Expressed sequence tag analysis of expression profiles in spleen and head kidney of olive flounder (*Paralichthys olivaceus*)

(넙치 (*Paralichthys olivaceus*) 비장과 두신 발현 양상의 발현유전자조각 (EST) 분석에 관한 연구)

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넙치 (*Paralichthys olivaceus*) 비장과 두신 발현 양상의 발현유전자조각 (EST) 분석에 관한 연구

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요 약

1991년에 Venter를 비롯한 연구자들은 cDNA 자원을 확보하기 위해 완전히 다른 개념의 연구방법을 도입하였다. 이른바 발현 유전자 조각 (EST; expressed sequence tag)을 통한 분석이 그것이다. 이후, EST 대량 확보를 위한 전 세계적인 노력이 이루어 졌으며 현재, 약 16,000,000 EST가 Genebank 내 dbEST에 등록되어 있다. EST 데이터베이스 사용자들에게 있어서 가장 큰 관심은 EST 자체의 비교적 짧은 염기 서열과 다른 유전자들과의 관계를 이해하는데 있다. 이를 위하여 NCBI는 염기서열의 유사성에 기초하여 UniGene 데이터베이스를 구축하였으며, 이들 중 30,000개가 염색체 상에 유전자지도로 작성되어 EST 데이터베이스를 위치 선택적 클로닝에 이용할 수 있게 되었다. 그러나 EST 데이터베이스의 대부분은 육상동물, 특히 포유류에 국한되어 있고, 어류와 관련한 EST는 극히 소수에 불과하다. 따라서 본 연구는 최근 주요 연구 대상으로 주목받고있는 어류 중 넙치 (*Paralichthys olivaceus*)의 비장과 두신으로 부터 총 348개의 EST를 이용하여 각 조직의 발현양상과 특성을 연구하였다. 먼저 넙치 비장과 두신으로 부터 cDNA library를 제조하여 각 1.2×10^7 pfu/ml, 1.3×10^6 pfu/ml로써 충분한 수준의 다양한 유전자를 포함하는 cDNA 자원을 확보하였다. 제작된 초기 library들로부터 증폭 library의 제조와 절단을 통해 얻어진 각 클론들의 삽입 cDNA의 평균 크기 1.9±0.6 kb, 1.3±0.5 kb로 조사됨에 따라 효과적인 크기별 분류에 의해 양질의 유전자를 확보되었음을 확인하였다. 총 348개(비장: 195, 두신: 153)개의 EST clone들의 염기서열을 결정하고, 생물정보학을 이용하여 확보된 대량의 유전자 정보의 기능을 해석함으로써 두 조직의 유전자 발현 양상과 발현 빈도 등을 조사하고, 각 조직의 유전자 발현특성을 밝히고자 하였다.

생물 정보학을 이용하여 348개의 EST를 분석한 결과 비장은 cluster 21개, sigleton은 174개로 구성되었으며, 발현 중복율은 15%로 나타났으며, 두신은 cluster 27개, sigleton은 146개로 구성되었으며, 발현 중복율은 29%로 나타났다. 또한 BLASTX를 이용하여 유전자검색 결과 331개 (95%)의 EST가

이미 알려진 유전자와 상동성을 가진 것으로 나타났고, 나머지 17개 (4.9%)의 EST는 전혀 알려지지 않은 유전자로 밝혀졌다. 이들 조직에서 전혀 밝혀지지 않은 유전자는 미지의 EST에 대한 좋은 자원으로 제공될 수 있으며, 반면 이미 밝혀진 95%는 기존의 유전자와 높은 상동성을 가짐에 따라 이미 밝혀진 EST에 대한 좋은 자원으로 사용될 수 있을 것으로 여겨진다. 또한 많은 EST가 다른 생물의 염기서열에 대한 유사성은 보이지만 기능이 전혀 알려져 있지 않음에 따라 비교 유전체학을 연구하는데 좋은 유전자 자원으로 이용될 것으로 여겨진다.

본 연구를 통해 비장과 두신의 cDNA library로부터 분석한 348개의 EST를 주요 기능에 따라 cell cycle and processing, cell rescure/ deference/ cell death, cellular communication/ signal transduction, cellular organization, cellular transport and transport mechanism, energy, metabolism, protein fate, protein synthesis, regulation/ interaction with cellular environment, transcription, unclassified proteins의 11개로 분류한 결과 비장과 두신은 비슷한 양상을 나타내었으며, 두 조직 모두 Cellular organization, cell cycle and DNA processing represent와 관련된 유전자가 높은 비율을 차지하였으며, cellular environment와 관련된 유전자는 낮은 비율을 나타내었다.

비장과 두신은 조혈 기관으로 EST 분석을 통하여 밝혀진 여러 유전자의 기능을 살펴본 결과, 어류 면역과 관련된 유전자들을 관찰할 수 있었다. 여러 면역 관련 유전자 중 granulin이라는 유전자가 비장과 두신의 EST에서 서로 다른 종류 (비장: granulinA precursor, 두신: granulin2) 로 zebra fish의 granulins와 높은 상동성을 지니는 것으로 밝혀졌다. 비장의 granulinA precursor는 3'말단의 단편을 포함하고 있으며, 두신의 granulin2는 전체 cDNA의 서열을 포함하고 있었다. RT-PCR을 이용하여 넙치의 각 조직에서 granulin 유전자의 발현 양상을 살펴본 결과 비장과 두신을 비롯한 면역관련 기관에서 강한 발현을 나타내는 것을 알 수 있었다.

본 연구는 다량의 넙치 유전자를 분석하고 규명된 유전자의 수를 증가시킴으로써 비교적 상대적으로 부족한 넙치 유전자 자원을 이용한 다른 연구에 기초적 자료를 제공할 뿐만 아니라 조혈 기관인 비장과 두신의 발현 유전자의 분석을 통해 어류 면역과 관련된 유용유전자의 특성을 밝히는데 그 목적이 있다. 본 연구를 통해 분석된 유전자가 축적됨에 따라 넙치와 관련한 유전학 분야와 발생 생물학 분야에 많은 도움을 줄 수 있을 것으로 기대 된다. 이들 유전자들의 응용분야로서는 유전자 발현 양상 및 기능 분석, 개체 및 조직 특이적인 유전자 표지 개발, 그리고 전체 cDNA 와 발현조절 부위 분리 등이 있다. 따라서 본 연구 결과를 통해 어류의 조직에서 특이하게 발현하는 유전자의 정보를 확보하고, 이를 이용하여 향후 어류 유전체학의 유용한 정보로 이용될 것으로 기대된다.

I . Introduction

The expressed sequence tag (EST) approach, first demonstrated in the human genome project (Adams *et al.*, 1991), is powerful in massive cloning of cDNAs as well as in large scale characterization of cDNA sequences for deciphering genome sequence. This approach is also valuable in studies of mRNA expression profiles at a single gene level from unbiased cDNA libraries. In general, two types of information can be obtained by this approach: the composition of expressed transcripts and the relative abundance of these transcripts. Both types of information are important to the understanding of molecular composition and function of source tissues and cells.

The olive flounder (*Paralichthys olivaceus*) is one of the more significant fish species in Korea and Japan due to human interests in aquaculture and fisheries. The olive flounder stocks currently used in aquaculture have become appreciably different to their wild counterparts and it seems likely that genetics will play an increasingly more important role in achieving further improvements in the performance of the brood stocks (Lie *et al.*, 1994). Traits that may be amenable to genetic improvement include growth, delayed maturity, sex determination, disease resistance and temperature tolerance. Despite this interest, relatively little information is available olive flounder genes and their sequences with less than 3,000 typical olive flounder gene

sequences currently deposited in the international DNA sequence database in the dbEST (Table 1). With the advancement of sequencing technology, it is now possible to produce large numbers of ESTs representing a large proportion of the overall transcriptional activity of an organism. ESTs have been shown to be and excellent and proven method of identification and characterizing novel genes. However, comprehensive information on steady state mRNA levels is not known for most known fish transcripts (Virlon *et al.*, 1999). This lack of knowledge may represent one obstacle to the effective use of genetics in aiding both fish aquaculture and conservation activities. Therefore, in order to increase the current database of fish genes, this study instigated olive flounder gene identification and expression analysis project following the EST-based strategy now commonly used for the identification of large numbers of genes in species of interest (Marra *et al.*, 1998)

Careful analyses of the sequence data have provided significant additional functional, structural, and evolutionary information (Quackenbush *et al.*, 2000). Large numbers of ESTs have been produced from a number of species (Adams *et al.*, 1991; Waterston *et al.*, 1992; Franco *et al.*, 1995; Azam *et al.*, 1996). ESTs represent 71% of all GenBank entries and 40% of the individual nucleotides (Quackenbush *et al.*, 2000). Currently, the number of fish-related ESTs in the public database is still small compared with mammalian sequences and there are relatively few tissue-specific cDNA libraries (Ton *et al.*, 2000). ESTs from teleosts account for only about 4% of the almost

sixteen million ESTs in the dbEST division of GenBank (Table 1). The greatest effort so far has been made in zebrafish (Gong, 1999), winter flounder (Douglas *et al.*, 1999), olive flounder (Inoue *et al.*, 1997; Aoki *et al.*, 1999; Nam *et al.*, 2000), medaka (Hirono and Aoki; 1997), channel cat fish (Ju *et al.*, 2000; Cao *et al.*, 2001; Karsi *et al.*, 2002), and salmon (Davey *et al.*, 2001; Martin *et al.*, 2002). Now, EST analysis has become a commonly used approach to identify genes involved in specific biological functions, and especially in organisms where genomic data are not available, like for instance, tolerance to osmotic stress in plant (Zhang *et al.*, 2001) or gene profiling during embryogenesis in ascidians (Satou *et al.*, 2002). The EST approach was used to identify genetic markers in salmon (Davey *et al.*, 2001), environmental stress indicators in American oyster (Jenny *et al.*, 2002) or to characterize immune genes in shrimp(Gross *et al.*, 2001). Indeed, in such marine organisms with economical interest, the access to genomic data may provide new insight into the management of aquaculture activities. Large-scale EST analysis is essential to adopt the cDNA microarray technology for comparative functional genomics, particularly to address the complex nature of gene expression involved in determination of performance traits such as feed conversion and behavioral traits. ESTs have also been great resources for genomic mapping (Boguski and Schuler, 1995; Hudson *et al.*, 1995; Schuler *et al.*, 1996).

In addition, infectious diseases in fish cause major losses in aquaculture

industry. As olive flounder is one of the most widely cultured fish species and considered to be a major source of protein in Korea and Japan, investigations into the immunological mechanisms are required for the establishment of new methods for prevention of diseases and its sustainable production. Gene cataloguing and profiling of the kidney is and essential part in EST analysis of immune organs. The immune system of fish is similar to that of mammals (Secombes *et al.*, 1983) consisting of non-specific defence barriers and specific immune functions; the latter includes T-and B-cell mediated cellular and humoral immunity (Partula *et al.*, 1995; Passer *et al.*, 1996; Yamaguchi *et al.*, 1996; Nishimura *et al.*, 1997). In addition to the thymus and spleen, which are also present in higher vertebrates, another lymphoid organ, the kidney, exists in fish (Pichappan, 1980; Chilmoczyk, 1992)and is the functional counterpart of mammalian bone marrow.

In this study, two cDNA libraries from the head kidney and spleen of adult olive flounder were constructed and all total of 348 expressed sequencing tag (EST) clones were generated. Various clones were identified as putative biodefense and immune response-related genes. The interesting genes among the putative immune-related genes can be found in the distribution of granulins between the head kidney and spleen libraries. Granulins are cysteine-rich polypeptide purified from human and rat hematopoietic cells and structurally related to the epithelin family of growth modulatory factors. A prototypic form of granulins was isolated from the hematopoietic organs of

teleost fish (Belcourt, D., and Bennett, H. P. J. (1998) J. Cell Biol. 107, 629(abstr.)) We cloned a partial cDNA fragment of a granulinA precursor and full length cDNA fragment granulin2 by an expressed sequences tag(EST) analysis of Olive flounder spleen and head kidney, and we characterized their expression patterns by RT-PCR.

