

# Expressed Sequence Tags Analysis of Black Rockfish (*Sebastes schlegeli*) Peripheral Leukocytes Stimulated with Con A/PMA or LPS

Gun Wook Baek, Ju Won Kim, Ki Hyuk Kim, Kwan Yong Jun,  
Geun Hee An and Chan Il Park<sup>†</sup>

Department of Marine Biology & Aquaculture, Institute of Marine Industry,  
College of Marine Science, Gyeongsang National University, 455, Tongyeong, 650-160, Korea

We constructed a black rockfish (*Sebastes schlegeli*) leukocyte cDNA library and a total of 386 expressed sequence tag (EST) clones were generated. Gene annotation procedures and homology searches of the sequenced ESTs were locally done by BLASTX for amino acid similarity comparisons. Of the 386 EST clones, 199 different ESTs showed significant homology to previously described genes while 97 ESTs were unidentified, hypothetical, or unnamed proteins. Encoding 38 different sequences were identified as putative bio-defense genes or genes associated with immune response.

*Key words:* Expressed sequence tags, ESTs, Black rockfish (*Sebastes schlegeli*), Leukocyte

## Introduction

Expressed sequence tags (ESTs) are single pass sequences generated from random sequencing of cDNA clones (Nam *et al.*, 2000). Large scale EST analysis is also an efficient way for identification of genes and for analysis of their expression by means of expression profiling (Franco *et al.*, 1995; Azam *et al.*, 1996; Lee *et al.*, 2000). It offers a rapid and valuable first look at genes expressed in specific tissue types, under specific physiological conditions, or during specific developmental stages. Currently, the number of fish-related ESTs in the public databases is still small compared with mammalian sequences and there are relatively few tissue-specific cDNA libraries (Ton *et al.*, 2000). ESTs have been shown to be an excellent and proven method of identification and characterizing novel genes.

The black rockfish (*Sebastes schlegeli*) is one of the more significant fish species in Korea due to human interests in aquaculture and fisheries. Traits that may be amenable to genetic improvement include growth, delayed maturity, disease resistance and temperature tolerance. Despite these interest, relatively little information is available about rockfish genes. To increase the efficiency of the detection of immune-response or bio-defence genes expressed during T-cell proliferation, the mitogen-induced T-cell system *in vitro* is a simple methodology (Miyamoto *et al.*, 2000; Studnicka, 2000). In this study, EST sequenced to screen for bio-defence or immune response related genes from black rockfish peripheral blood leukocytes (PBL) after the treatment with Con A/PMA or LPS as an *in vitro* activation system.

This lack of knowledge may represent one obstacle

<sup>†</sup>Corresponding Author : Chan Il Park, Tel : 82-55-640-3103  
Fax : 82-55-642-4509, E-mail : vinus96@hanmail.net

to the effective use of genetics in aiding both black rockfish aquaculture and conservation activities.

## Materials and Methods

Peripheral blood samples were taken from a black rockfish as previously described (Nam *et al.*, 2000). Isolated leukocytes were adjusted to a concentration of  $10^7$  cells/ml and cultured in RPMI1640 containing 10% fetal bovine serum (FBS),  $70 \mu\text{g/ml}$  Con A and  $0.35 \mu\text{g/ml}$  PMA or  $500 \mu\text{g/ml}$  LPS at  $20^\circ\text{C}$  for 1, 3, 6, and 12 h. Following each incubation, leukocytes were harvested, washed twice with phosphate buffered saline (PBS) and frozen at  $-80^\circ\text{C}$  until use. mRNA was isolated from the Con A/PMA or LPS induced leukocytes using a micro mRNA purification kit (Amersham-Pharmacia, U.S.A.). The purified mRNAs of three different time periods were pooled to ensure complete coverage of expressed genes in the allotted time frame and used to construct a cDNA library. cDNA was synthesised using a cDNA synthesis kit (Amersham-Pharmacia, U.S.A.) with an oligo-dT primer. The cDNA library was constructed in Uni-ZAP XR cloning systems (Stratagene, U.S.A.) according to the instructions of the manufacturer.

Conversion of the recombinant Uni-ZAP XP into the Bluescript plasmid was carried out by *in vivo* excision according to the manufacturer's protocol

(Stratagene, U.S.A.). The 5'-termini base sequencing of selected cDNA clones in phagemid form were performed using the ABI 3100 automatic DNA sequencer (PE Applied Biosystems, CA, U.S.A.) and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, U.S.A.).

