04	unkown	3.674
ND_384.08.N.22	LSASL_96.001.G.11	unkown	2.256
ND_384.03.N.13	GOF175	vitelline coat protein 41	6.466
ND_384.06.I.02	ABLSL_96.002.E.01	unkown	3.989
ND_384.06.J.01	ABLSL_96.003.E.01	RPL18A protein	3.106
ND_384.06.F.17	ABLSL_96.003.C.09	unkown	4.918
ND_384.06.A.04	ABLSL_96.002.A.02	unkown	3.099
ND_384.09.N.21	LSASL_96.004.G.11	AMP deaminase 3	3.347
ND_384.09.C.17	LSASL_96.002.B.09	unkown	5.061
ND_384.09.H.10	LSASL_96.005.D.05	unkown	2.502
ND_384.09.J.17	LSASL_96.004.E.09	unkown	4.064
ND_384.09.C.21	LSASL_96.002.B.11	unkwon	2.172
ND_384.04.K.21	GOM071	CG10510-PA	2.311
ND_384.09.I.08	LSASL_96.003.E.04	unkown	8.913
ND_384.09.N.13	LSASL_96.004.G.07	unkown	7.599
ND_384.10.A.05	VHP195	hypothetical protein	2.928

Table 7. (*Continued*)

Systematic Name	**Clone no.**	**Putative identification**	**Fold chage**
ND_384.02.D.16	SKN116	unkown	6.007
ND_384.09.P.22	LSASL_96.005.H.11	unkown	4.148
ND_384.02.L.13	SKN067	Mcm5-prov protein	8.605
ND_384.03.D.05	GOF111	Ubiquitin-activating enzyme E1 1	7.06
ND_384.08.H.11	ABHSL_96.004.D.06	Hypothetical protein F41G3.12	4.65
ND_384.09.M.08	LSASL_96.003.G.04	unkown	2.249
ND_384.03.D.01	GOF109	ArcA1 arginine deiminase	9.121
ND_384.06.C.13	ABLSL_96.001.B.07	ribosomal protein P1	4.527
ND_384.07.C.18	ABLSL_96.006.B.09	unnamed protein product	2.046
ND_384.09.H.09	LSASL_96.004.D.05	integrin beta 5	5.199
ND_384.03.A.04	GOF002	vitelline coat protein 41	2.874
ND_384.03.C.02	GOF013	vitelline coat protein 41	7.415
ND_384.04.L.22	GIL071	Mcl1b	3.028
ND_384.03.O.02	GOF085	Serine/threonine phosphatase 4 regulatory subunit 1	4.952
ND_384.03.C.17	SKN223	unkown	5.053
ND_384.06.I.05	ABLSL_96.001.E.03	unkown	2.1
ND_384.06.D.13	ABLSL_96.003.B.07	unkown	3.123
ND_384.06.M.03	ABLSL_96.001.G.02	unkown	2.113
ND_384.07.I.03	ABLSL_96.005.E.02	unkown	3.898
ND_384.06.P.16	ABLSL_96.004.H.08	unkown	2.017
ND_384.07.D.17	ABLSL_96.007.B.09	unkown	14.66
ND_384.07.G.01	ABLSL_96.005.D.01	unkown	3.985
ND_384.04.K.17	GOM065	sperm lysin	3.221
ND_384.04.K.18	GOM165	sperm lysin	8.121
ND_384.07.D.11	ABLSL_96.007.B.06	Beta-Ig-H3/fasciclin	2.092
ND_384.07.F.11	ABLSL_96.007.C.06	unkown	2.066
ND_384.02.H.08	SKN136	pannexin 5	2.159
ND_384.02.D.13	SKN019	Hypothetical protein LOC283078	3.837
ND_384.01.D.19	DGT214	similar to Poly	3.222
ND_384.02.D.18	SKN117	heat shock protein gp96	2.064
ND_384.02.D.02	SKN109	gp164	2.398
ND_384.02.D.07	SKN016	unnamed protein product	2.267
ND_384.03.O.12	GOF090	Vitellogenin precursor (VTG)	2.665
ND_384.03.F.01	GOF121	CTP SYNTHASE	2.212
ND_384.03.N.21	GOF179	ubiquitin ligase	2.156
ND_384.03.F.09	GOF125	vitelline coat protein 41	2.015
ND_384.03.H.01	GOF133	similar to kinesin family member 18A	6.288
ND_384.03.E.20	GOF034	vitelline coat protein 41	2.619
ND_384.03.F.03	GOF122	vitelline coat protein 41	2.281
ND_384.03.G.20	GOF046	vitelline coat protein 41	2.29
ND_384.03.M.18	GOF081	dihydrolipoamide dehydrogenase precursor	2.01
ND_384.03.K.04	GOF062	vitelline envelope sperm lysin receptor	2.029
ND_384.03.L.09	GOF161	Hypothetical protein CBG12915	2.304
ND_384.03.M.20	GOF082	vitelline coat protein 41	2.424
ND_384.03.N.01	GOF169	vitelline coat protein 41	2.217

