

## Immune Gene Discovery by Expressed Sequence Tags Generated from Olive Flounder (*Paralichthys olivaceus*) Kidney

Jeong-Ho Lee, Young-Ok Kim<sup>1</sup>, Jong-Hyun Kim, Jae Koo Noh,  
Hyun Chul Kim, Kyung-Kil Kim\* and Kyu-Won Kim<sup>2</sup>

Fish Genetics and Breeding Research Center, National Fisheries Research and  
Development Institute, Geoje 656-842, Korea

<sup>1</sup>Biotechnology Research Center, National Fisheries Research and  
Development Institute, Busan 619-900, Korea

<sup>2</sup>Research Institute of Pharmaceutical Sciences, College of Pharmacy,  
Seoul National University, Seoul 151-742, Korea

**Expressed sequence tag (EST) analysis was conducted using a complementary DNA (cDNA) library made from the kidney mRNA of olive flounder (*Paralichthys olivaceus*). In the survey of 390 ESTs chosen from the kidney cDNA library, 250 ESTs showed significant homology to previously described genes while 140 ESTs were unidentified or novel. Comparative analysis of the 250 identified ESTs showed that 14 (5.6%) clones were representing 11 unique genes identified as homologous to the previously reported olive flounder ESTs, 198 (79.2%) clones representing 160 unique genes were identified as orthologs of known genes from other organisms, and orthologs were established for 38 (15.2%) clones representing 37 genes of known sequences with unknown functions. We also identified several kinds of immune associated proteins, indicating EST as a powerful method for identifying immune-related genes of fish as well as identifying novel genes. Further studies using cDNA microarrays are needed to identify the differentially expressed transcripts after disease infection.**

**Key words : expressed sequence tags (ESTs), expression profile, olive flounder (*Paralichthys olivaceus*), kidney, immune gene**

### 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.

Recently, 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

\*Corresponding author: k48722@momaf.go.kr

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 flounder (Aoki *et al.*, 1999; Nam *et al.*, 2000) and 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.

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 cataloging and profiling of the kidney is an 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 defense barriers and specific immune functions; the latter includes T- and B-cell mediated cellular and humoral immunity (Partula *et al.*, 1995; Yamaguchi *et al.*, 1996; Passer *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. The objectives of this study were to identify genes and their expression profiles in the kidney, and to develop EST resources for functional genomic studies. As part of immune gene discovery by expressed sequence tag analysis, we report analysis of 390 clones from the olive flounder (*Paralichthys olivaceus*) kidney.

## Materials and Methods

### 1. Fish and tissue preparation

Olive flounders used in this study were maintained in 6 tons flow-through tank at  $12 \pm 1^\circ\text{C}$  under a natural photoperiod. Kidney tissues from 10 fishes were collected and cut into as small pieces as possible. Pooled kidney 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 (Chomczynski and Sacchi, 1987).

### 2. Construction of kidney cDNA library

Total RNA was extracted using the TRIzol reagent (Gibco BRL Life Technologies Ltd., Renfrewshire, UK), and mRNA was enriched by oligo-(dT) cellulose chromatography using the PolyA Tract mRNA isolation kit (Promega, WI, USA). cDNA synthesis was carried out using an oligo-(dT)<sup>18</sup> primer for the reverse transcription of approximately 5 µg of mRNA, and the library was constructed by directional cloning approach using Stratagene's Uni-ZAP XR cloning systems. The primary library was amplified to a titer of  $1.0 \times 10^7$  pfu/mL and stored at both 4 and  $-70^\circ\text{C}$ . Mass excision was performed and the cDNA inserts from the amplified Uni-ZAP XR library were rescued as pBluescript phagemids in SOLR *Escherichia coli*. A total of 1,000 colonies were randomly picked and rearranged in 96-well plates.

### 3. Plasmid preparation and sequencing

The plasmid cDNA library was plated to a density appropriate for picking individual colonies. Random clones were grown in 1.5 mL LB medium overnight in  $12 \times 75$  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 µg) were used in sequencing reactions. Single-pass sequencing of the 5'-termini of selected kidney cDNA clones in phagemid form was performed using the ABI 3,100 automatic DNA sequencer (PE Applied Biosystems, CA, USA) and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

### 4. Bioinformatic analysis

Bioinformatic analysis was conducted to determine gene identities using GeneMaster software (Ensoltek, Korea). Briefly, vector sequences were removed and database search were limited to ESTs > 100 bp in length. ESTs were then assembled in clusters of contiguous 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 e-values less than  $1.0 \times 10^{-3}$  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.

## Results and Discussion

### 1. EST sequencing and general characteristics

The kidney cDNA library was constructed from the poly-adenylated fraction of mRNA from the olive flounder kidney. 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 1. A total of 390 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 100 bp after elimination of vector sequence. The average insert size was estimated to be  $1.7 \pm 0.3$  kb by PCR amplification of inserts from 20 randomly selected clones. We used the assembly program ICAtools software (Parsons, 1995) to organize the redundant ESTs into overlapping contigs. The results showed that the 390 kidney ESTs were composed of 27 clusters and 317 singletons, suggesting that the overall redundancy of the library was 11.8%.