Bioinformatic analysis was conducted to determine gene identities using Genetyx ver. 8.0 software (SDC software, Japan). Briefly, vector sequences were removed and database search were limited to ESTs > 400 bp in length. The sequence of each cDNA was compared with sequences in the peptide sequence databases at the National Center for Biotechnology Information (NCBI) using the BLAST network service. Nucleotide sequence comparisons were carried out using the program BLASTX (Gish and David, 1993).

## Results and Discussion

A total of 386 randomly selected clones were single-pass sequenced from the 5' end, resulting in the characterization of cDNA clones that were longer than 400 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 1.

Gene annotation procedures and homology

**Table 1.** Summary of sequences and clones represented

Numbers of ESTs (n)	386
Putatively identified clones (n)	289 (74.8%)
Unidentified clones (n)	97 (25.2%)
Putatively identified different genes (n)	199
Immune related genes (n)	38
Total nucleotides (bp)	333,284
Average of sequenced length (bp)	863

searches of the sequenced ESTs have been locally done by BLASTX for amino acid similarity comparisons. The ESTs with significant similarities to known proteins were evaluated to determine if the significant similarities were caused by simple amino acid matches (Table 2). Of the 386 clones, 289 (74.8%) were identified as orthologues of known genes from other organisms. The remaining 97 (25.2%) clones could not be identified by BLASTX for amino acid similarity comparisons;

most of them are expected to derive from hitherto uncharacterized or novel genes, whereas some of them may be due to the fact that the homology is too low to detect in the region that is sequenced. Among the 199 identified EST clones, 38 clones were immune related genes identified as homologous to the previously reported genes from other organisms (Table 2).

Lymphocyte proliferation is an essential process of a specific immune response where activated lym-

**Table 2.** List of identified ESTs from leukocyte cDNA of rockfish.

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	s <sup>b</sup>	bp
BRF-D-6	Asteroid homolog 1	<i>Homo sapiens</i>	EAW79214.1	3e-09	51	65.5	677
BRF-D-11	Annexin max2	<i>Oryzias latipes</i>	NP_001098345.1	2e-120	85	435	1000
BRF-E-128	Annexin A3b	<i>Danio rerio</i>	NP_001007303.1	1e-101	75	372	928
BRF-D-31	Aminolevulinate, delta-, synthetase 2	<i>Danio rerio</i>	AAH67149	9e-105	64	383	988
BRF-D-41	Adrenomedullin-5	<i>Takifugu rubripes</i>	NP_001027913	2e-36	62	155	699
BRF-E-17	ATP synthase F0 subunit 6	<i>Sebastes schlegelii</i>	NP_976183.1	1e-88	96	330	930
BRF-E-25	Aldolase a, fructose-bisphosphate, b	<i>Danio rerio</i>	NP_998380.1	6e-143	91	508	929
BRF-E-33	ATPase, H <sup>+</sup> transporting, 1 ysosomal 70kDa, V1 subunit A	<i>Xenopus tropicalis</i>	NP_001007512.1	9e-24	48	114	916
BRF-E-73	Atp6v0c-like protein	<i>Danio rerio</i>	NP_991117.1	1e-59	97	233	930
<b>BRF-D-33</b>	<b>Beta-thymosin</b>	<i>paracentrotus lividus</i>	<b>CAD9144.1</b>	<b>4e-12</b>	<b>87</b>	<b>74.3</b>	<b>549</b>
BRF-E-87	Beta actin	<i>Xestia cnigrum</i>	ABW03226.1	2e-07	100	58.5	500
BRF-A-53	Coronin, actin binding protein, 1A	<i>Danio rerio</i>	NP_957408.1	8e-137	84	489	974
<b>BRF-D-14</b>	<b>C-Jun protein</b>	<i>Takifugu rubripes</i>	<b>CAD56856.1</b>	<b>1e-72</b>	<b>84</b>	<b>276</b>	<b>928</b>
BRF-D-29	Cyclin G1	<i>poecilia reticulata</i>	ABN80454.1	3e-108	78	359	923
BRF-D-32	Cytochrome c oxidase subunit I	<i>Zebrafoma flavescens</i>	YP_001491515.1	2e-133	96	478	927
BRF-E-28	Cytochrome c oxidase subunit 2	<i>Sebastes ruberrimus</i>	ABS88953.1	2e-94	94	348	681
BRF-A-47	Cytochrome c oxidase subunit 3	<i>Sebastes ruberrimus</i>	ABS88955	3e-113	93	411	921
BRF-D-69	Collagenase	<i>Oryzias latipes</i>	NP_001098185.1	4e-25	73	118	690
BRF-D-40	Chromobox-like 5	<i>Takifugu rubripes</i>	ABF22449.1	2e-35	89	153	924