Table 7. (*Continued*)

Systematic Name	**Clone no.**	**Putative identification**	**Fold chage**
ND_384.03.I.06	GOF051	vitelline coat protein 41	2.115
ND_384.03.N.09	GOF173	vitelline coat protein 41	2.058
ND_384.03.H.21	GOF143	vitelline coat protein 41	2.704
ND_384.02.N.07	SKN076	unkwon	3.49
ND_384.02.P.01	SKN085	sperm lysin	9.832
ND_384.04.C.09	GOM017	unkown	2.609
ND_384.04.C.07	GOM016	70 kDa heat shock protein	12.12
ND_384.02.L.16	SKN164	unkown	15.23
ND_384.02.L.19	SKN070	lysin	29.78
ND_384.04.A.09	GOM005	Arginine kinase (AK)	2.869
ND_384.04.A.12	GOM102	KARG_NORMA Arginine kinase (AK)	2.937
ND_384.04.A.18	GOM105	Arginine kinase (AK)	2.93
ND_384.04.A.10	GOM101	hypothetical protein XP_683358	3.319
ND_384.04.A.05	GOM003	Arginine kinase (AK)	2.97
ND_384.03.P.21	GOF191	ENSANGP00000000511	5.816
ND_384.03.C.08	GOF016	70 kDa heat shock protein	3.088
ND_384.06.G.13	ABLSL_96.001.D.07	agmatinase, putative	2.824
ND_384.02.P.23	SKN096	immediate-early protein	2.028
ND_384.04.A.24	GOM108	Arginine kinase (AK)	2.803
ND_384.04.C.04	GOM110	lysin	12.25
ND_384.08.C.20	ABHSL_96.003.B.10	heat shock protein 70	8.08
ND_384.08.N.17	ABHSL_96.004.G.09	unkown	2.768
ND_384.08.C.01	ABHSL_96.002.B.01	unkown	2.958
ND_384.09.C.18	LSASL_96.003.B.09	unkown	7.049
ND_384.08.K.11	ABHSL_96.002.F.06	heat shock protein 70	7.405
ND_384.09.D.18	LSASL_96.005.B.09	actin	2.564
ND_384.08.H.19	ABHSL_96.004.D.10	unkown	3.812
ND_384.07.L.18	ABHSL_96.001.F.09	SJCHGC01954 protein	3.703
ND_384.09.E.10	LSASL_96.003.C.05	unkown	3.757
ND_384.07.B.22	ABHSL_96.001.A.11	unkown	6.75
ND_384.08.E.18	ABHSL_96.003.C.09	similar to TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor	15.6
ND_384.08.K.17	ABHSL_96.002.F.09	unkown	2.891