Gene annotation procedures and homology searches of the sequenced ESTs have been locally done by BLASTX for amino acid similarity comparisons. The ESTs with significant similarities ( $E < 1 \times 10^{-3}$ ) to known proteins were evaluated to determine if the significant similarities were caused by simple amino acid matches. Of the 390 clones, 250 (64.1%) were identified as orthologues of known genes from other organisms. The remaining 140 (35.9%) clones could not be identified by similarity comparisons ( $E \geq 1 \times 10^{-3}$ ). Among the 250 EST clones, 14 (5.6%) clones were representing 11 unique genes identified as homologous to the previously reported olive floun-

**Table 1.** General characteristics of olive flounder kidney ESTs

Total cDNA sequenced <sup>a</sup>	390
Average insert size <sup>b</sup>	1.7 kb $\pm$ 0.3
Average EST length	539 bp
EST clusters <sup>c</sup>	27
Singletons <sup>d</sup>	317
Redundancy <sup>e</sup>	11.8%
ESTs with e-value $< 1 \times 10^{-3}$ (matched)	250 (64.1%)
ESTs with e-value $\geq 1 \times 10^{-3}$ (unknown)	140 (35.9%)

<sup>a</sup>Length of sequence used for comparison after editing (inserts  $< 100$  base pairs were excluded).

<sup>b</sup>The average insert size was calculated for 20 randomly selected cDNA clones.

<sup>c</sup>ESTs with 90% or greater identity over a 100 bp region were clustered together forming 27 EST clusters.

<sup>d</sup>317 sequences did not sufficiently match any sequence in the data set to allow assembly.

<sup>e</sup>Redundancy = The number of genes/total ESTs.

der ESTs, 198 (79.2%) clones representing 160 unique genes were identified as orthologs of known genes from other organisms (Table 2). The fact that the majority of EST clones could be identified by similarity comparisons suggests that high-quality EST analysis is an efficient way for gene annotation in less-well studied species. Thirty-eight (15.2%) clones representing 37 unique genes showed significant similarities to known sequences of unknown functions from model systems such as *Homo sapiens* and *Mus musculus*. Although functions are not yet known, their conservation in fish demonstrated the existence of many gene families through evolution. Once a gene is characterized in any one of these species, comparative functional genomics will allow annotation to these orthologous genes.

### 2. Expression profile and gene identification

Expression profiles of the EST clones identified from the olive flounder kidney is shown in Fig. 1. Of the 390 EST clones identified by BLASTX, 317 (81.3%) were singletons. Although redundancy will increase as the number of sequenced clones increases, the high percentage of singletons indicated that the complexity and coverage of this olive flounder liver cDNA library was good. Among 250 identified distinct known genes, 183 genes (73.2%) were sequenced only once; 57 genes (22.8%) were sequenced 2~5 times; 10 genes (4.0%) were sequenced over five times.