**Table 2.** (Continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	s <sup>b</sup>	bp
BRF-E-32	Chaperonin containing TCP-1 delta	<i>Takifugu rubripes</i>	NP_001027851.1	1e-149	91	523	930
BRF-E-49	Cathepsin S, a	<i>Danio rerio</i>	NP_956720.1	6e-45	64	184	930
BRF-E-55	Cathepsin	<i>Paralabidochromis chilotes</i>	AAQ01147.1	4e-68	82	261	708
BRF-E-82	Cathepsin D	<i>Lates calcarifer</i>	ABV59077.1	1e-130	89	371	928
BRF-E-96	Cathepsin S cysteine protease	<i>Paralichthys olivaceus</i>	AAX51229.1	5e-132	83	474	928
BRF-E-40	Novel protein similar to vertebrate cathepsin L	<i>Danio rerio</i>	CAK11015.1	3e-06	34	43.1	794
BRF-A-72	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase	<i>Danio rerio</i>	AAH56559.1	5e-73	86	276	645
BRF-E-56	DNA-directed RNA Polymerase I Subunit M	<i>Encephalitozoon cuniculi GB-M1</i>	NP_597262.1	9.4	38	34.7	930
BRF-D-2	Elongation factor 1 alpha	<i>Oryzias latipes</i>	NP_001098132.1	1e-160	96	569	928
BRF-D-85	Elongation factor 1-gamma (EF-1-gamma)	<i>Carassius auratus</i>	BAB64568.1	3e-137	82	491	923
BRF-D-44	Exosome component 10	<i>Gallus gallus</i>	NP_001012748.1	4e-112	69	407	927
BRF-D-48	Eukaryotic translation elongation factor 2	<i>Ornithorhynchus anatinus</i>	XP_001515708.1	1e-125	87	452	927
BRF-D-75	Envelope glycoprotein	<i>Human immunodeficiency virus type 1</i>	AAD05089.1	7.1	36	35.0	924
BRF-D-90	Extracellular matrix protein 1	<i>Rattus norvegicus</i>	NP_446334.1	1e-18	27	97.4	922
BRF-E-70	Enolase 1 alpha-like	<i>Oreochromis mossambicus</i>	ABE98235.1	1e-81	95	306	1000
<b>BRF-B-6</b>	<b>Ferritin heavy chain</b>	<b><i>Chionodraco rastrispinosus</i></b>	<b>CAL92185.1</b>	<b>2e-86</b>	<b>87</b>	<b>322</b>	<b>924</b>
<b>BRF-A-49</b>	<b>G-protein-coupled receptor GPR34 type 1</b>	<b><i>Tetraodon nigroviridis</i></b>	<b>AAP04329.1</b>	<b>3e-22</b>	<b>70</b>	<b>70.1</b>	<b>922</b>
BRF-B-94	GTP-binding protein, HSR1-related	<i>Exiguobacterium sibiricum 255-15</i>	ZP_00538697.1	4.4	34	34.3	565
BRF-E-137	GP91phox	<i>Siniperca chuatsi</i>	ABC72118.1	5e-160	98	567	927
BRF-B-68	Heme oxygenase (decycling) 1	<i>Danio rerio</i>	NP_955972.1	1e-94	70	349	924
BRF-D-5	Heat shock protein 70	<i>Oreochromis mossambicus</i>	CAA04673.1	2e-148	88	528	929
BRF-D-16	Heat shock protein 70 isoform 2	<i>Fundulus heteroclitus macrolepidotus</i>	ABB17042.1	4e-142	92	468	929
BRF-E-38	Hsd3b7 protein	<i>Danio rerio</i>	AAI55182.1	6e-114	67	414	931
BRF-E-90	Heterogeneous nuclear ribonucleoprotein A/B	<i>Danio rerio</i>	AAH48898.1	1e-04	91	50.8	928
BRF-E-65	Heat shock protein 90 alpha	<i>Paralichthys olivaceus</i>	ABG56393.1	2e-146	94	514	931