Table 8. List of down-regulated gene by hyposalinity

Systematic Name	**Clone no.**	**Putative identification**	**Fold chage**
ND_384.05.G.10	GIL233	Hypothetical protein LOC406376	0.152
ND_384.09.N.24	LSASL_96.005.G.12	SocE	0.352
ND_384.08.E.03	ABHSL_96.002.C.02	MK2 non-allergic IgE heavy chain IGHV2-5	0.494
ND_384.07.H.12	ABHSL_96.001.D.06	unkown	0.47
ND_384.09.A.17	LSASL_96.002.A.09	hypothetical protein 3	0.404
ND_384.08.L.03	ABHSL_96.004.F.02	MHC II invariant chain	0.39
ND_384.04.D.22	GIL023	asparaginyl-tRNA synthetase	0.125
ND_384.09.K.22	LSASL_96.003.F.11	unkown	0.457
ND_384.09.C.13	LSASL_96.002.B.07	ribosomal protein P2, putative	0.421
ND_384.06.D.03	ABLSL_96.003.B.02	unkown	0.421
ND_384.06.D.15	ABLSL_96.003.B.08	hypothetical protein LOC550606	0.449
ND_384.07.H.20	ABHSL_96.001.D.10	unkown	0.416
ND_384.01.P.03	DGT278	undecaprenyl-PP-N-acetylmuramic acid-pentapeptide N-acetylglucosamine transferase	0.444
ND_384.01.P.01	DGT277	similar to 5 nucleotidase, partial	0.477
ND_384.01.B.10	RM005	unkown	0.45
ND_384.01.J.11	DGT246	unkown	0.488
ND_384.01.J.17	DGT249	hypothetical protein	0.399
ND_384.01.L.07	DGT256	unkown	0.456
ND_384.09.P.21	LSASL_96.004.H.11	transcription factor Crx	0.271
ND_384.08.O.05	ABHSL_96.002.H.03	glyceraldehyde phosphate dehydrogenase	0.462
ND_384.09.P.13	LSASL_96.004.H.07	unkown	0.407
ND_384.01.F.24	RM036	putative sugar transporter	0.372
ND_384.09.G.03	LSASL_96.002.D.02	similar to Calcium-binding protein 2 (CaBP2)	0.449

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SUMMARY

In chapter II, EST analysis was conducted using seven cDNA libraries made from gill, digestive gland, heptopancreas, skin, muscle, testis, and ovary. The assembly program ICAtools software was used to organize the redundant ESTs into overlapping contigs. The results showed that the 1,393 ESTs were composed of 135 clusters and 951 singletons, suggesting that the overall redundancy of the library was 22%. Of the 1,086 clones 1,278 clones (91.7%) were identified as known genes by BLAST searches and 115 clones (8.3%) did not match to any previously described genes. Based on major function of their encoded proteins, the identified clones are classified into 16 broad categories. Sequence analysis of ESTs revealed the presence of microsatellite-containing genes that may be valuable for further mapping studies.

In chpater III, to analyze expressed genes for the temperature and salinity change, suppression subtractive hybridization (SSH) method was used and constructed three cDNA libraries from abalone exposed to heat-shock, cold-shock or hyposalinity stress. Putative function could be assigned to 75.8% of the 1,536 sequenced cDNAs. Based on sequence similarities, candidate genes was identified that may be implicated in stress response or immune function. Among them, several stress- and immune-related genes were identified including HSC, HSP, major histocompatibility complex (MHC) class IIa chain, CD45 homolog, and IRF7 from three subtracted cDNA libraries. The expressions of

these genes were investigated in abalone exposed to stress. They were induced in response to stress, supporting their involvement in abalone immunity.

In the chpater IV, to investigate the response of abalone to environmental stress by heat-shock, cold-shock or hyposalinity using a cDNA microarray consisting of over 2,993 different amplicons was done. Reverse transcription-RCR assays were used to verify the differential expression of candiate genes. The differentially expressed genes were previously revealed by suppression subtractive hybridization and EST surveys and were recognized to encode in components of the stress or immune system. Some of genes identified in this study were not previously recognized as a stress-associated genes. In addition, a number of genes with no known homologs were uncovered. Determination of their specific roles during stress condition may lead to a better understanding of stress response system.

Overall, this series of experiments greatly expand our knowledge of the shellfish stress response and immune system at the DNA molecular level. In addition, the accumulation of a large number of identified cDNA clones is invaluable for abalone genetics and developmental biology. The cDNA clone tagging approach will rapidly build up the resource of the abalone genes and be feasible to clone most, if not all, of the abundantly expressed genes. Among the many possibilities and applications, these identified clones will be useful for selection of tissue-specific, cell type specific, stress-specific or disease-specific markers, isolation of full-length clones and gene promoters, and analysis of the gene expression pattern and gene function.

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