The putative amino acid sequence deduced from

**Table 2.** List of identified ESTs from liver cDNA of olive flounder

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	F <sup>b</sup>
kidney-3-E6	elongation factor 1-alpha	[ <i>Sparus aurata</i> ]	AAD56406	0.00E+00	97	1
kidney-2-D12	IgM precursor	[ <i>Paralichthys olivaceus</i> ]	AAF35884	0.00E+00	96	1
kidney-3-G2	SEC23-related protein B	[ <i>Mus musculus</i> ]	Q9D662	0.00E+00	92	1
kidney-2-C3	immunoglobulin M	[ <i>Paralichthys olivaceus</i> ]	BAB60868	0.00E+00	91	1
kidney-2-E1	cytochrome c oxidase subunit I	[ <i>Paralichthys olivaceus</i> ]	NP_037584	0.00E+00	89	1
KIDNEY-1-F8	hypothetical protein FLJ13258	[ <i>Homo sapiens</i> ]	NP_071921	0.00E+00	85	1
kidney-2-G11	UDP-glucuronosyl transferase	[ <i>Pleuronectes platessa</i> ]	Q91280	0.00E+00	83	1
kidney-2-A5	KIAA0687 protein	[ <i>Homo sapiens</i> ]	BAA31662	0.00E+00	82	1
KIDNEY-1-C12	cytochrome-b	[ <i>Paralichthys olivaceus</i> ]	NP_037594	3.00E-98	88	1
kidney-2-F8	glycyl-tRNA synthetase	[ <i>Homo sapiens</i> ]	NP_002038	4.00E-98	78	1
kidney-2-F7	receptor for activated protein kinase C	[ <i>Oreochromis niloticus</i> ]	O42249	1.00E-97	96	2
KIDNEY-1-F9	S6 ribosomal protein	[ <i>Oncorhynchus mykiss</i> ]	Q9YGF2	3.00E-97	96	1
kidney-3-D2	cathepsin D	[ <i>Chionodraco hamatus</i> ]	CAA07719	3.00E-96	89	1
kidney-2-A2	GK001 protein	[ <i>Homo sapiens</i> ]	XP_044382	1.00E-95	85	1
kidney-2-D11	band 3 anion transport protein	[ <i>Oncorhynchus mykiss</i> ]	S24318	2.00E-94	79	1
KIDNEY-1-E8	myosin regulatory light chain	[ <i>Gallus gallus</i> ]	P24032	3.00E-93	95	1
kidney-2-C7	major histocompatibility class I receptor	[ <i>Stizostedion vitreum</i> ]	AAL11413	2.00E-92	73	3
kidney-2-C6	eukaryotic translation initiation factor 3	[ <i>Mus musculus</i> ]	NP_542366	5.00E-91	77	1
KIDNEY-1-F1	cytochrome oxidase subunit-3	[ <i>Paralichthys olivaceus</i> ]	NP_037588	4.00E-90	95	1
kidney-3-E	translation initiation factor eIF4A I	[ <i>Xenopus laevis</i> ]	CAA73167	1.00E-89	90	2
kidney-3-B9	MER receptor tyrosine kinase	[ <i>Homo sapiens</i> ]	XP_057391	1.00E-88	90	1
kidney-2-B2	TNF receptor associate factor-2	[ <i>Carassius auratus</i> ]	CAC82653	2.00E-87	86	1
kidney-3-H3	N-ethylmaleimide sensitive fusion protein	[ <i>Mus musculus</i> ]	NP_080174	3.00E-86	79	1
kidney-3-H9	brain acidic ribosomal phosphoprotein P0	[ <i>Rana sylvatica</i> ]	AAG09233	4.00E-86	87	2
kidney-3-C7	HSC71	[ <i>Oncorhynchus mykiss</i> ]	P08108	1.00E-85	80	1
kidney-3-D9	ribosomal protein L5b	[ <i>Ictalurus punctatus</i> ]	AAK95129	8.00E-85	92	1