Table 1. The number of fish ESTs deposited in the dbEST.

(as of May 23, 2003)

Species	dbEST
Danio rerio (Zebrafish)	318,552
Oryzias latipes (Japanese medaka)	103,098
Oncorhynchus mykiss (Rainbow trout)	101,845
Salmo salar (Atlantic salmon)	58,330
Ictalurus punctatus (Channel catfish)	15,562
Cyprinus carpio(Common carp)	7,477
***Paralichthys olivaceus* (Japanese flounder)**	**2,735**
Pleuronectes americanus (Winter flounder)	938
Oreochromis niloticus (Nile tilapia)	294
Tetraodon fluviatilis (Puffer fish)	99
Hippoglossus hippoglossus (Atlantic halibut)	32
***Paralichthys olivaceus* (Olive flounder ; *this study*)**	**2,518**

Ⅱ Materials and Methods

1. Fish and tissue preparation

Olive flounders (*Paralichthys olivaceus*) were obtained from Koje Hatchery of National Fisheries Research and Development Institute (NFRDI) and maintained in 6 tons flow-through tank at 12±1℃ under a natural photoperiod. Spleen and head kidney tissues were collected and cut into as small pieces as possible. Pooled tissues were rapidly frozen with liquid nitrogen and were ground with a mortar/pestle, and then homogenized with a hand-held tissue tearor in RNA extraction buffer following the guanidium thiocyanate method (Chomcynski and Sacchi, 1987).

2. Construction of olive flounder cDNA libraries

Olive flounder spleen and head kidney cDNA libraries were constructed from then individual fish depending on tissue type using the Uni-ZAP XR cDNA synthesis/Gigapack cloning kit (Stratagene Cloning Systems). Total RNA was extracted using the TRIzol reagent(Gibco BRL Life Technologies Ltd, Renfrewshire, UK), and mRNA isolation kit (Promega, WI, USA). cDNA synthesis was carried out using an oligo-$(dT)_{18}$ primer for the revers

transcription of approximately 5㎍ of mRNA and the libraries were constructed by directional cloning based on the manufacturers instruction manual.

All primary libraries were amplified and aliquots of each amplifid library were stored at both 4 and -70℃. Mass excision was performed and the cDNA inserts from the amplified Uni-ZAP XR libraries were rescued as pBluescript phagemids in SOLR Escherichia coli.

3. Plasmid preparation and sequencing

The plasmid cDNA libraries were plated to a density appropriate for picking individual colonies. Random clones were grown in 1.5-ml LB medium overnight in 12×75-mm culture tubes. Plasmid DNA was prepared by the alkaline lysis method (Sambrook *et al.*,1989) using the Qiagen Spin Column Mini-plasmid kits. Three microliters of plasmid DNA(about 0.5~1.0㎍) were used in sequencing reactions. Single-pass sequencing of 5′-termini of selected cDNA clones in phagemid from was performed using the ABI 3100 automatic DNA sequencer (PE Applied Biosystems, CA, USA) and the ABI Prism Big Dye Terminatior Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

4. Bioinformatic analysis

Bioinformatic analysis was conducted to determine gene identities using GeneMaster software (Ensoltek, Korea). Procedures for establishing orthologs are shown in Fig. 2. Briefly, vector sequences were removed and database search were limited to ESTs>100bp in length. ESTs were then assembled in clusters of contigous sequences (contig) using ICAtools program(Parsons, 1995). Gene annotation procedures and homology searches of the sequenced ESTs have been locally done by BLASTX for amino acid similarity comparisons (Altschul *et al.*, 1997). Matches with the Expect value (E) less than $1.0 \times e^{-4}$ were considered to be significant. After the BLAST searches, a visual inspection was made to determine if the significant similarity was caused by simple sequences. ESTs with significant similarities in searches were considered orthologs of known genes only when the similarities were not caused by simple sequences. All ESTs that were not identified as orthologs of known genes were designated as unknown EST clones.

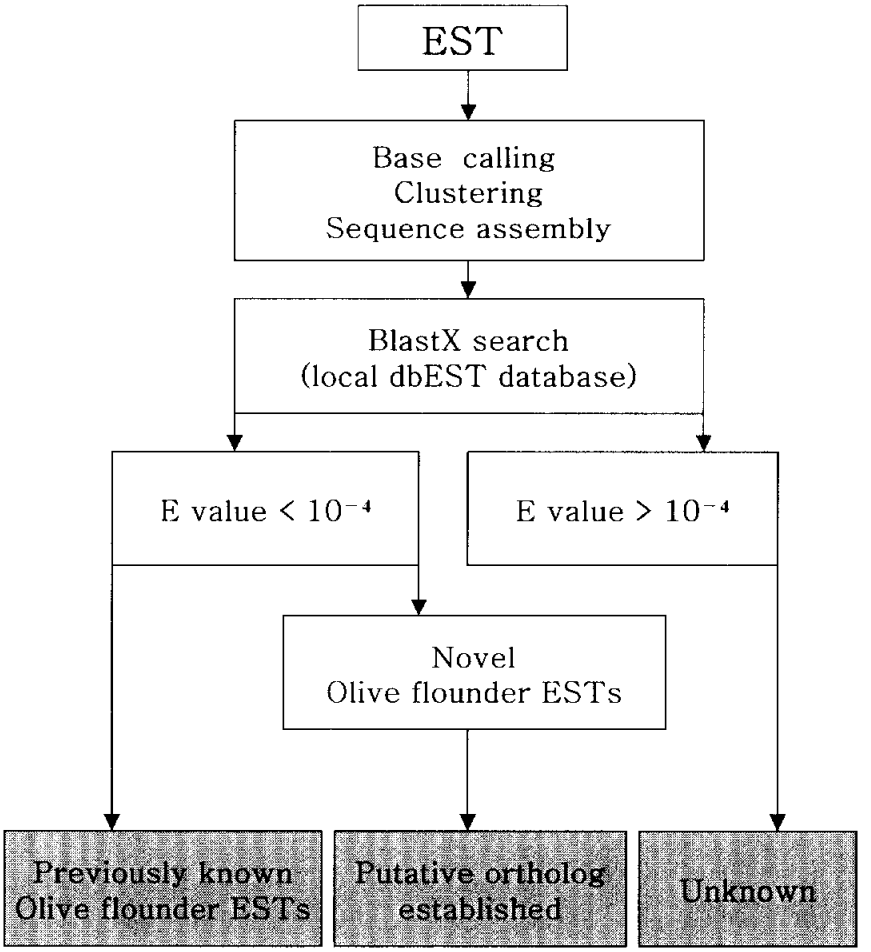

Fig. 1. Schematic presentation of sequence analysis and gene annotation using BlastX searches.

5. RT PCR analysis

Total RNA was extracted from healthy Olive flounder gill, liver, stomach, spleen, pyloric caeca, intestine, rectum, head kidney, body kidney, heart, brain and muscle using Trizol (Life Technologies). The purified total RNA(10㎍) was treated with DNase and then reverse transcribed into cDNA using an AMV Reverse Transcriptase First-Strand cDNA Synthesis kit(Life Sciences). The final volume of the cDNA synthesis reaction was 25㎕. The reverse-transcribed sample(1㎕) was used in 50㎕ of PCR reaction mixture. The PCR primers used in this study were grnA-F, 5′-cacgaggccagacatagtaca-3′, grnA-R, 5′-cacacagtgggtgtaagagta-3′, grn2-F, 5′-agggacaagctctgtcatca-3′ and grn2-R, 5′-tatttactctggagctgggtc-3′. PCR was performed with an initial denaturation step of 2min at 95℃, and then 20 cycles were run as follows: 30s of denaturation at 95℃, 30s of annelaing at 55℃, and 1min of extension at 72℃. The reacted products were electrophoresed on a 2.0% agarose gel.

Ⅲ. Results and Discussion

1. Summary of EST clones in olive flounder spleen cDNA library

The spleen cDNA library was constructed from the poly-adenylated fraction of mRNA from the olive founder spleen. The number of clones sequenced from the cDNA library, the average size of inserts, and the redundancy of the obtained seqeunces, are given in Table 2. A total of 229 randomly selected clones, in phagemid form, were single-pass sequenced from the 5′ end, resulting in the characterization of cDNA clones that were longer than 100bp after elimination of vector sequence. The average insert size was estimatoed to be 1.9 ± 0.6kb by PCR amplification of inserts from 20 randomly selectted clones. The assembly program ICAtools software(Parsons, 1995) was used to organize the redundant ESTs into overlapping contigs. The results showed that the 229 spleen ESTs were composed of 21 clusters and 174 sigletons, suggesting that the overall redundancy of the library was 15%.

2. Summary of EST clones in olive flounder head kidney cDNA library

In the process of EST analysis from the olive flounder head kidney, 216 cDNA clones were single-pass sequenced from the 5′ end, resulting in the characterization of cDNA clones that were longer than 100 bp after elimination of vector sequence. The number of clones sequenced from the cDNA library, the average size of inserts, and the redundancy of the obtained sequences, are given in Table 2. The average insert size was estimated to be 1.3 ± 0.5 kb by PCR amplification of insert from 20 randomly selected clones. The assembly program ICAtools software(Parsons, 1995) was used to organize the redundant ESTs into overlapping contigs. The results showed that the 244 head kidney ESTs were composed of 27 clusters and 146 singletons, suggesting that the overall redundancy of the library was 29%.

Table 2. General characteristics of olive flounder spleen and head kidney ESTs

	Tissue	
	spleen	**head kidney**
Primary library size (pfu/ml)	1.2×107	1.3×106
Average Length of insert(kb)	1.9±0.6	1.3±0.5
Number of clones filed	195	153
No. of unigene	174	124
Redundancy(%)	15	29

3. Distribution of the identified clones in two libraries

The complete list of identified clones is available upon request. Based on major function of their encoded proteins, the identified clones are classified into eleven broad categories ; cell cycle and processing, cell rescure/ deference/ cell death, cellular communication/ signal transduction, cellular organization, cellular transport and transport mechanism, energy, metabolism, protein fate, protein synthesis, regulation/ interaction with cellular environment, transcription, unclassified proteins. The distribution of identified clones in the spleen and head kidney cDNA libraries is summarized Fig 2. and Fig 3.

Between the spleen and head kidney cDNA libraries is not a notable difference. A related clones of Cellular organization, cell cycle and DNA processing represent high percentage. However, the reverse trend is that the regulation and cellular environment clones are relatively low percentage.

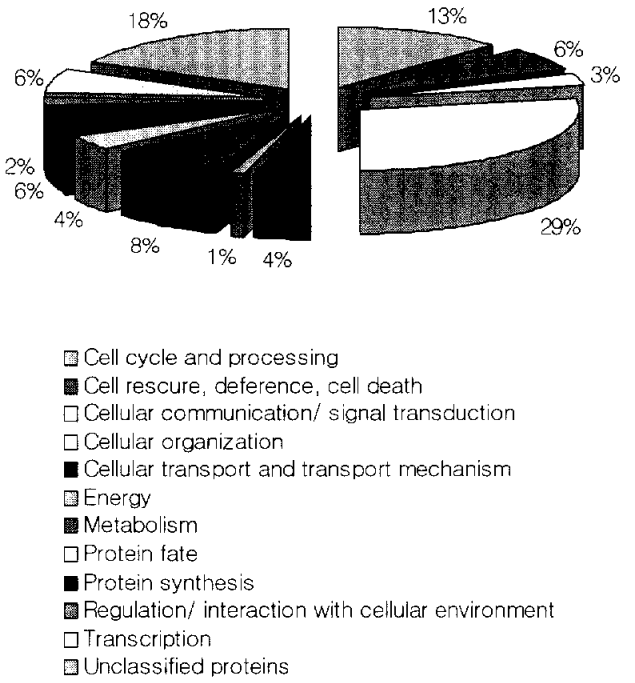

Fig. 2. Functional categorization statistics of olive flounder speen ESTs.