**Table 2.** (Continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	s <sup>b</sup>	bp
BRF-E-6	Hydroxyacylglutathione hydrolase	<i>Gallus gallus</i>	NP_001012807.1	6e-89	64	330	922
<b>BRF-C-5</b>	<b>Interferon-stimulated gene 15</b>	<i>Channa argus</i>	<b>ABK63480</b>	<b>4e-41</b>	<b>56</b>	<b>172</b>	<b>926</b>
<b>BRF-B-14</b>	<b>Hntegrin beta 1 binding protein 3</b>	<i>Gallus gallus</i>	<b>NP_001025721.1</b>	<b>5e-70</b>	<b>73</b>	<b>268</b>	<b>923</b>
<b>BRF-E-61</b>	<b>Interleukin-8-like protein</b>	<i>Siniperca chuatsi</i>	<b>AAAY79282.2</b>	<b>2e-10</b>	<b>62</b>	<b>70.5</b>	<b>931</b>
<b>BRF-E-117</b>	<b>Immunoglobulin light chain</b>	<i>Dicentrarchus labrax</i>	<b>CAC16852.1</b>	<b>4e-48</b>	<b>86</b>	<b>194</b>	<b>658</b>
BRF-D-72	Iroquois homeobox protein 1, a isoform 2	<i>Danio rerio</i>	NP_997068.1	5.5	27	35.4	930
BRF-D-25	Kelch-like 6 (Drosophila)	<i>Mus musculus</i>	EDK97558.1	2e-27	73	124	377
BRF-E-67	KRIT1, ankyrin repeat containing	<i>Danio rerio</i>	NP_955866.1	9e-101	79	367	929
<b>BRF-D-63</b>	<b>Leptin receptor</b>	<i>Oryzias melastigma</i>	<b>ABC86922.1</b>	<b>2e-23</b>	<b>43</b>	<b>113</b>	<b>925</b>
BRF-E-18	Lactate dehydrogenase-A; NAD <sup>+</sup> :lactate oxidoreductase	<i>Chromis xanthochira</i>	AAP44528.1	9e-45	98	184	929
<b>BRF-E-78</b>	<b>LBP (LPS binding protein)</b>	<i>Oncorhynchus mykiss</i>	<b>BAB91243.1</b>	<b>2e-99</b>	<b>79</b>	<b>365</b>	<b>930</b>
<b>BRF-A-57</b>	<b>MHC class II antigen alpha chain</b>	<i>Dicentrarchus labrax</i>	<b>ABH09448.1</b>	<b>3e-62</b>	<b>72</b>	<b>242</b>	<b>728</b>
<b>BRF-D-26</b>	<b>MOB1, Mps One Binder kinase activator-like 2A</b>	<i>Xenopus tropicalis</i>	<b>NP_001011080.1</b>	<b>2e-103</b>	<b>88</b>	<b>379</b>	<b>929</b>
<b>BRF-D-83</b>	<b>Myeloid cell leukemia sequence 1-like</b>	<i>Danio rerio</i>	<b>NP_919379.1</b>	<b>3e-37</b>	<b>52</b>	<b>159</b>	<b>930</b>
<b>BRF-D-9</b>	<b>Membrane protein p24B</b>	<i>Oncorhynchus mykiss</i>	<b>CAF25504.1</b>	<b>2e-83</b>	<b>71</b>	<b>312</b>	<b>923</b>
<b>BRF-E-114</b>	<b>Membrane protein, palmitoylated 5</b>	<i>Danio rerio</i>	<b>CAK04945.1</b>	<b>2e-134</b>	<b>86</b>	<b>481</b>	<b>930</b>
BRF-E-50	Mdm4 protein	<i>Danio rerio</i>	AAH97069.1	4e-39	85	165	930
<b>BRF-E-72</b>	<b>Mitogen-activated protein kinase 3</b>	<i>Homo sapiens</i>	<b>EAX00346.1</b>	<b>3e-109</b>	<b>76</b>	<b>398</b>	<b>931</b>
BRF-E-118	Matrix metalloproteinase	<i>Oncorhynchus mykiss</i>	BAB19131.1	4e-109	68	397	929
BRF-E-138	Mitochondrial ATP synthase F0 complex subunit c isoform 1	<i>Takifugu rubripes</i>	ABF22409.1	1e-52	97	209	748
<b>BRF-A-69</b>	<b>NADPH-cytochrome P450 oxidoreductase</b>	<i>Danio rerio</i>	<b>AAX37331.1</b>	<b>3e-23</b>	<b>72</b>	<b>112</b>	<b>922</b>
<b>BRF-E-122</b>	<b>NADPH oxidase cytosolic protein p47phox</b>	<i>Oncorhynchus mykiss</i>	<b>BAD60781.1</b>	<b>2e-117</b>	<b>74</b>	<b>425</b>	<b>930</b>
<b>BRF-D-57</b>	<b>Neurotoxin/C59/Ly-6-like protein</b>	<i>Ctenopharyngodon idella</i>	<b>AAR20998.1</b>	<b>4e-07</b>	<b>33</b>	<b>58.2</b>	<b>624</b>
<b>BRF-D-60</b>	<b>nephrosin</b>	<i>Oryzias latipes</i>	<b>NP_001098206.1</b>	<b>1e-27</b>	<b>81</b>	<b>125</b>	<b>504</b>
BRF-D-52	N-acetylgalactosamine-6-sulfate sulfatase	<i>Lentisphaera araneosa</i> <i>HTCC2155</i>	ZP_01873551	8.2	48	32.7	189
BRF-E-83	Nudix (nucleoside diphosphate linked moiety X)-type motif 9	<i>Danio rerio</i>	NP_998517.1	7e-83	74	310	929
BRF-E-54	Omega class glutathione-S-transferase	<i>Takifugu rubripes</i>	AAL08414.1	3e-83	80	311	863