kidney-2-F9	mitochondrial ATP synthase gamma-subunit	[ <i>Cyprinus carpio</i> ]	BAB47390	2.00E-84	79	1
kidney-2-C4	ribosomal protein L9	[ <i>Mus musculus</i> ]	NP_035422	3.00E-84	89	1
kidney-3-G6	peptide chain release factor subunit 1 (eRF1)	[ <i>Xenopus laevis</i> ]	P35615	7.00E-84	91	1
KIDN2E02	isocitrate dehydrogenase subunit 1	[ <i>Bos taurus</i> ]	O77784	4.00E-83	83	1
KIDNEY-1-C9	ribosomal protein L10a	[ <i>Ictalurus punctatus</i> ]	AAK95136	2.00E-82	84	1
kidney-2-F4	alcohol dehydrogenase I	[Baltic cod]	P26325	2.00E-82	78	2
KIDNEY-1-F3	TM21	[ <i>Takifugu rubripes</i> ]	Q90515	3.00E-81	86	1
kidney-3-F5	gelsolin	[ <i>Danio rerio</i> ]	AAF99088	7.00E-81	77	2
KIDN1G09	ubiquitin-conjugating enzyme	[ <i>Mus musculus</i> ]	NP_003330	2.00E-80	94	1
kidney-3-B6	40S ribosomal protein S13	[ <i>Ictalurus punctatus</i> ]	AAK95195	1.00E-79	98	1
KIDNEY-1-B7	proteasome subunit, beta type	[ <i>Rattus norvegicus</i> ]	NP_058980	1.00E-79	81	1
kidney-3-C8	cytochrome c oxidase subunit II	[ <i>Paralichthys olivaceus</i> ]	NP_037585	4.00E-79	82	3
kidney-2-C10	40S ribosomal protein S9	[ <i>Ictalurus punctatus</i> ]	AAK95191	6.00E-79	98	1
KIDN1F09	hypothetical protein FLJ10718	[ <i>Homo sapiens</i> ]	NP_060662	1.00E-78	83	1
KIDNEY-1-C6	heat shock protein hsp90beta	[ <i>Danio rerio</i> ]	AAC21566	3.00E-78	71	1
kidney-2-E10	NADH dehydrogenase subunit 1	[ <i>Paralichthys olivaceus</i> ]	NP_037582	5.00E-78	72	1
kidney-2-E2	cathepsin D	[ <i>Oncorhynchus mykiss</i> ]	AAC60301	9.00E-78	87	1
KIDNEY-1-H1	Unknown (protein for MGC : 2976)	[ <i>Homo sapiens</i> ]	AAH18847	9.00E-78	79	1
KIDNEY-1-F4	chicken-type lysozyme	[ <i>Paralichthys olivaceus</i> ]	BAB17215	2.00E-77	92	2
kidney-2-G7	ferritin heavy chain	[ <i>Danio rerio</i> ]	NP_571660	2.00E-77	88	1
KIDNEY-1-H3	ribosomal protein L12	[ <i>Ictalurus punctatus</i> ]	AAK95138	2.00E-76	88	1
kidney-2-E3	MHC class II protein	[ <i>Morone saxatilis</i> ]	AAA49379	3.00E-76	70	3
kidney-2-C2	apolipoprotein E	[ <i>Scophthalmus maximus</i> ]	CAB65356	9.00E-76	79	1
kidney-2-A3	Unknown (protein for IMAGE : 3894870)	[ <i>Homo sapiens</i> ]	AAH10731	1.00E-75	73	1
kidney-3-D10	40S ribosomal protein S10	[ <i>Ictalurus punctatus</i> ]	Q90YR4	1.00E-73	94	1
kidney-2-A4	sorting nexin 4	[ <i>Homo sapiens</i> ]	NP_003785	1.00E-72	73	1
KIDN2F01	cytochrome P450	[ <i>Fundulus heteroclitus</i> ]	AAD54014	3.00E-72	81	1
KIDN1E04	ribosomal protein L21	[ <i>Ictalurus punctatus</i> ]	AAK95147	4.00E-72	90	1
KIDN1H12	ribosomal protein S7	[ <i>Takifugu rubripes</i> ]	P50894	6.00E-72	94	2

Table 2. Continued.