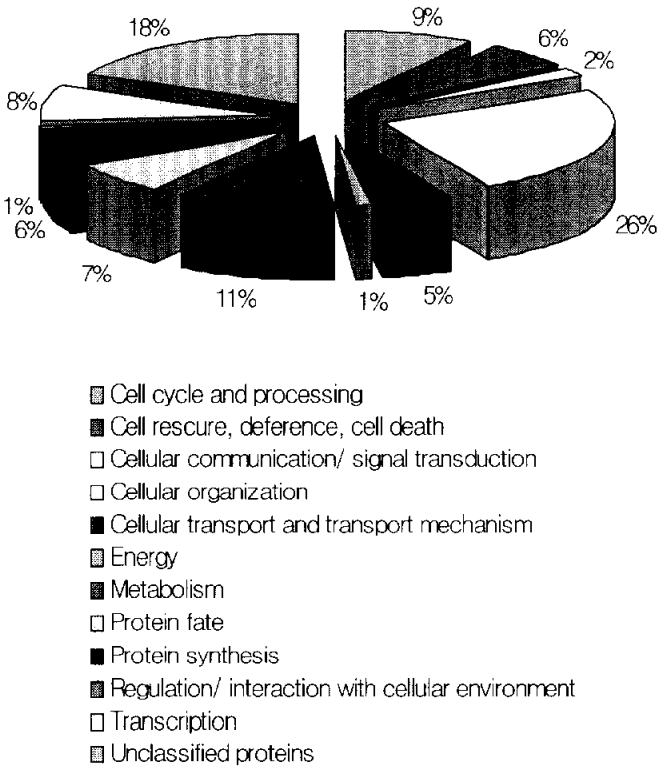

Fig. 3. Functional categorization statistics of olive flounder head kidney ESTs.

4. Isolation of immune response-related genes from the two cDNA libraries

Another aim of this study is to identify cDNA clones that can be investigated for immune system in Olive flounder hematopoietic tissue. Database searches identified putative immune response-related genes that were expressed. (table 5)

Lymphocytes undergo high rates of apoptosis during development and immune response proliferation. The putative amino acid sequence deduced from one cDNA clone, SPLEEN-03-B06, was identified as BCL2-associated athanogene, that is a cell death inhibitory protein. During apoptosis, the mitochondira swell, allowing cytochrome c to leak into the cytosol, wich in turn leads to DNA fragmentation. BCL2 interacts with the mitochondrial outer membrane, which blocks the mitochondrial swelling. (Cosulich, S. F. ,1997)

Cytokine genes and chemokine genes were identified in this study. One of these was TNF family B cell activiation factor (HEAD KIDNEY-01-A06), whose plays roles in immune and inflammatory responses and in the phathogenesis of many diseases. (Carswell, E. A., 1975) Chemokines are small cytokines, and are subdivided into four subfamilies by their cysteine signature motifs. (Hedrick, J. A., 1996) SPLEEN-01-C02 and SPLEEN-03-B06 show similarities to chemokine (C-X-C motif) represneted by IL-8 and CC chemokine eotaxin, respectively. The action of chemokines are mediated by

seven transmembrane domain (7 TMD) receptors (Murphy, P. M., 1994), which utilise heterotrimeric GTP-binding proteins as signal transducers.

The amino acid sequence of HEAD KIDNEY-02-F11 (HEAD KIDNEY-03-B08) was 94%(100%) identical through the IgM precursor (immunoglobulin M) of the oliver flounder.

The putative amino acid sequence deduced from cDNA clones, SPLEEN-02-D03 and HEAD KIDNEY-03-E07 were identified as granulins (spleen cDNA clones; granulin A precusor, head kidney cDNA clones; granulin 2). (Fig. 4) The association of granulins with haematopoietic tissues spans the entire vertebrate kingdom, as granulins have also been isolate from the spleen and head kidney of a teleost fish, the carp (Belcourt *et al.*, 1993). Ohter investigators purified and partially sequenced two growth regulatory peptides from rat kidneys, which they called epithelins 1 and 2 (Shoyab *et al.*, 1990). A comparison of the structures of epithelins 1 and 2 with the rat peptide purified from bone marrow showed that rat granulin A and epithelins 1 are identical, with the exception that the first residue of the amino terminus is absent in the epithelin 1. At present, only granulin A/ epithelin 1 and granulin B/ epithelin 2 have been shown to have biological activities, and the actions of the other granulins, if any, are unknown.

The expression of flounder granulins was studied by RT-PCR. The mRNA of granulin A precursor was mainly detected in spleen and weakly detected in the other organs.

Granulin 2 expression was notably detected in immune-related organs such as spleen, and head kidney. Signal were also detected in gill and body kidney. However, low expression of granulin 2 mRNA was found in liver, stomach, pyloric caeca, intestine, rectum, heart, and brain. (Fig. 6)

Granulins are cysteine-rich polypeptides. (Fig. 5) These structures play an important role in the immune response of fish and function as clearing units for invading organisms, antigen presentation, and possibly act as germinal centers for different classes of lymphocytes.

These indicate the importance of granulins in immune regulation that may also be functioning in fish.

5. Evaluation of the olive flounder spleen and head kidney ESTs.

When a cDNA library is evaluated for and EST project, two factors are worth noting. First, what is the potential of a cDNA library to supply identifiable clones? This was important at the beginnig of our fish EST project as our focus was on the number of identified cDNA clones in order to rapidly characterize a large number of olive flounder cDNAs. Secondly, particularly at the present stage of the olive flounder EST project, what is potential of a cDNA library to supply non-redundant or novel cDNA clones?

Of the 348 EST clones identified by BLASTX, 298 (85.6%, 174; spleen EST, 124; head kidney) were singletons. Although redundancy will increase as the number of sequenced clones increases, the high percentage of singleton indicated that the complexity and coverage of those olive flounder spleen and head kidney cDNA library were good.

The number of this type of clones will indicate how well the library can provide novel genes and whether a further exploration of the cDNA library is worthy.

6. Olive flounder EST and its applications

Because of the relative scarcity of olive flounder gene resources, this study aims to increase the number of characterized olive flounder genes to facilitate other studies. The accumulation of a large number of identified cDNA clones is invaluable for olive flounder genetics and developmental biology. The cDNA clone tagging approach will rapidly build up the resource of the olive flounder 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 or cell type specific markers, isolation of full-length clones and gene promoters, and analysis of the gene expression pattern and gene function.

An immediate application of these tagged clones is to construct dense genetic and physical maps for the olive flounder genome (Postlethwait *et al.*, 1994; Knapik *et al.*, 1996), which are valuable to analysis of olive flounder mutants and an olive flounder genome project. There are several approaches to map the tagged clones to the olive flounder genome map. One is by means of single-strand conformation polymorphism (SSCP)(Beier, 1993). The 3′ untranslated sequences, which are generally polymorphic, are readily obtainable by sequencing from the 3′ end of the tagged clones, that are interesting for mapping. Another approach, which is independent of DNA polymorphism, is to construct a radiation hybrid map (Cox *et al.*, 1990). The

tagged clones can be used as probes either to hybridize the radiation panel DNAs or to use the sequence information to design PCR primers to screen the radiation hybrid panel. Similarly, these tagged clones can be used to screen olive flounder YAC, BAC and PAC libraries for construction of a physical map. Finally, these tagged clones can be used for chromosome in-situ hybirdization when the technique is available in olive flounder.

Fig. 4. Nucleotide and deduced amino acid sequences of the olive flounder granulin 2 gene

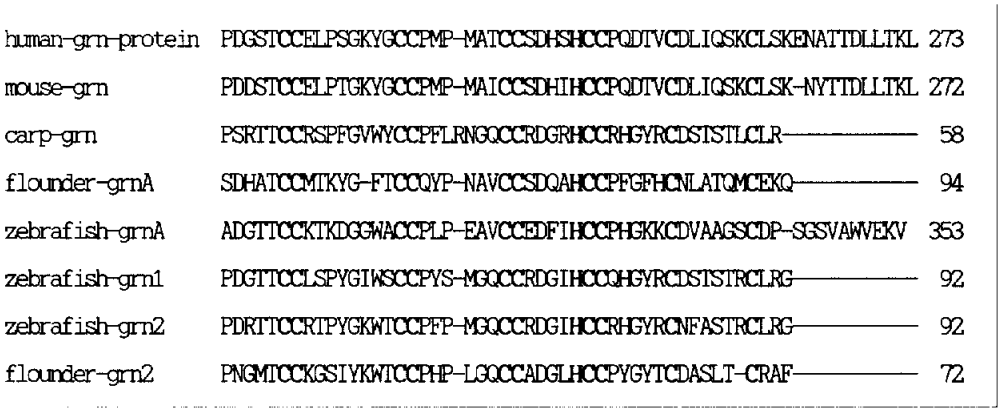

Fig. 5. Multiple alignments of the predicted flounder granulins amino acid sequence with other known granulin proteins.

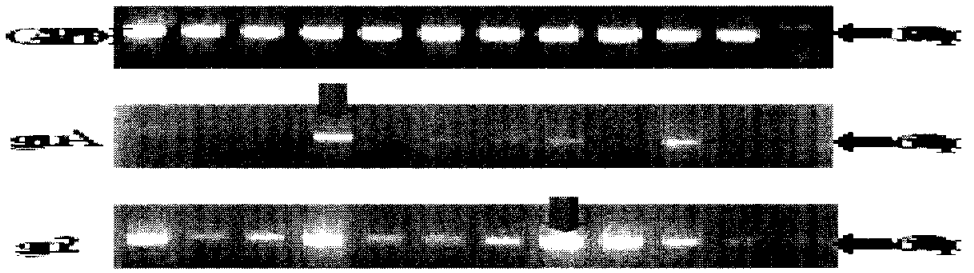

Fig. 6. Detection of mRNAs of the Olive flounder granulinA and granulin2 by RT-PCR, *lane 1* gill, *lane 2* liver, *lane 3* stomach, *lane 4* spleen, *lane 5* pyloric caeca, *lane 6* intestine, *lane* rectum, *lane 8* head kidney, *lane 9* body kidney, *lane 10* heart, *lane 11* brain, *lane 12* muscle

Table 3. List of identified ESTs from the olive flounder spleen cDNA library.

Clone no.	Genes	Closest species	Accession number	length (aa)	E-value	a.a. Homology	Identity (%)
SPLEEN-01-H09		[unknown]		0	0.00E+00	0|0	0
SPLEEN-02-A09		[unknown]		0	0.00E+00	0|0	0
SPLEEN-02-A12		[unknown]		0	0.00E+00	0|0	0
SPLEEN-02-H01		[unknown]		0	0.00E+00	0|0	0
SPLEEN-02-H09		[unknown]		0	0.00E+00	0|0	0
SPLEEN-03-C06	CSP precursor	[Locusta migratoria]	AAO16796.1	109	2.60E+00	21|89	23
SPLEEN-02-D08	536aa long hypothetical acylamino-acid-releasing enzyme	[Sulfolobus tokodaii]	NP_377716.1	536	3.20E+00	31|131	23
SPLEEN-01-G08	protein F15H18.15 [imported] - Arabidopsis thaliana	[Arabidopsis thaliana]	AAF25986.1	590	3.50E+00	30|118	25
SPLEEN-02-B08	NADH dehydrogenase subunit 5	[Cyanidioschyzon merolae]	NP_059362.1	666	8.60E-01	29|114	25
SPLEEN-02-D12	hypothetical protein	[Plasmodium falciparum 3D7]	NP_705576.1	2825	2.50E+00	17|68	25
SPLEEN-02-E10	hypothetical protein	[Staphylococcus aureus]	NP_372740.1	315	7.40E-01	24|91	26
SPLEEN-01-E06	DNA polymerase 1, putative	[Plasmodium falciparum 3D7]	NP_703911.1	1444	4.50E+00	19|72	26
SPLEEN-02-E11	lysine and histidine specific transporter	[Arabidopsis thaliana]	AAC49885.1	446	6.90E+00	16|59	27
SPLEEN-03-E06	hypothetical protein	[Schizosaccharomyces pombe]	NP_595526.1	309	1.10E+00	28|103	27
SPLEEN-03-H05	GLP_435_15509_25057	[Giardia lamblia ATCC 50803]	EAA38907.1	3182	2.60E+00	21|77	27
SPLEEN-02-F08	G surface protein	[Paramecium primaurelia]	A23475	2718	1.30E+00	23|80	28
SPLEEN-03-C01	ENSANGP00000024385	[Anopheles gambiae str. PEST]	XP_309273.1	350	8.80E+00	17|60	28
SPLEEN-02-A02	hypothetical protein	[Mus musculus]	XP_153701.1	303	5.10E+00	17|57	29
SPLEEN-03-C02	putative AMINOACID TRANSPORTER	[Encephalitozoon cuniculi]	NP_597041.1	420	1.20E+00	24|81	29
SPLEEN-03-H03	prosaposin	[Gallus gallus]	AAF05899.1	518	1.00E-14	57|195	29
SPLEEN-03-E02	Indy-2-PA	[Drosophila pseudoobscura]	Indy-2-PA	505	5.70E+00	19|62	30
SPLEEN-02-A08	hypothetical protein	[Homo sapiens]	XP_212167.1	168	1.20E-01	18|60	30
SPLEEN-02-C12	hypothetical protein	[Mus musculus]	XP_206288.1	399	6.30E+00	18|59	30
SPLEEN-03-A03	Kruppel-associated box protein	[Rattus norvegicus]	XP_225081.1	635	3.50E+00	20|63	31

Table 3. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-02-C10	hypothetical protein hc1 - mouse (fragment)	[Mus musculus]	S26689	118	6.30E-02	17|54	31
SPLEEN-03-E04	ORF YGR221c	[Saccharomyces cerevisiae]	NP_011737.1	622	2.40E+00	21|66	31
SPLEEN-03-G01	putative protein	[Arabidopsis thaliana]	NP_192794.1	703	4.70E+00	14|44	31
SPLEEN-01-D05	hypothetical protein	[Plasmodium falciparum 3D7]	NP_702134.1	1431	8.40E+00	17|52	32
SPLEEN-01-F01	T-cell receptor alpha	[unknown]		136	1.00E-06	39|119	32
SPLEEN-01-F09	Cell cycle protein MesJ	[Fusobacterium nucleatum]	EAA24225.1	446	5.60E+00	18|55	32
SPLEEN-01-H12	Hypothetical protein Y75B8A.31	[Caenorhabditis elegans]	NP_499604.1	313	2.70E+00	16|49	32
SPLEEN-02-E01	NICAL protein	[Homo sapiens]	AAH09972.2	981	3.00E+00	20|61	32
SPLEEN-02-F07	similar to B lymphocyte cell adhesion molecule	[Rattus norvegicus]	XP_218523.1	970	4.00E-11	49|150	32
SPLEEN-02-F10	Unknown (protein for IMAGE:4815093)	[Homo sapiens]	AAH50356.1	224	1.70E+00	17|52	32
SPLEEN-03-B02	mannose-binding protein associated serine protease-3	[Cavia porcellus]	CAD29748.1	496	3.00E-19	60|183	32
SPLEEN-03-E03	hypothetical protein XP_156393	[Mus musculus]	XP_156393.2	636	2.00E-03	22|65	33
SPLEEN-01-A06	predicted protein	[Neurospora crassa]	XP_324921.1	483	4.00E-01	25|75	33
SPLEEN-01-G02	A/B over-sized serum albumin	[Sphenodon punctatus]	AAM46106.1	400	1.10E-01	20|59	33
SPLEEN-01-H04	integral membrane protein	[Lactococcus lactis]	AAK58893.1	270	2.20E+00	19|57	33
SPLEEN-02-D01	interleukin-8 receptor	[Paralichthys olivaceus]	BAB97378.1	356	4.00E-17	48|143	33
SPLEEN-01-B08	maturase	[Allium grayi]	BAA84045.1	375	8.80E+00	22|63	34
SPLEEN-01-C02	chemokine CCL4/MIP-1BETA	[Macaca mulatta]	AAN76071.1	92	1.00E-07	26|76	34
SPLEEN-01-E09	ORF1	[TT virus]	BAB19320.1	720	6.00E-01	16|46	34
SPLEEN-01-G03	death associated protein 4	[Homo sapiens]	NP_004696.2	761	7.00E-20	49|143	34
SPLEEN-01-B11	ferrochelatase	[Synechocystis sp. PCC 6803]	NP_442453.1	387	2.90E-01	24|68	35
SPLEEN-01-D02	conserved hypothetical protein	[Cryptosporidium parvum]	CAD98393.1	1399	3.90E+00	25|70	35
SPLEEN-02-E06	hypothetical protein	[Thermobifida fusca]	ZP_00059624.1	417	2.30E+00	21|59	35
SPLEEN-02-H11	332L [Invertebrate iridescent virus 6]	[Chilo iridescent virus]	NP_149795.1	234	8.30E+00	13|37	35
SPLEEN-01-A10	NADH dehydrogenase subunit 1	[Trypanosoma vespertilionis]	AAM75878.1	212	2.00E+00	23|63	36

Table 3. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-01-C03	ORF3	[TTV-like mini virus]	BAA86949.1	130	3.20E+00	21|58	36
SPLEEN-01-D10	Similar to RIKEN cDNA E430025L02 gene	[Homo sapiens]	AAH44233.1	692	3.00E-18	65|177	36
SPLEEN-02-B05	CG14934-PA	[Drosophila melanogaster]	NP_609522.1	584	1.70E+00	27|74	36
SPLEEN-01-A05	complement component 1,	[Homo sapiens]	NP_758957.1	245	1.00E-20	57|153	37
SPLEEN-01-E03	Similar to RIKEN cDNA 3000004N20 gene	[Danio rerio]	AAH45946.1	314	2.00E-19	57|152	37
SPLEEN-01-E10	JU0132 acylaminoacyl-peptidase (EC 3.4.19.1)	[swine, liver, Peptide, 732 aa]	AAB36056.1	732	5.90E-01	19|51	37
SPLEEN-02-D07	perforin	[Paralichthys olivaceus]	BAC76420.1	587	6.00E-39	77|208	37
SPLEEN-01-H07	hypothetical protein	[Plasmodium falciparum 3D7]	NP_704843.1	4530	7.20E-01	22|57	38
SPLEEN-02-D03	granulin-A precursor	[Danio rerio]	AAM00265.2	700	5.00E-29	61|159	38
SPLEEN-02-E04	Unknown (protein for IMAGE:6799173)	[Danio rerio]	AAH54640.1	472	1.00E-15	55|143	38
SPLEEN-03-F06	cytokine receptor common gamma chain	[Oncorhynchus mykiss]	CAC09429.2	343	1.00E-42	99|254	38
SPLEEN-02-B02	pristinamycin I synthase 2	[Streptomyces pristinaespiralis]	T30288	2591	6.90E+00	20|51	39
SPLEEN-02-G10	KIAA0924 protein	[Homo sapiens]	NP_055712.1	606	1.00E-17	62|156	39
SPLEEN-02-E05	hypothetical protein L7610.9	[Leishmania major]	T18318	148	6.30E+00	18|45	40
SPLEEN-01-C04	transcription factor nrf2	[Danio rerio]	BAC10573.1	586	4.00E-23	70|174	40
SPLEEN-02-B10	conserved hypothetical protein	[Streptococcus mutans UA159]	NP_720954.1	179	4.40E+00	16|40	40
SPLEEN-02-E09	hypothetical protein	[Plasmodium falciparum 3D7]	NP_703631.1	719	1.60E+00	19|47	40
SPLEEN-03-A02	CR3	[unknown]		70	7.20E-01	18|45	40
SPLEEN-01-C07	similar to phenylalanine-tRNA synthetase	[Rattus norvegicus]	XP_236223.1	605	2.00E-15	37|90	41
SPLEEN-01-C11	invariant chain-like protein 14-1	[Oncorhynchus mykiss]	AAL58575.1	201	2.00E-24	42|99	42
SPLEEN-01-A12+A56	HUMAN Phosducin-like protein (PHLP)	[Homo sapiens]	Q13371	301	8.00E-29	72|171	42
SPLEEN-01-B05	erythrocyte membrane protein 1 (PfEMP1)	[Plasmodium falciparum 3D7]	NP_704249.1	2595	3.80E+00	17|40	42
SPLEEN-01-D01	invariant chain-like protein 14-1	[Oncorhynchus mykiss]	AAL58575.1	201	7.00E-33	76|180	42
SPLEEN-03-A01	Similar to general transcription factor IIA, 1, 19/37kDa	[Danio rerio]	AAH48894.1	369	5.00E-28	82|191	42
SPLEEN-03-B08	ribonuclease inhibitor, hepatic - pig	[pig]	P10775	456	4.00E-17	51|120	42

Table 3. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-01-E07	Hypothetical protein	[Sulfolobus solfataricus]	NP_342744.1	692	4.20E+00	21|47	44
SPLEEN-02-F12	unnamed protein product	[Mus musculus]	BAC34644.1	76	2.00E-07	33|74	44
SPLEEN-01-A07	NADH dehydrogenase subunit 3	[Paralichthys olivaceus]	NP_037589.1	116	7.00E-14	44|96	45
SPLEEN-03-G04	ORF103R	[necrosis virus]	NP_612325.1	133	9.00E-09	26|56	46
SPLEEN-03-D01	unnamed protein product	[Mus musculus]	BAC26716.1	802	4.00E-50	96|205	46
SPLEEN-03-A06	Unknown (protein for MGC:799)	[Homo sapiens]	AAH09930.1	250	5.00E-28	61|125	48
SPLEEN-03-F05	Similar to chromosome 20 open reading frame 149	[Xenopus laevis]	AAH41266.1	113	5.00E-12	32|65	49
SPLEEN-02-E02	tetraspan NET-5	[Homo sapiens]	NP_006666.1	239	5.00E-22	48|97	49
SPLEEN-01-F10	smarce1-related protein	[Homo sapiens]	AAG01174.1	317	4.00E-50	104|204	50
SPLEEN-02-H04	homeobox protein XHox11	[Xenopus laevis]	AAG14453.1	312	2.00E-46	111|220	50
SPLEEN-03-A04	salivary gland secretion protein 4	[Drosophila melanogaster]	CAA88613.1	378	2.00E-21	41|81	50
SPLEEN-03-E01	sphingosine-1-phosphatase	[Homo sapiens]	NP_110418.1	441	1.00E-10	27|53	50
SPLEEN-03-H02	hypothetical protein T01B11.1	[Caenorhabditis elegans]	T25847	361	5.30E+00	15|30	50
SPLEEN-03-B03	similar to RIKEN cDNA 1110004B13	[Rattus norvegicus]	XP_213332.1	140	3.00E-21	54|105	51
SPLEEN-03-F01	putative membrane-associated oxidoreductase	[Streptomyces coelicolor A3(2)]	NP_624409.1	503	4.60E+00	14|27	51
SPLEEN-03-G03	beta-2 microglobulin precursor	[Ictalurus furcatus]	AAC64994.1	108	5.00E-23	30|57	52
SPLEEN-02-H02	ubiquitin-like protein	[Oncorhynchus mykiss]	AAO14689.1	156	4.00E-37	79|150	52
SPLEEN-03-D02	RIKEN cDNA 9430067K14 gene	[Mus musculus]	XP_136665.2	761	4.80E-02	15|28	53
SPLEEN-01-F04	novel protein	[Homo sapiens]	AAH54692.1	374	2.00E-54	109|201	54
SPLEEN-02-B01	Unknown (protein for MGC:66368)	[Danio rerio]	AAH54692.1	458	1.00E-33	64|117	54
SPLEEN-01-C06	sorting nexin 10	[Homo sapiens]	NP_037454.2	201	4.00E-43	85|152	55
SPLEEN-02-F02	F5-2	[Mus musculus]	BAA89662.1	305	2.00E-17	45|81	55
SPLEEN-01-G09	RIKEN cDNA 0610041D24	[Mus musculus]	NP_079617.2	336	1.00E-34	70|124	56
SPLEEN-02-D06	swelling dependent chloride channel	[Danio rerio]	NP_571499.1	249	2.00E-49	109|194	56
SPLEEN-02-E03	UMP synthase	[Homo sapiens]	BAA19921.1	480	6.00E-63	64|113	56