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	F <sup>b</sup>
KIDNEY-1-D3	Aromatic-L-amino-acid decarboxylase	[ <i>Cavia porcellus</i> ]	P22781	8.00E-72	71	1
KIDNEY-1-A5	hemoglobin beta-A chain	[ <i>Seriola quinqueradiata</i> ]	Q9PVM2	3.00E-71	87	2
kidney-2-H6	high mobility group protein	[ <i>Mus musculus</i> ]	CAA56631	1.00E-70	74	1
KIDNEY-1-D8	hypothetical protein CAB56184	[ <i>Homo sapiens</i> ]	AAH14592	8.00E-70	78	1
kidney-2-C9	carbonic anhydrase	[ <i>Danio rerio</i> ]	NP_571185	8.00E-70	74	1
KIDNEY-1-B3	unnamed protein product	[ <i>Mus musculus</i> ]	BAB28647	1.00E-69	66	1
KIDNEY-1-C10	Similar to hypothetical protein FLJ10856	[ <i>Mus musculus</i> ]	AAH18491	3.00E-68	62	1
KIDNEY-1-E5	cyclin G1	[ <i>Homo sapiens</i> ]	NP_004051	3.00E-68	59	1
kidney-3-E7	protein tyrosine kinase 9	[ <i>Mus musculus</i> ]	XP_110123	2.00E-67	67	1
kidney-2-B8	aldose reductase	[ <i>Homo sapiens</i> ]	AAA51714	1.00E-66	60	1
kidney-2-C8	ribosomal protein L18	[ <i>Oreochromis mossambicus</i> ]	AAF64457	2.00E-66	85	1
KIDNEY-1-G7	proline dehydrogenase	[ <i>Mus musculus</i> ]	XP_110168	2.00E-66	61	1
KIDN2E09	aldehyde dehydrogenase	[ <i>Danio rerio</i> ]	AAK49120	8.00E-66	76	1
kidney-2-F3	unnamed protein product	[ <i>Homo sapiens</i> ]	BAB13861	1.00E-65	75	1
kidney-3-E2	palmitoyl-protein thioesterase	[ <i>Mus musculus</i> ]	NP_032943	4.00E-65	73	1
KIDNEY-1-A8	ribosomal protein L27a	[ <i>Epinephelus coioides</i> ]	AAM27202	1.00E-64	86	1
KIDN1E08	N-ethylmaleimide sensitive fusion protein	[ <i>Rattus norvegicus</i> ]	NP_542152	1.00E-63	86	1
KIDN1E06	glutathione S-transferase	[ <i>Pleuronectes platessa</i> ]	CAA64495	2.00E-63	79	1
KIDN1H05	steroid dehydrogenase	[ <i>Anas platyrhynchos</i> ]	O57314	3.00E-63	68	1
kidney-3-A12	chaperonin subunit 7	[ <i>Mus musculus</i> ]	NP_031664	1.00E-62	92	1
KIDNEY-1-F11	dendritic cell protein	[ <i>Homo sapiens</i> ]	XP_034431	1.00E-62	89	1
KIDN1B05	ubiquitin polyprotein (heat shock related)	[ <i>Gallus gallus</i> ]	I50438	2.00E-61	95	3
kidney-2-E12	peroxiredoxin 5	[ <i>Homo sapiens</i> ]	NP_036226	2.00E-61	68	1
KIDN2B03	unnamed protein product	[ <i>Mus musculus</i> ]	BAB22439	2.00E-60	83	1
KIDNEY-1-E1	immunoglobulin light chain precursor	[ <i>Seriola quinqueradiata</i> ]	BAB59086	5.00E-60	75	1
kidney-2-E4	NADH dehydrogenase subunit 2	[ <i>Paralichthys olivaceus</i> ]	NP_037583	8.00E-59	62	1
KIDNEY-1-D4	IQ motif containing GTPase activating protein 1	[ <i>Mus musculus</i> ]	NP_057930	8.00E-59	61	1
KIDNEY-1-H2	beta-tubulin	[ <i>Bombyx mori</i> ]	BAB86852	5.00E-58	98	1
KIDNEY-1-A11	hypothetical protein	[ <i>Mus musculus</i> ]	XP_143698	5.00E-58	59	1
kidney-2-C11	phospholipase C, gamma 2	[ <i>Mus musculus</i> ]	AAH19654	6.00E-58	63	1
kidney-2-C12	proteasome 26S subunit, non-ATPase	[ <i>Homo sapiens</i> ]	NP_002800	6.00E-57	79	1
KIDN1D07	cathepsin B	[ <i>Homo sapiens</i> ]	AAH10240	4.00E-56	72	1
kidney-2-G6	translationally controlled tumor protein	[ <i>Labeo rohita</i> ]	Q98SJ7	6.00E-56	59	1
KIDN2C01	DGCR6 protein	[ <i>Gallus gallus</i> ]	O73770	1.00E-55	75	2
kidney-3-A1	major histocompatibility class I receptor	[ <i>Stizostedion vitreum</i> ]	AAL11412	1.00E-55	67	2
KIDN1G02	nephrosin precursor	[ <i>Cyprinus carpio</i> ]	AAB62737	1.00E-55	67	2
KIDNEY-1-B12	CGI-49 protein	[ <i>Homo sapiens</i> ]	NP_057086	2.00E-54	52	1
KIDNEY-1-B1	glutathione peroxidase	[ <i>Platichthys flesus</i> ]	CAC27424	3.00E-53	91	1
KIDN2D12	ribosomal protein L34	[ <i>Homo sapiens</i> ]	NP_000986	9.00E-53	88	1
kidney-3-A9	DSPA alpha-1	[ <i>Desmodus rotundus</i> ]	P98119	1.00E-52	53	1
KIDNEY-1-H4	NADPH-ferrihemoprotein reductase	[ <i>Salmo trutta</i> ]	A28577	2.00E-52	78	1
KIDN2F03	Glycine cleavage system H protein	[ <i>Gallus gallus</i> ]	P11183	1.00E-51	70	1
kidney-2-G1	RIKEN cDNA 2610001E01	[ <i>Mus musculus</i> ]	NP_080194	1.00E-51	54	1
KIDN2F06	matrix metalloproteinase	[ <i>Oncorhynchus mykiss</i> ]	BAB19131	9.00E-51	58	1
KIDN1A09	ribosomal protein L6	[ <i>Ictalurus punctatus</i> ]	AAK95130	4.00E-50	78	1
KIDNEY-1-H7	alpha hemoglobin B	[ <i>Seriola quinqueradiata</i> ]	Q9PVM3	4.00E-50	68	10
KIDN2D06	acidic ribosomal protein P0	[ <i>Rattus rattus</i> ]	R5RT10	3.00E-49	93	1
kidney-2-G8	alpha-L-fucosidase fucosyltransferase	[ <i>Homo sapiens</i> ]	CAB53746	5.00E-49	57	1
kidney-3-G10	Integral membrane protein 2B	[ <i>Gallus gallus</i> ]	O42204	3.00E-48	75	2
KIDN2B05	transgelin; SM-22 alpha	[ <i>Mus musculus</i> ]	NP_035656	8.00E-48	65	1
KIDNEY-1-H11	PAS1	[ <i>Takifugu rubripes</i> ]	AAC34392	2.00E-47	72	1
KIDN1E03	Similar to CGI-36 protein	[ <i>Mus musculus</i> ]	AAH13538	4.00E-47	62	1
KIDN1B06	Ubiquitin-protein ligase G2	[ <i>Mus musculus</i> ]	XP_036087	2.00E-46	87	1