Table 3. *(continued)*

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-02-A10	lens epithelium-derived growth factor - human	[erived growth factor - human]	JC7168	530	6.00E-48	92|161	57
SPLEEN-03-C05	immunoglobulin light chain L2	[Oncorhynchus mykiss]	AAB41310.1	247	6.00E-48	101|171	59
SPLEEN-01-B03	pleckstrin homology domain, family B (evectins) member 2	[Danio rerio]	AAH45398.1	223	2.00E-55	107|178	60
SPLEEN-01-D04	dolichyl-phosphate mannosyltransferase polypeptide 3	[Rattus norvegicus]	XP_215615.1	92	4.00E-24	55|91	60
SPLEEN-01-F02	ribokinase	[Homo sapiens]	NP_071411.1	322	8.00E-66	128|210	60
SPLEEN-02-H12	biliverdin-IX beta reductase isozyme I {EC 1.3.1.24}	[human, liver, Peptide, 204 aa]	AAB29537.1	204	1.00E-59	119|195	61
SPLEEN-03-E05	ribonuclease P (14kD)	[Homo sapiens]	NP_008973.1	124	2.00E-35	74|120	61
SPLEEN-01-F07	endothelial cell growth factor 1	[Homo sapiens]	NP_001944.1	482	2.00E-64	124|197	62
SPLEEN-03-B06	BCL2-associated athanogene	[Homo sapiens]	NP_004314.2	345	4.00E-51	84|134	62
SPLEEN-03-B05	Unknown (protein for MGC:64255)	[Xenopus laevis]	AAH54148.1	211	3.00E-63	81|127	63
SPLEEN-01-D06	unnamed protein product	[Homo sapiens]	BAB14193.1	800	4.00E-74	89|138	64
SPLEEN-03-A08	mutant lysosomal beta-galactosidase	[Felis catus]	AAB86405.1	669	1.00E-16	40|62	64
SPLEEN-03-H04	H-protein	[Gallus gallus]	P11183	164	1.00E-48	62|94	65
SPLEEN-01-E02	40S RIBOSOMAL PROTEIN S15	[Xiphophorus maculatus]	P70066	145	3.00E-46	97|145	66
SPLEEN-01-G11	HSCH5 histone H5	[chicken]	HSCH5	189	4.00E-22	52|78	66
SPLEEN-02-E07	major histocompatibility class I receptor	[Stizostedion vitreum]	AAL11413.1	378	2.00E-67	123|183	67
SPLEEN-01-A03	matrix metalloproteinase	[Oncorhynchus mykiss]	BAB19131.1	475	3.00E-23	50|74	67
SPLEEN-02-B03	Cofilin 2, muscle	[Mus musculus]	NP_031714.1	166	7.00E-59	111|164	67
SPLEEN-02-H05	RIKEN cDNA 2410013I23	[Mus musculus]	XP_136156.1	518	8.00E-67	104|154	67
SPLEEN-03-C04	1-acylglycerol-3-phosphate O-acyltransferase 3	[Danio rerio]	AAH49474.1	377	4.00E-64	94|140	67
SPLEEN-01-D03	H+ transporting lysosomal ATPase subunit 1	[Danio rerio]	NP_775372.1	459	5.00E-18	42|61	68
SPLEEN-01-H01+A112	Hemoglobin alpha-B chain	[Seriola quinqueradiata]	Q9PVM3	144	7.00E-50	99|144	68
SPLEEN-03-A05	Unknown (protein for MGC:64143)	[Danio rerio]	AAH53279.1	280	7.00E-95	104|149	69
SPLEEN-02-F09	MHC class II protein	[Morone saxatilis]	AAA49380.1	250	2.00E-81	129|182	70
SPLEEN-01-D12+A84	TAP1 protein	[Oncorhynchus mykiss]	AAD53033.1	739	5.00E-85	156|220	70

Table 3. *(continued)*

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-02-C04	similar to RIKEN cDNA 2610018L09	[Rattus norvegicus]	XP_224622.1	148	1.00E-19	39|55	70
SPLEEN-01-F06	nin one binding protein	[Homo sapiens]	NP_054781.1	412	9.00E-74	129|181	71
SPLEEN-02-H06	Similar to CGI-135 protein	[Homo sapiens]	AAH03540.1	152	2.00E-44	86|121	71
SPLEEN-01-H05	immunoglobulin light chain precursor	[Seriola quinqueradiata]	BAB59096.1	245	9.00E-57	102|140	72
SPLEEN-01-C10	Similar to neuronal protein	[Danio rerio]	AAH44160.1	201	3.00E-81	144|198	72
SPLEEN-03-D03	ribosomal protein L6	[Ictalurus punctatus]	AAK95130.1	260	5.00E-66	126|173	72
SPLEEN-01-C01	liver annexin-like protein	[Rattus norvegicus]	AAF36518.1	472	2.00E+00	14|19	73
SPLEEN-01-C08	Protective protein for beta-galactosidase	[Mus musculus]	AAH18534.1	474	2.00E-93	159|216	73
SPLEEN-02-G03	Similar to cyclin G1	[Danio rerio]	AAH52125.1	299	2.00E-91	166|225	73
SPLEEN-03-B04	hypothetical protein FLJ10830	[Homo sapiens]	Q96KP4	475	3.00E-99	160|217	73
SPLEEN-03-C03	Rho family small GTPase	[Homo sapiens]	Q9HBH0	211	4.00E-41	60|80	75
SPLEEN-01-G04	matrix metalloproteinase	[Oncorhynchus mykiss]	BAB19131.1	475	7.00E-97	127|163	77
SPLEEN-03-H01	chemokine (C-X-C motif) ligand 12	[Danio rerio]	NP_840092.1	99	1.00E-24	49|63	77
SPLEEN-02-C01	p100 co-activator variant 1	[Danio rerio]	AAP23062.1	888	1.00E-75	145|182	79
SPLEEN-02-D04	carboxypeptidase N	[Mus musculus]	NP_109628.1	457	3.00E-07	25|31	80
SPLEEN-02-G02	glycyl-tRNA synthetase	[Homo sapiens]	NP_002038.1	685	7.00E-92	157|196	80
SPLEEN-02-G08	similar to KIAA1033 protein	[Homo sapiens]	XP_035313.5	1173	5.00E-97	171|212	80
SPLEEN-02-G11	UDP-glucuronic acid decarboxylase 1	[Danio rerio]	NP_775349.1	418	5.00E-38	78|97	80
SPLEEN-02-B06	dihydropyrimidine dehydrogenase	[Mus musculus]	NP_740748.1	1025	0.00E+00	188|230	81
SPLEEN-03-G06	Pleckstrin	[Oncorhynchus mykiss]		352	7.00E-19	43|53	81
SPLEEN-02-G09	immunoglobulin D	[Paralichthys olivaceus]	BAB41204.1	998	4.00E-95	177|214	82
SPLEEN-02-D02	B-cell associated protein	[Homo sapiens]	NP_009204.1	299	8.00E-71	133|161	82
SPLEEN-03-C07	Similar to splicing factor 30	[Danio rerio]	AAH45967.1	237	2.00E-46	97|118	82
SPLEEN-01-A04	heat shock protein hsp90 beta	[Salmo salar]	AAD30275.1	722	4.00E-98	183|218	83
SPLEEN-01-D07	Glycerol-3-phosphate dehydrogenase 1	[Danio rerio]	AAH53116.1	349	0.00E+00	176|212	83

Table 3. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-02-D09	Elongation factor 2 (EF-2)	[unknown]	Q90705	858	3.00E-95	169|200	84
SPLEEN-01-B01	cytochrome c oxidase subunit II	[Paralichthys olivaceus]	NP_037585.1	230	1.00E-94	175|208	84
SPLEEN-01-F12	cAMP-specific phosphodiesterase isoform PDE4B4	[Rattus norvegicus]	AAL31764.1	659	0.00E+00	190|225	84
SPLEEN-02-H08+A180	similar to rab11-binding protein	[Homo sapiens]	AAH28697.1	913	0.00E+00	163|193	84
SPLEEN-03-A07	60S ribosomal protein L10a	[Ictalurus punctatus]	Q90YV8	216	2.00E-95	129|152	84
SPLEEN-01-E12	Similar to chaperonin subunit 7 (eta)	[Xenopus laevis]	AAH42312.1	561	9.00E-93	174|204	85
SPLEEN-03-B07	ornithine decarboxylase 1	[Danio rerio]	NP_571876.1	461	1.00E-59	110|128	85
SPLEEN-03-D05	Similar to RIKEN cDNA 1200015K23 gene	[Homo sapiens]	AAH41699.1	659	1.00E-23	49|57	85
SPLEEN-01-D11	phosphogluconate dehydrogenase	[Homo sapiens]	NP_002622.1	483	0.00E+00	186|214	86
SPLEEN-01-F05	ribosomal protein L3	[Ictalurus punctatus]	AAK95126.1	402	0.00E+00	196|227	86
SPLEEN-02-F01	helios	[Raja eglanteria]	AAF87270.1	522	3.50E+00	13|15	86
SPLEEN-01-H10	Unknown (protein for MGC:64097)	[Danio rerio]	AAH53250.1	552	0.00E+00	159|181	87
SPLEEN-03-B01	cytochrome b	[Paralichthys olivaceus]	NP_037594.1	380	8.00E-94	174|197	88
SPLEEN-01-B10	ferritin heavy subunit	[Oreochromis mossambicus]	AAP22046.1	157	2.00E-69	119|134	88
SPLEEN-01-D09	SI:bZ1G13.1 (novel protein)	[Danio rerio]	CAD60628.1	282	0.00E+00	196|222	88
SPLEEN-01-G01	Unknown (protein for MGC:24596)	[Homo sapiens]	AAH26296.1	137	1.00E-28	60|68	88
SPLEEN-02-G01	cytochrome c oxidase subunit III	[Paralichthys olivaceus]	AAH26296.1	261	5.00E-95	176|198	88
SPLEEN-02-G12	oligosaccharyltransferase 48 kDa subunit	[dog]	Q05052	445	2.00E-70	129|145	88
SPLEEN-01-E05	Similar to hypothetical protein LOC215449	[Xenopus laevis]	AAH44988.1	184	2.00E-73	139|155	89
SPLEEN-02-C02	ribosomal protein L15	[Lateolabrax japonicus]	AAP35254.1	204	1.00E-85	154|172	89
SPLEEN-02-H10	ribosomal protein L5a	[Ictalurus punctatus]	AAK95128.1	296	4.00E-96	172|192	89
SPLEEN-03-H06	Similar to aspartyl-tRNA synthetase	[Xenopus laevis]	AAH42227.1	530	5.00E-68	123|137	89
SPLEEN-02-D11	60S acidic ribosomal protein P0	[Rana sylvatica]	Q9DG68	315	8.00E-99	181|200	90
SPLEEN-01-B04	ribosomal protein L8	[Xenopus laevis]	P41116	257	0.00E+00	193|211	91
SPLEEN-01-F11	mitogen-activated protein kinase kinase 1 interacting protein 1	[Homo sapiens]	NP_068805.1	124	2.00E-56	110|120	91

Table 3. *(continued)*

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
SPLEEN-02-A01	ribosomal protein L17	[Paralichthys olivaceus]	AAF61071.1	184	5.00E-71	132|145	91
SPLEEN-02-D05	syntaxin binding protein 2	[Mus musculus]	NP_035633.1	593	3.00E-11	33|36	91
SPLEEN-02-E08	heat shock factor binding protein 1	[Homo sapiens]	NP_001528.1	76	3.00E-24	56|61	91
SPLEEN-01-G06	Similar to eukaryotic translation initiation factor 3	[Danio rerio]	AAH49046.1	293	0.00E+00	208|223	93
SPLEEN-02-F04	src-family tyrosine kinase SCK	[Salmo salar]	AAG38611.1	502	0.00E+00	192|206	93
SPLEEN-02-G04	Similar to cell division cycle 42 homolog (S. cerevisiae)	[Danio rerio]	AAH48035.1	191	0.00E+00	179|191	93
SPLEEN-02-H03	Homo sapiens ubiquitin-conjugating enzyme E2G 2	[synthetic construct]	AAP36286.1	166	3.00E-88	154|165	93
SPLEEN-02-C08	beta prime COP	[Rattus norvegicus]	NP_068533.1	905	0.00E+00	206|217	94
SPLEEN-03-D04+A207	Unknown (protein for MGC:64142)	[Danio rerio]	AAH53278.1	254	6.00E-03	18|19	94
SPLEEN-01-H08	receptor for activated protein kinase C	[Oreochromis niloticus]	O42249	317	0.00E+00	202|211	95
SPLEEN-01-B02	unnamed protein product	[Mus musculus]	BAC38743.1	451	0.00E+00	197|204	96
SPLEEN-02-D10	similar to ribosomal protein S26	[Rattus norvegicus]	XP_221359.1	138	7.00E-38	75|78	96
SPLEEN-01-F03	cytosolic malate dehydrogenase A	[Oryzias latipes]	AAO26197.1	333	1.00E-52	104|107	97
SPLEEN-02-F05	Similar to signal sequence receptor, gamma	[Danio rerio]	AAH47859.1	185	1.00E-59	117|120	97
SPLEEN-01-C12	Similar to DC2 protein	[Danio rerio]	AAH49047.1	149	1.00E-79	147|149	98
SPLEEN-02-A04	chicken-type lysozyme	[Paralichthys olivaceus]	BAB17215.1	143	2.00E-76	129|130	99
SPLEEN-02-F03	40S ribosomal protein S5	[Ictalurus punctatus]	AAK95187.1	203	0.00E+00	201|203	99
SPLEEN-03-F04	ribosomal protein S3	[Mus musculus]	NP_036182.1	243	2.00E-92	173|174	99
SPLEEN-02-C11	Beta-2-microglobulin precursor	[Paralichthys olivaceus]	Q8AYH8	115	1.00E-54	99|99	100
SPLEEN-02-H07	polyubiquitin	[Homo sapiens]	BAA09860.1	611	0.00E+00	222|222	100
SPLEEN-02-C03	calmodulin 2 (phosphorylase kinase, delta)	[Mus musculus]	NP_001734.1	149	1.00E-79	149|149	100

Table 4. List of identified ESTs from the olive flounder head kidney cDNA library.

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-03-D02		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-02-G10		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-01-B05		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-01-D02		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-02-B10		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-02-D07		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-02-F10		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-03-A08		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-03-E04		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-03-E06		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-03-F05		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-03-F06		[unknown]		0	0.00E+00	0|0	0
HEADKIDNEY-03-D06	CSP precursor	[Locusta migratoria]	AAO16796.1	109	2.80E+00	20|89	22
HEADKIDNEY-02-G02	hypothetical protein	[Plasmodium falciparum 3D7]	NP_702209.1	1518	7.10E+00	22|95	23
HEADKIDNEY-02-B11	unknown	[Homo sapiens]	AAL55824.1	400	1.30E+00	23|93	24
HEADKIDNEY-02-E09	hypothetical protein	[Plasmodium falciparum 3D7]	NP_704180.1	3351	8.00E+00	31|126	24
HEADKIDNEY-03-C04	hypothetical protein	[Plasmodium falciparum 3D7]	NP_703824.1	1680	3.50E+00	15|54	27
HEADKIDNEY-02-B04	ENSANGP00000018854 [	[Anopheles gambiae str. PEST]	XP_310402.1	603	5.00E+00	25|88	28
HEADKIDNEY-03-A03	similar to tweety homolog 2 (Drosophila)	[Rattus norvegicus]	XP_221081.1	770	9.00E+00	21|73	28
HEADKIDNEY-03-E01	hypothetical protein	[Ralstonia metallidurans]	ZP_00026693.1	144	9.30E+00	20|68	29
HEADKIDNEY-02-C06	similar to HSP60 protein (AA 1-547)	[Rattus norvegicus]	XP_223918.1	809	9.40E+00	21|72	29
HEADKIDNEY-02-E06	similar to mannose receptor, C type 1	[Rattus norvegicus]	XP_225585.1	835	6.00E-07	40|131	30
HEADKIDNEY-01-G09	predicted protein	[Neurospora crassa]	XP_324612.1	1001	6.40E-01	22|71	30
HEADKIDNEY-02-G01	putative retroelement	[Oryza sativa]	AAM74403.1	1844	3.70E-01	15|50	30

Table 4. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-02-D09	hypothetical protein	[Plasmodium falciparum 3D7]	NP_702830.1	4832	7.50E+00	18|58	31
HEADKIDNEY-01-B12	Sodium phosphate transporter family member	[Caenorhabditis elegans]	NP_506066.1	377	4.80E+00	26|82	31
HEADKIDNEY-01-H05	zinc finger protein Gli2	[Xenopus laevis]	AAD28180.1	1354	4.20E+00	29|89	32
HEADKIDNEY-02-B01	protein kinase ATR	[Homo sapiens]	NP_001175.1	2644	5.40E+00	14|43	32
HEADKIDNEY-02-C07	Similar to profilin 2	[Danio rerio]	AAH45843.1	139	7.00E-16	46|140	32
HEADKIDNEY-02-E04	Dihydrodipicolinate synthase/N-acetylneuraminate lyase	[Vibrio vulnificus CMCP6]	NP_762672.1	299	1.30E+00	16|50	32
HEADKIDNEY-03-G07	apoptosis-associated speck-like protein containing CARD	[Mus musculus]	Q9EPB4	193	2.00E-05	37|112	33
HEADKIDNEY-01-G06	leucine-rich repeat extensin family	[Arabidopsis thaliana]	NP_175372.1	847	2.00E-04	25|74	33
HEADKIDNEY-01-H10	unnamed protein product	[Mus musculus]		219	4.00E-15	47|131	35
HEADKIDNEY-01-C10	expressed protein	[Arabidopsis thaliana]	NP_194947.2	1399	7.40E+00	18|49	36
HEADKIDNEY-02-F08	similar to HYPOTHETICAL PROTEIN DJ845O24.1	[Rattus norvegicus]	XP_223251.1	951	5.90E-01	18|50	36
HEADKIDNEY-02-H08	similar to seven transmembrane helix receptor	[Homo sapiens]	XP_115093.3	483	2.20E-01	23|63	36
HEADKIDNEY-02-F09	invariant chain like protein 1	[Cyprinus carpio]	BAC53767.1	234	2.00E-34	89|235	37
HEADKIDNEY-01-A09	PALS2-beta splice variant	[Mus musculus]	Q9JLB0	553	5.10E+00	15|40	37
HEADKIDNEY-01-D06	fls485	[Homo sapiens]	NP_057015.1	353	1.00E-46	90|240	37
HEADKIDNEY-02-A09	hypothetical protein XP_151231	[Mus musculus]	XP_151231.1	267	9.70E+00	10|27	37
HEADKIDNEY-02-B05	putative beta-N-Acetylglucosaminidase	[Streptomyces avermitilis]	NP_824570.1	545	1.30E+00	24|64	37
HEADKIDNEY-02-C01	interferon alpha-7	[Mus musculus]		190	1.50E+00	14|37	37
HEADKIDNEY-03-F02	Similar to profilin II	[Xenopus laevis]	AAH46719.1	140	1.60E-02	22|59	37
HEADKIDNEY-02-F01	unknown	[Fusobacterium nucleatum]	NP_602682.1	64	5.80E+00	19|50	38
HEADKIDNEY-03-C06	ENSANGP00000012903	[Anopheles gambiae str. PEST]	XP_306147.1	70	6.00E-05	25|64	39
HEADKIDNEY-01-F11	Similar to complement component 1	[Mus musculus]	AAH27183.1	694	1.00E-20	54|132	40
HEADKIDNEY-02-D08	hypothetical protein	[Cytophaga hutchinsonii]	ZP_00117949.1	302	8.00E+00	16|40	40
HEADKIDNEY-03-F01	chorion class A protein L12 precursor	[Bombyx mori]	P08825	132	3.90E-01	17|42	40
HEADKIDNEY-01-F09	hypothetical protein FLJ32028	[Homo sapiens]	NP_689893.1	183	2.00E-04	23|55	41

Table 4. *(continued)*

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-03-H04	invariant chain-like protein 14-1	[Oncorhynchus mykiss]	AAL58575.1	201	3.00E-14	46|108	42
HEADKIDNEY-03-A02	hypothetical protein	[Plasmodium falciparum 3D7]	NP_472954.2	1353	1.50E+00	15|34	44
HEADKIDNEY-03-B03	RIKEN cDNA 2210418J09	[Mus musculus]	XP_133265.2	124	2.00E-20	50|112	44
HEADKIDNEY-03-H06	hypothetical protein	[Plasmodium falciparum 3D7]	NP_702818.1	2418	6.70E+00	20|45	44
HEADKIDNEY-02-H10	unknown protein	[Arabidopsis thaliana]	NP_200204.1	529	5.30E+00	20|44	45
HEADKIDNEY-03-B05	matrix metalloproteinase 17	[Homo sapiens]	NP_057239.2	606	5.00E-12	31|66	46
HEADKIDNEY-01-H08	complement subcomponent C1q chain C precursor	[human]	C1HUQC	245	5.00E-25	58|126	46
HEADKIDNEY-02-E12	hypothetical protein	[Neurospora crassa]	CAB91386.2	338	1.80E+00	19|41	46
HEADKIDNEY-01-G08	conserved hypothetical protein	[Pyrobaculum aerophilum str. IM2]	NP_560577.1	247	7.20E-01	16|34	47
HEADKIDNEY-02-B02	FcR gamma subunit	[Ictalurus punctatus]	AAN38001.1	87	1.00E-13	33|69	47
HEADKIDNEY-01-G10	mitochondrial ribosomal protein L16	[Homo sapiens]	NP_060310.1	251	2.00E-30	63|128	49
HEADKIDNEY-03-E07	granulin 2	[Danio rerio]	AAK58709.1	147	5.00E-17	37|74	50
HEADKIDNEY-02-H09	similar to hypothetical protein MGC7434	[Rattus norvegicus]	XP_227711.1	791	4.00E-01	18|35	51
HEADKIDNEY-03-E05	rhamnose binding lectin STL2	[Oncorhynchus mykiss]	BAA92256.2	218	1.00E-20	48|92	52
HEADKIDNEY-03-H07	phospholipase C	[Homo sapiens]	NP_002652.1	1252	1.00E-28	68|129	52
HEADKIDNEY-03-C05	Similar to hypothetical protein FLJ11273	[Danio rerio]	AAH50177.1	282	2.00E-19	42|79	53
HEADKIDNEY-01-H06	unnamed protein product	[Homo sapiens]	BAC04597.1	323	3.20E-02	24|44	54
HEADKIDNEY-01-G11	histone H1	[Oncorhynchus mykiss]	P06350	207	1.00E-20	50|88	56
HEADKIDNEY-02-H03	CD45	[Takifugu rubripes]	CAB96212.1	1245	2.00E-57	114|202	56
HEADKIDNEY-01-C12	similar to mitochondrial ribosomal protein 63	[Rattus norvegicus]	XP_214181.1	102	1.00E-13	32|55	58
HEADKIDNEY-02-C12	Id2 protein	[Oncorhynchus mykiss]	CAA69657.1	135	2.00E-07	30|51	58
HEADKIDNEY-02-G06	neutrophil cytosolic factor 1	[Rattus norvegicus]	AAO32681.1	389	1.00E-01	14|24	58
HEADKIDNEY-01-A04	leukemia virus-b receptor	[Felis catus]	AAD08995.1	681	3.00E-63	121|204	59
HEADKIDNEY-01-A06	TNF family B cell activation factor	[Gallus gallus]	AAM90951.2	288	4.00E-24	53|89	59
HEADKIDNEY-02-D03	HLA class II region expressed gene KE2	[Homo sapiens]	NP_055075.1	129	3.00E-32	68|109	62