Table 2. Continued.

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	F <sup>b</sup>
KIDNEY-1-H5	protein-tyrosine-phosphatase	[ <i>Mus musculus</i> ]	S17671	7.00E-46	55	1
kidney-3-B1	Catalase	[ <i>Danio rerio</i> ]	Q9PT92	1.00E-44	83	1
KIDNEY-1-H6	hsp90 binding protein	[ <i>Oryctolagus cuniculus</i> ]	AAA31439	1.00E-44	47	1
kidney-2-H10	hypothetical protein MGC3133	[ <i>Homo sapiens</i> ]	NP_112577	2.00E-44	92	1
KIDN1F06	capping protein, gelsolin-like	[ <i>Mus musculus</i> ]	AAH03480	3.00E-44	54	1
KIDN1C05	c-src kinase	[ <i>Gallus gallus</i> ]	P41239	4.00E-44	82	1
kidney-3-D3	RIKEN cDNA 1500005J14 gene	[ <i>Homo sapiens</i> ]	XP_060025	2.00E-43	53	1
kidney-2-B1	RIKEN cDNA 1110014J22	[ <i>Mus musculus</i> ]	NP_079647	2.00E-41	68	1
KIDN2B10	keratin	[ <i>Carassius auratus</i> ]	AAC38007	7.00E-41	61	1
KIDNEY-1-H10	tetraspan 3	[ <i>Homo sapiens</i> ]	AAH00704	2.00E-40	73	1
KIDN1D10	peptidyl-prolyl cis-trans isomerase	[ <i>Xenopus laevis</i> ]	O42123	1.00E-39	68	1
KIDNEY-1-G4	40S ribosomal protein S26-2	[ <i>Ictalurus punctatus</i> ]	AAK95209	6.00E-39	97	1
KIDNEY-1-D2	H3 histone, family 3A	[ <i>Homo sapiens</i> ]	NP_002098	6.00E-38	100	1
KIDN2A02	target of myb1	[ <i>Homo sapiens</i> ]	NP_005479	2.00E-37	78	1
kidney-2-D4	sirtuin 5, isoform 1	[ <i>Homo sapiens</i> ]	NP_036373	2.00E-37	78	1
KIDNEY-1-C5	Calcineurin-like phosphoesterase precursor	[ <i>Caenorhabditis elegans</i> ]	NP_506191	2.00E-36	40	1
KIDN1G12	Unknown (protein for MGC : 9788)	[ <i>Homo sapiens</i> ]	AAH11964	1.00E-35	52	1
kidney-2-G10	granzyme A	[ <i>Mus musculus</i> ]	NP_034500	6.00E-35	42	1
KIDN2H05	CD97 protein	[ <i>Bos taurus</i> ]	CAC94754	7.00E-35	46	1
KIDNEY-1-E10	RIKEN cDNA 3110001A13	[ <i>Mus musculus</i> ]	NP_079902	2.00E-34	61	1
KIDNEY-1-A1	Unknown (protein for MGC : 37327)	[ <i>Mus musculus</i> ]	AAH26598	5.00E-34	46	1
KIDNEY-1-C7	renal organic anion transporter	[ <i>Pseudopleuronectes americanus</i> ]	CAB09724	8.00E-34	50	1
KIDNEY-1-E2	RIKEN cDNA 2700085M18 gene	[ <i>Homo sapiens</i> ]	AAH05879	4.00E-33	74	1
kidney-3-G11	ribosomal protein P1	[ <i>Ictalurus punctatus</i> ]	AAK95124	1.00E-32	64	1
KIDN1C11	desmoplakin	[ <i>Homo sapiens</i> ]	XP_004463	5.00E-31	71	1
kidney-3-D5	uncharacterized bone marrow protein BM046	[ <i>Homo sapiens</i> ]	XP_059419	9.00E-31	55	1
KIDNEY-1-G9	unnamed protein product	[ <i>Mus musculus</i> ]	BAB25109	1.00E-30	87	2
KIDN2E06	beta-2 microglobulin precursor	[ <i>Ictalurus furcatus</i> ]	AAC64994	1.00E-30	61	4
kidney-2-G5	eukaryotic translation elongation factor 1	[ <i>Danio rerio</i> ]	AAM21716	2.00E-30	86	1
KIDN1B02	unnamed protein product	[ <i>Homo sapiens</i> ]	BAB71093	4.00E-30	51	1
KIDN2A11	aspartyl aminopeptidase	[ <i>Mus musculus</i> ]	NP_077296	2.00E-29	66	2
KIDNEY-1-A12	Unknown (protein for IMAGE : 5102170)	[ <i>Mus musculus</i> ]	AAH21603	2.00E-29	63	1
KIDN1H08	transferrin receptor	[ <i>Canis familiaris</i> ]	AAG24850	5.00E-29	52	1
KIDN1E10	NRD2 convertase	[ <i>Homo sapiens</i> ]	NP_002516	1.00E-28	55	1
kidney-3-C12	simple type I keratin	[ <i>Oncorhynchus mykiss</i> ]	CAA74664	2.00E-28	77	1
KIDN2A01	beta-galactoside-binding lectin	[ <i>Electrophorus electricus</i> ]	A28302	4.00E-28	46	1
KIDNEY-1-D7	selectin, lymphocyte	[ <i>Rattus norvegicus</i> ]	NP_062050	6.00E-28	48	2
kidney-2-H4	beta-2 microglobulin precursor	[ <i>Ictalurus lupus</i> ]	AAC64992	7.00E-28	60	2
KIDN1E02	x-box binding protein 1B	[ <i>Danio rerio</i> ]	AAL05527	7.00E-28	51	1
KIDNEY-1-D10	hypothetical protein MGC11242	[ <i>Homo sapiens</i> ]	NP_077296	1.00E-26	66	1
KIDNEY-1-E7	My021 protein	[ <i>Homo sapiens</i> ]	AAG43135	1.00E-26	65	1
KIDN1F12	cis-retinol/androgen dehydrogenase type 3	[ <i>Mus musculus</i> ]	AAM21318	3.00E-26	60	1
KIDN1F05	bromodomain containing protein 3	[ <i>Homo sapiens</i> ]	NP_031397	4.00E-26	58	1
KIDNEY-1-A2	receptor tyrosine kinase flk-1/VEGFR-2	[ <i>Danio rerio</i> ]	AAL16381	6.00E-26	67	1
kidney-3-E12	neuropsin type2	[ <i>Homo sapiens</i> ]	BAA82666	1.00E-25	42	1
KIDN1G11	CRSP70	[ <i>Danio rerio</i> ]	AAK61395	8.00E-25	77	1
KIDN2D05	SET domain, bifurcated 1	[ <i>Homo sapiens</i> ]	NP_036564	2.00E-24	39	1
KIDN2F07	RIKEN cDNA 2600013N14	[ <i>Mus musculus</i> ]	XP_131301	3.00E-24	52	1
KIDN1A12	HSPC267	[ <i>Homo sapiens</i> ]	AAF28945	3.00E-23	65	1
KIDN2C06	Carboxypeptidase A2 precursor	[ <i>Rattus norvegicus</i> ]	P19222	3.00E-23	51	1
KIDN1A07	unknown	[ <i>Homo sapiens</i> ]	AAG17266	4.00E-23	75	1
kidney-3-A5	alpha hemoglobin B	[ <i>Seriola quinqueradiata</i> ]	BAA86219	4.00E-23	50	1
kidney-3-D7	Chain H, Cytochrome Bc1 Complex	[ <i>Gallus gallus</i> ]	1BCC	5.00E-22	71	1
KIDNEY-1-E3	Similar to CGI-49 protein	[ <i>Homo sapiens</i> ]	AAH26185	4.00E-21	74	1

Table 2. Continued.

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	F <sup>b</sup>
KIDNEY-1-G10	GABA (A) receptor-associated protein like 1	[ <i>Homo sapiens</i> ]	NP_065615	2.00E-20	50	1
kidney-3-F9	RIKEN cDNA 2210418J09	[ <i>Mus musculus</i> ]	XP_133265	2.00E-20	45	1
KIDN1B11	ubiquitin carboxyl-terminal esterase L3	[ <i>Homo sapiens</i> ]	NP_005993	5.00E-20	70	1
kidney-3-G1	Cytochrome c oxidase polypeptide VIC-2	[ <i>Rattus norvegicus</i> ]	P11951	5.00E-20	61	1
KIDN2F09	unnamed protein product	[ <i>Mus musculus</i> ]	BAB30020	6.00E-19	58	1
KIDN2A08	bridging integrator-2	[ <i>Homo sapiens</i> ]	NP_057271	7.00E-19	55	1
KIDN1C04	coagulation factor XIIIa	[ <i>Gallus gallus</i> ]	CAC10657	7.00E-18	36	1
KIDN1G04	immunoglobulin light chain variable region	[ <i>Oncorhynchus mykiss</i> ]	CAB72438	8.00E-18	66	1
kidney-2-A8	RIKEN cDNA 2610524G09	[ <i>Mus musculus</i> ]	NP_084520	1.00E-17	77	1
KIDN1C09	mouse double minute 4 homolog	[ <i>Homo sapiens</i> ]	NP_002384	3.00E-17	52	1
kidney-2-F12	estrogen-responsive B box protein	[ <i>Homo sapiens</i> ]	AAC79080	7.00E-17	29	1
KIDN2D11	small Rho-like GTPase RhoA	[ <i>Xenopus laevis</i> ]	AAD40671	1.00E-16	90	1
KIDNEY-1-B10	P2Y1 receptor	[ <i>Homo sapiens</i> ]	XP_062888	1.00E-16	49	1
kidney-3-G12	cytokine-like protein C17	[ <i>Homo sapiens</i> ]	NP_061129	6.00E-16	41	1
KIDN2G03	annexin A13	[ <i>Danio rerio</i> ]	NP_571849	2.00E-15	90	1
kidney-3-A10	CGI-10 protein	[ <i>Homo sapiens</i> ]	XP_007436	3.00E-15	54	1
KIDNEY-1-D1	hypothetical protein	[ <i>Homo sapiens</i> ]	XP_032129	3.00E-15	48	1
kidney-3-B8	profilin 1	[ <i>Homo sapiens</i> ]	NP_005013	2.00E-14	32	2
kidney-3-E5	ATPase inhibitor precursor	[ <i>Homo sapiens</i> ]	AAH04955	1.00E-13	76	2
KIDNEY-1-C1	invariant chain-like protein 2	[ <i>Danio rerio</i> ]	NP_571447	1.00E-13	33	5
KIDN2H06	mannose receptor C type 1 precursor	[ <i>Homo sapiens</i> ]	NP_002429	2.00E-13	28	1
KIDN1C03	BCL2/adenovirus E1B 19 kDa-interacting protein 3	[ <i>Rattus norvegicus</i> ]	NP_445872	4.00E-13	73	1
kidney-2-A6	VHSV-induced protein-6	[ <i>Oncorhynchus mykiss</i> ]	AAM18471	1.00E-12	61	1
kidney-3-B2	junctional adhesion molecule JAM	[ <i>Rattus norvegicus</i> ]	NP_446248	2.00E-12	31	1
kidney-3-D8	XFEN1b	[ <i>Xenopus laevis</i> ]	AAB08478	3.00E-12	30	1
kidney-2-A7	macrolide-binding protein FKBP12	[ <i>Cryptococcus neoformans</i> ]	AAD16171	6.00E-12	73	1
kidney-2-D9	ADP-ribosylation factor 4	[ <i>Mus musculus</i> ]	NP_031505	9.00E-12	74	1
kidney-2-E7	RIKEN cDNA 5830413L19	[ <i>Mus musculus</i> ]	NP_083775	2.00E-11	34	1
KIDN1B10	NADH dehydrogenase subunit 3	[ <i>Paralichthys olivaceus</i> ]	NP_037589	3.00E-11	93	1
kidney-3-C6	heat shock protein 90 beta	[ <i>Platichthys flesus</i> ]	CAC27523	6.00E-11	86	1
KIDN2H09	selenoprotein T	[ <i>Homo sapiens</i> ]	Q9NZJ3	6.00E-11	63	1
KIDN1F08	molybdenum cofactor synthesis-step 1 protein B	[ <i>Drosophila melanogaster</i> ]	AAF67856	2.00E-10	76	1
kidney-3-F10	Unknown (protein for IMAGE : 4222343)	[ <i>Mus musculus</i> ]	AAH26424	2.00E-09	58	1
KIDN2G09	neutrophil cytosolic factor 2	[ <i>Mus musculus</i> ]	NP_035007	7.00E-09	80	1
KIDN2B11	CD45	[ <i>Cyprinus carpio</i> ]	BAA92179	4.00E-08	35	1
kidney-2-E9	monocyte chemoattractant protein-1	[ <i>Canis familiaris</i> ]	P52203	3.00E-07	37	1
KIDN1C02	NADPH oxidase cytosolic protein p40phox	[ <i>Bison bison</i> ]	AAL11887	4.00E-07	64	1
kidney-2-B5	hypothetical protein	[ <i>Homo sapiens</i> ]	XP_084823	5.00E-07	85	1
kidney-2-E11	RIKEN cDNA 1300013J15	[ <i>Mus musculus</i> ]	NP_080459	1.00E-06	56	1
kidney-2-D7	fibrinogen-binding protein A	[ <i>Staphylococcus aureus</i> ]	NP_373997	2.00E-06	25	1
KIDN2C03	hypothetical protein SB143	[ <i>Homo sapiens</i> ]	AAK67634	3.00E-06	48	1
kidney-3-B4	hypothetical protein	[ <i>Homo sapiens</i> ]	XP_069911	3.00E-05	34	1
KIDNEY-1-F6	Ran binding protein 1	[ <i>Danio rerio</i> ]	AAK61352	1.00E-04	80	1
KIDNEY-1-D11	Niemann-Pick disease, type C1	[ <i>Homo sapiens</i> ]	NP_000262	3.00E-04	73	1
kidney-2-D1	saxitoxin binding protein2	[ <i>Takifugu pardalis</i> ]	BAB55583	3.00E-04	24	1
KIDNEY-1-A10	transmembrane protein TIARP	[ <i>Mus musculus</i> ]	NP_473439	8.00E-04	56	1