Table 4. *(continued)*

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-03-G02	nephrosin precursor	[Cyprinus carpio]	AAB62737.1	273	1.00E-64	118|183	64
HEADKIDNEY-01-A12	AF401552_1 ribosomal protein P1	[Ictalurus punctatus]	AAK95124.1	113	2.00E-32	73|113	64
HEADKIDNEY-01-D10	HEM1 protein	[Homo sapiens]	AAH01604.2	875	1.00E-82	149|229	65
HEADKIDNEY-02-H11	Hemoglobin alpha-B chain	[Seriola quinqueradiata]	Q9PVM3	144	8.00E-50	100|144	69
HEADKIDNEY-01-D11	unnamed protein product	[Mus musculus]	BAB22140.1	171	4.00E-53	116|168	69
HEADKIDNEY-01-A05	apolipoprotein E	[Scophthalmus maximus]	CAB65356.1	224	6.00E-05	22|31	70
HEADKIDNEY-01-H07	Similar to protein kinase C substrate 80K-H	[Danio rerio]	AAH46883.1	529	5.00E-87	153|216	70
HEADKIDNEY-03-D07	hypothetical protein [Frankia sp. CpI1]	[Frankia sp. CpI1]	NP_085458.1	396	2.80E+00	12|17	70
HEADKIDNEY-03-H02	unnamed protein product	[Mus musculus]	BAC31585.1	216	2.00E-51	94|131	71
HEADKIDNEY-03-F07	AAL09414.1STAT1	[Tetraodon fluviatilis]	AAL09414.1	758	3.00E-63	122|162	75
HEADKIDNEY-02-E01	Similar to hypothetical protein MGC3067	[Danio rerio]	AAH45413.1	256	1.00E-27	58|77	75
HEADKIDNEY-01-G05	stomatin	[Danio rerio]	NP_571833.1	284	4.00E-32	74|96	77
HEADKIDNEY-03-H05	Unknown (protein for MGC:64056)	[Danio rerio]	AAH53229.1	369	8.00E-71	130|167	77
HEADKIDNEY-03-B06	Carbonmonoxy Hemoglobin	[unknown]	1SPG	144	4.00E-29	63|80	78
HEADKIDNEY-01-D03	Similar to cyclin G1	[Danio rerio]	AAH52125.1	299	3.00E-58	113|144	78
HEADKIDNEY-02-B12	Unknown (protein for MGC:64027)	[Danio rerio]	AAH53210.1	584	3.00E-39	73|93	78
HEADKIDNEY-03-H03	Similar to high mobility group box 1	[Danio rerio]	AAH45917.1	205	2.00E-73	131|167	78
HEADKIDNEY-01-G03	Unknown (protein for MGC:55316)	[Danio rerio]	AAH44366.1	348	2.00E-57	102|129	79
HEADKIDNEY-01-H04	40S ribosomal protein S30	[Ictalurus punctatus]	AAK95215.1	133	4.00E-53	106|133	79
HEADKIDNEY-01-A10	Similar to chromosome 15 open reading frame 15	[Danio rerio]	AAH51780.1	161	4.00E-50	96|118	81
HEADKIDNEY-01-E10	NADH dehydrogenase:ubiquinone Fe-S protein 8	[Mus musculus]	AAM34451.1	212	1.00E-30	63|77	81
HEADKIDNEY-03-B01	unnamed protein product	[Mus musculus]	BAB27540.1	79	4.00E-33	62|76	81
HEADKIDNEY-01-C09	Unknown (protein for MGC:55303)	[Danio rerio]	AAH44363.1	402	9.00E-94	169|204	82
HEADKIDNEY-01-E06	mitochondrial ATP synthase gamma-subunit	[Cyprinus carpio]	BAB47390.1	292	1.00E-85	155|187	82
HEADKIDNEY-03-A05	peptidylprolyl isomerase (EC 5.2.1.8)	[Drosophila melanogaster]	P25007	165	1.00E-75	133|161	82

Table 4. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-01-C04	phosphoglycerate mutase 1	[Homo sapiens]	NP_002620.1	254	0.00E+00	149|179	83
HEADKIDNEY-02-B07	annexin max2	[Oryzias latipes]	CAA72123.1	317	1.00E-32	69|83	83
HEADKIDNEY-02-C08	immunoglobulin light chain	[Dicentrarchus labrax]	CAC16852.1	244	5.00E-20	45|54	83
HEADKIDNEY-02-D06	Crystal Structure Of A Mammalian 2-Cys Peroxiredoxin, Hbp23	[unknown]	1QQ2	199	2.00E-94	165|198	83
HEADKIDNEY-03-G05	hypothetical protein DKFZp566A1524	[Danio rerio]	AAH45305.1	323	0.00E+00	198|233	84
HEADKIDNEY-01-E12	hypothetical protein	[Homo sapiens]	NP_056230.1	127	1.00E-36	72|85	84
HEADKIDNEY-02-D12	unnamed protein product	[Mus musculus]	BAB27648.1	562	0.00E+00	177|210	84
HEADKIDNEY-02-G08	catalase	[Danio rerio]	Q9PT92	526	0.00E+00	183|217	84
HEADKIDNEY-02-A03	phosphogluconate dehydrogenase	[Mus musculus]	NP_080077.1	483	1.00E-47	87|102	85
HEADKIDNEY-02-H06	carbonic anhydrase	[Danio rerio]	NP_571185.1	260	8.00E-57	107|125	85
HEADKIDNEY-01-H03	HUMAN Very hypothetical protein	[unknown]	Q16465	122	8.00E-07	23|27	85
HEADKIDNEY-02-B08	glutamate-cysteine ligase, catalytic subunit	[Danio rerio]	AAH45303.1	631	0.00E+00	197|231	85
HEADKIDNEY-02-C03	aldehyde dehydrogenase 2 precursor	[Danio rerio]	AAM19352.1	516	2.00E-36	73|85	85
HEADKIDNEY-03-A07	40S ribosomal protein S4	[chicken]	P47836	263	4.00E-74	135|158	85
HEADKIDNEY-03-B07	ribosomal protein L27a	[Epinephelus coioides]	AF502247_1	148	2.00E-68	124|145	85
HEADKIDNEY-03-E02	cytochrome c oxidase subunit II	[Paralichthys olivaceus]	NP_037585.1	230	0.00E+00	188|220	85
HEADKIDNEY-01-G01	eukaryotic translation initiation factor 4A1	[Xenopus laevis]	AAH45237.1	404	0.00E+00	174|202	86
HEADKIDNEY-03-D05	Hemoglobin beta-A chain	[unknown]	Q9PVM2	148	7.00E-71	129|148	87
HEADKIDNEY-03-D04	Hemoglobin beta-A chain	[unknown]	Q9PVM2	148	9.00E-71	129|148	87
HEADKIDNEY-01-F10	ribosomal protein L18	[Oreochromis mossambicus]	AAF64457.1	188	8.00E-92	167|188	88
HEADKIDNEY-01-C02	cytochrome c oxidase subunit III	[Paralichthys olivaceus]	NP_037588.1	261	1.00E-99	184|208	88
HEADKIDNEY-02-D04	Similar to CG5057 gene product	[Homo sapiens]	AAH03353.1	135	2.00E-29	61|69	88
HEADKIDNEY-02-H02	Unknown (protein for MGC:64154)	[Danio rerio]	AAH54628.1	104	5.00E-46	90|102	88
HEADKIDNEY-03-G06	syntaxin 18	[Mus musculus]	NP_081235.1	308	2.00E-04	22|25	88
HEADKIDNEY-01-C08	cytochrome b	[Paralichthys olivaceus]	NP_037594.1	380	0.00E+00	207|232	89

Table 4. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-01-F05	solute carrier family 25 alpha, member 5	[Danio rerio]	NP_775354.1	298	0.00E+00	205|230	89
HEADKIDNEY-03-D03	Similar to acyl-Coenzyme A dehydrogenase	[Danio rerio]	AAH45911.1	424	2.00E-12	35|39	89
HEADKIDNEY-03-C07	chicken-type lysozyme	[Paralichthys olivaceus]	BAB17215.1	143	1.00E-75	131|143	91
HEADKIDNEY-01-B08	ubiquitin fusion degradation 1-like protein	[Xenopus laevis]	AAG25922.1	305	6.00E-98	174|190	91
HEADKIDNEY-01-B11	PTP-IV1 gene product	[unknown]	AAB39331.1	167	5.00E-71	126|136	92
HEADKIDNEY-02-A04	ribosomal protein S8	[Mus musculus]	NP_001003.1	208	0.00E+00	187|199	93
HEADKIDNEY-01-E04	Similar to chromobox homolog 1	[Danio rerio]	AAH45443.1	210	2.00E-34	70|75	93
HEADKIDNEY-02-F07	>methionyl aminopeptidase 2	[Homo sapiens]	NP_006829.1	478	4.00E-50	92|98	93
HEADKIDNEY-02-A06	similar to ribosomal protein S26	[Rattus norvegicus]	XP_221359.1	138	1.00E-35	72|76	94
HEADKIDNEY-02-F11	IgM precursor	[Paralichthys olivaceus]	AAF35884.1	579	0.00E+00	201|212	94
HEADKIDNEY-01-E08	myosin regulatory light chain, isoform L20-B1	[Gallus gallus]	P24032	172	6.00E-93	165|172	95
HEADKIDNEY-02-A12	RAS-related C3 botulinum substrate 2	[Mus musculus]	NP_033034.1	192	2.00E-98	172|181	95
HEADKIDNEY-03-B04	40S ribosomal protein S7	[Mus musculus]	NP_001002.1	194	1.00E-94	176|183	96
HEADKIDNEY-03-E03	60S ribosomal protein L18a	[Danio rerio]	AAH49045.1	176	1.00E-89	158|164	96
HEADKIDNEY-02-E05	receptor for activated protein kinase C	[Oreochromis niloticus]	O42249	317	0.00E+00	216|224	96
HEADKIDNEY-03-F03	ribosomal protein S12	[Oreochromis niloticus]	O13019	132	3.00E-25	54|56	96
HEADKIDNEY-02-A01	serine/threonine kinase Cdc2	[Oryzias luzonensis]	Q9DG98	303	0.00E+00	194|198	97
HEADKIDNEY-02-G11	40S ribosomal protein S3	[Ictalurus punctatus]	Q90YS2	245	1.00E-81	151|155	97
HEADKIDNEY-02-D11	40S ribosomal protein S2	[Ictalurus punctatus]	Q90YS3	277	0.00E+00	212|215	98
HEADKIDNEY-01-F07	proteasome activator subunit 2	[Paralichthys olivaceus]	AAN40737.1	242	8.00E-94	172|174	98
HEADKIDNEY-02-B09	alpha 4 subunit of 20S proteasome	[Carassius auratus]	BAA89276.1	251	0.00E+00	185|187	98
HEADKIDNEY-02-E02	ribosomal protein L37a	[Homo sapiens]	NP_000989.1	92	9.00E-48	91|92	98
HEADKIDNEY-01-E11	histone deacetylase	[Takifugu rubripes]	AF411956_6	477	4.00E-99	173|174	99
HEADKIDNEY-03-B08	immunoglobulin M	[Paralichthys olivaceus]	BAB60868.1	578	0.00E+00	185|185	100
HEADKIDNEY-01-F12	proteasome activator subunit 2	[Paralichthys olivaceus]	AAN40737.1	242	8.00E-75	140|140	100