<sup>a</sup>Identity (%)<sup>b</sup>Frequency of the clones in the sequenced pool.

one cDNA clone, KIDNEY-1-D7, was identified as selectin, a superfamily of C-type lectin. The

alignment showed that the putative sequence is 48.7, 47.0 and 46.2% identical to the carbohy-





with MHC class II  $\alpha/\beta$  molecules in endoplasmic reticulum (Wright *et al.*, 1990). It is thought to play a role in antigen presentation by binding to the antigenic peptide-binding groove of the  $\alpha$  and  $\beta$  chains shortly after their synthesis in the endoplasmic reticulum (ER). This association has multiple effects on class II molecules, all of which optimize the binding and presentation of foreign peptides derived from extra-cellular environment (Sant and Miller, 1994). This indicates the importance of li in immune regulation that may also be functioning in fish.

In conclusion, this work identified 250 known genes and 140 novel clones from the olive flounder kidney. Expression profiles of these genes were revealed by their frequency in a cDNA library. We also identified several kinds of immune associated proteins, indicating EST as a powerful method for identifying immune-related genes of fish as well as identifying novel genes. Further studies using cDNA microarrays are needed to identify the differentially expressed transcripts after disease infection.

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### 넙치 (*Paralichthys olivaceus*) 신장에서 생성된 ESTs (Expressed Sequence Tags)로부터 면역관련 유전자의 탐색

이정호 · 김영옥<sup>1</sup> · 김종현 · 노재구  
김현철 · 김경길\* · 김규원<sup>2</sup>

국립수산과학원 어류육종연구센터, <sup>1</sup>국립수산과학원 생명공학연구단,

<sup>2</sup>서울대학교 약학대학

넙치 (*Paralichthys olivaceus*) 신장에서 추출한 mRNA로부터 cDNA library를 제작하고 이를 이용하여 EST (Expressed sequence tag) 분석을 하였다. 넙치의 신장 cDNA library에서 무작위로 선별한 390개의 EST를 조사한 결과, 250개의 EST는 이미 밝혀진 유전자와 유사성이 있는 것으로 나타났으며, 140개의 EST는 새로운 유전자로 밝혀졌다. 유전자의 기능이 밝혀진 250개의 EST 중 14 (5.6%)개의 EST는 이미 알려진 넙치 EST와 상동성이 있는 유전자로 확인되었고, 198 (79.2%)개의 EST는 다른 생물에서 알려진 유전자와 상동성이 있는 것으로 나타났다. 그러나 38 (15.2%)개의 EST는 전혀 기능이 알려지지 않은 새로운 유전자로 밝혀졌다. EST 분석은 새로운 유전자 뿐만 아니라 기능적으로 중요한 유전자를 탐색하는데도 아주 강력한 연구 방법이다. 이에 따라 본 연구에서는 넙치 신장 EST 분석을 통해 C-, L-type lectin과 MHC class II-invariant chain like proteins (Ii) 같은 면역기능과 관련이 있는 여러 개의 유전자를 확인하였고, 이들 유전자들은 질병감염 후 유전자 발현에서 나타나는 차이를 분석하는데 이용되는 cDNA microarray 연구에 유용하게 사용될 것으로 보인다.