Table 4. (*continued*)

Clone no.	Genes	Closest species	Accession number	length (a.a)	E-value	a.a. homology	Identity (%)
HEADKIDNEY-02-A08	Beta-actin 1	[Dicentrarchus labrax]	P53484	375	0.00E+00	1	100
HEADKIDNEY-02-D10	calmodulin 2 (phosphorylase kinase, delta)	[Mus musculus]	CAA43674.1	149	2.00E-74	1	100
HEADKIDNEY-02-F03	splicing factor arginine/serine-rich 3	[Paralichthys olivaceus]	AAO45173.1	168	4.00E-48	8	100
HEADKIDNEY-03-G04	40S ribosomal protein S15A	[Paralichthys olivaceus]	AAF61072.1	130	6.00E-68	127|127	100

Table 5. Putative immune-related genes isolated spleen and head kidney cDNA libraries of olive flounder.

Clone no.	Genes	Closest species	Identity (%)
SPLEEN-01-F01	T-cell receptor alpha	[*unknown*]	32
SPLEEN-02-F07	similar to B lymphocyte cell adhesion molecule	[*Rattus norvegieus*]	32
SPLEEN-02-D01	interlukin-8-receptor	[*Paralichthys olivaceus*]	33
SPLEEN-01-C02	Chemokine	[*Macaca mulatta*]	34
SPLEEN-02-D07	Peforin	[*Paralichthys olivaceus*]	37
SPLEEN-02-D03	granulin A precursor	[*Danio rerio*]	38
SPLEEN-03-F06	cytokine receptor common gamma chain	[*Oncorthynchus mykiss*]	38
SPLEEN-01-C04	transcription factor nrf2	[*Danio rerio*]	40
SPLEEN-02-A10	lens epithelim-derived growth factor human	[*Derived growth factor human*]	57
SPLEEN-01-B03	immunoglobulin light chain L2	[*Oncorthynchus mykiss*]	59
SPLEEN-01-F07	endothelial cell growth factor 1	[*Homo sapiens*]	62
SPLEEN-03-B06	BCL2-associated athanogene	[*Homo sapiens*]	62
SPLEEN-02-F09	MHC class II protein	[*Morone szxatilis*]	70
SPLEEN-01-H05	immunoglobulin light chain precursor	[*Seriola quinqueradiata*]	72
SPLEEN-03-H01	Chemokine (C-X-C motif) ligand 12	[*Danio rerio*]	77
SPLEEN-02-G09	immunoglobulin D	[*Paralichthys olivaceus*]	82
SPLEEN-02-D02	B-cell associated protein	[*Homo sapiens*]	82
SPLEEN-01-B01	cytochrome c oxidase subunit II	[*Paralichthys olivaceus*]	84
SPLEEN-03-B01	cytochrome b	[*Paralichthys olivaceus*]	88
SPLEEN-02-G01	cytochrome c oxidase subnit III	[*Paralichthys olivaceus*]	88
SPLEEN-01-F11	mitogne-activaged protein kinase I interacting protein	[*Homo sapiens*]	91
SPLEEN-02-E08	heat shock factor binding protein 1	[*Homo sapiens*]	91
SPLEEN-02-A04	chicken-type lysozyme	[*Paralichthys olivaceus*]	99

Table 5. (*continued*)

Clone no.	Genes	Closest species	Identity (%)
HEADKIDNEY-03-G07	apoptosis-associated speck-like protein containing CARD	[*Rattus norvegieus*]	32
HEADKIDNEY-02-F09	invariant chain-like protein 14-1	[*Cyprinus carpio*]	37
HEADKIDNEY-02-C01	interferon alpha-7	[*Musmusculus*]	37
HEADKIDNEY-03-F02	similar to profolin II	[*Macaca mulatta*]	37
HEADKIDNEY-03-H04	invariant chain-like protein 14-1	[*Oncorthynchus mykiss*]	42
HEADKIDNEY-03-E07	granulin2	[*Danio rerio*]	50
HEADKIDNEY-02-H03	CD45	[*Takifugu rubripes*]	56
HEADKIDNEY-01-A06	TNF family B cell activation factor	[*Gallus gallus*]	59
HEADKIDNEY-02-C08	imunoglobulin light chain	[*Dicentrarchus labrax*]	83
HEADKIDNEY-03-E02	cytochrome c oxidase subunit II	[*Paralichthys olivaceus*]	85
HEADKIDNEY-01-C02	cytochrome c oxidase subunit III	[*Paralichthys olivaceus*]	88
HEADKIDNEY-01-C08	cytochrome b	[*Paralichthys olivaceus*]	89
HEADKIDNEY-03-C07	chicken-type lysozyme	[*Paralichthys olivaceus*]	91
HEADKIDNEY-02-F11	IgM precursor	[*Paralichthys olivaceus*]	94
HEADKIDNEY-03-B08	immunoglobulin M	[*Paralichthys olivaceus*]	100

Ⅳ. Summary

A total of 348 random clones from the two olive flounder (*Paralichthys olivaceus*) cDNA libraries were partially sequenced. Theses included 195 from the spleen library, 153 from the head kidney library. The assembly program ICAtools software was used to organize the redundant ESTs into overlapping contigs. The results showed that the 348 ESTs were composed of 48 clusters (spleen : 21, head kidney : 27) and 320 singletons (spleen : 174, head kidney : 146), suggesting that the overall redundancy of the library was 22%.

Based on major function of their encoded proteins, the identified clones are classified into eleven broad categories. A related clones of Cellular organization, cell cycle and DNA processing represent high percentage. However, the reverse trend is that the regulation and cellular environment clones are relatively low percentage.

The most abundant cDNA was genes related of immune system. Particularly, the putative amino acid sequence deduced from cDNA clones, SPLEEN-02-D03 and HEAD KIDNEY-03-E07 were identified as granulins (spleen cDNA clones; granulin A precusor, head kidney cDNA clones; granulin 2). The widespread occurrence of granulin mRNA in cells of the hematopoietic system and in epithelia implies important functions in these tissues.

Because of the relative scarcity of olive flounder gene resources, this study

aims to increase the number of characterized olive flounder genes to facilitate other studies. The accumulation of a large number of identified cDNA clones is invaluable for olive flounder genetics and developmental biology. The cDNA clone tagging approach will rapidly build up the resource of the olive flounder 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 or cell type specific markers, isolation of full-length clones and gene promoters, and analysis of the gene expression pattern and gene function.

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감사의 글

본 학위논문을 마무리하면서 지금까지 많은 도움과 격려를 아끼지 않으셨던 모든 분들에게 감사의 마음을 전합니다.

지금의 논문이 있기까지 부족한 저를 가르쳐주시고 이끌어주신 공인수 교수님께 머리 숙여 감사드립니다. 그리고 많은 도움을 주셨던 공재열 교수님, 홍용기 교수님, 이형호 교수님, 김중균 교수님, 박남규 교수님, 김성구 교수님께 진심으로 감사드립니다.

가까운 곳에서 많은 관심과 격려를 아끼지 않으셨던 생명공학 연구단 이상준 단장님과 항상 인자한 미소로 대해 주셨던 지영주 연구관님께 감사드립니다. 그리고 본 논문을 수행하기 위해서 많은 지도와 격려를 아끼지 않으셨던 선배님이신 김영옥 박사님, 학문의 길로 들어선 부족한 저에게 따끔한 질책과 격려를 아끼지 않으셨던 이정호 박사님, 항상 밝은 모습으로 언니처럼 다정하신 남보혜 박사님, 문은정 박사님께 진심으로 감사드립니다.

그리고 언제나 다정한 미소로 아침마다 사무실까지 태워주시고 많은 가르침을 주셨던 박중연 박사님, 생명공학 연구단 생활을 하면서 항상 정겹게 대해 주셨던 김우진 박사님께 감사드립니다.

항상 부족한 저에게 많은 조언을 주셨던 윤호동 연구관님, 박희연 연구관님, 김봉석 연구관님, 한현섭 박사님, 안혜숙 박사님, 강정하 박사님, 박진일 박사님, 김연계 박사님께도 감사를 드립니다.

생명공학 연구단 생활에 있어 많은 도움을 주었던 김호경 선생님, 이영자 선생님께도 감사를 드립니다.

그리고 유전공학 연구실의 든든한 기둥이었던 김한우 박사님, 이제는 교수님이 되신 홍수희 박사님, 아무 것도 모르던 저를 따뜻하게 가르쳐 주고 지금까지도 저의 큰 그늘이 되어준 상숙이 언니, 항상 열심히 하는 경진 선배, 무뚝뚝하지만

정겨운 갑민 선배, 우리 실험실 막내둥이 인숙이 에게도 감사의 마음을 전하고 싶습니다.

나의 학문의 동지이자 친언니처럼 다정하게 보살펴 주는 가정이 언니, 언제나 부족한 동생을 아껴주는 려진이 언니에게도 감사의 마음을 전하며 앞으로의 미래에 보다 좋은 일들만이 생기기를 바랍니다.

학위 과정 중에 많은 가르침을 주셨던 이종희 선배님, 안선희 선배님, 김선회 선배님, 부족한 동기를 많이 챙겨주었던 이은미, 귀여운 후배 승하, 지현이, 지영이를 비롯한 실험실 식구들에게도 깊은 감사를 드립니다.

그리고 나의 평생 친구가 될 오랜 벗인 혜영이, 마음의 잘 통하는 유진이, 이제는 새댁이 된 혜정이, 언제나 나의 든든한 친구 민주, 모든 일에 열심히 하는 멋진 친구 지성이, 언제 봐도 항상 그대로인 복선이, 이제는 일터의 동기가 된 착한 영화, 언제나 유쾌한 기분을 주는 재원, 좋은 충고를 아끼지 않는 친구 현종이 에게도 감사의 마음을 전하며, 밝은 앞날을 기대합니다.

나의 실험실이자 일터인 생명공학 연구단에서 제게 따뜻한 격려를 해주었던 이경언니, 태희 언니, 지훈이 오빠, 용국이, 대심이를 비롯한 언니, 오빠, 동기들에게 고마움을 표합니다.

끝으로 항상 변함없는 믿음과 사랑으로 가장 큰 버팀목이 되어준 나의 가족, 은애 언니, 동생 현규, 그리고 세상에서 가장 사랑하는 부모님께 감사를 드립니다. 지금의 제가 있기까지 많은 분들의 도움이 있었지만 저의 존경하는 아버지, 어머니의 은혜가 가장 큰 것 같습니다. 항상 건강하시길 바랍니다. 작지만 소중한 저의 노력이 담긴 이 논문을 부모님께 바칩니다.