**Table 2.** (Continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	s <sup>b</sup>	bp
BRF-D-53	Plrg1 protein	<i>Danio rerio</i>	AAH85620.1	5e-118	78	427	925
BRF-D-64	Phospholipase D	<i>Clostridium phytofermentans</i> ISDg	YP_001559557.1	1.5	29	36.6	715
<b>BRF-D-80</b>	<b>Predicted neutrophil cytosolic factor 1</b>	<b><i>Tetraodon nigroviridis</i></b>	<b>FAA00355.1</b>	<b>2e-117</b>	<b>69</b>	<b>425</b>	<b>928</b>
BRF-D-17	Pancreatic zymogen granule	<i>Danio rerio</i>	XP_696654.2	7.0	29	35	918
BRF-D-42	PDZ and LIM domain 5	<i>Danio rerio</i>	NP_001002654.1	2e-87	54	326	922
BRF-D-51	Phosphoglucomutase 3	<i>Danio rerio</i>	NP_001007054.1	4.8	23	33.5	484
BRF-E-84	Pentraxin	<i>Salmo salar</i>	CAA67765.1	5e-46	44	188	928
BRF-E-99	Processing of precursor 4, ribonuclease P/MRP subunit	<i>Danio rerio</i>	NP_001018585.1	5e-19	31	98.6	931
BRF-E-102	P47phox protein	<i>Takifugu rubripes</i>	NP_001027718.1	2e-126	76	455	929
BRF-E-135	Patatin	<i>Ralstonia eutropha</i> JMP134	YP_294422.1	8.0	37	33.9	642
<b>BRF-A-78</b>	<b>Ras-related C3 botulinum toxin substrate 2</b>	<b><i>Pagrus major</i></b>	<b>AAP20195.1</b>	<b>2e-36</b>	<b>95</b>	<b>156</b>	<b>834</b>
<b>BRF-E-45</b>	<b>Ras homolog gene family, member G</b>	<b><i>Danio rerio</i></b>	<b>NP_956334.1</b>	<b>5e-25</b>	<b>73</b>	<b>118</b>	<b>929</b>
<b>BRF-B-5</b>	<b>Regulator of G protein signaling 3</b>	<b><i>Gallus gallus</i></b>	<b>AAM94022.1</b>	<b>8e-54</b>	<b>88</b>	<b>214</b>	<b>925</b>
<b>BRF-B-40</b>	<b>Resistance protein RGC2</b>	<b><i>Lactuca sativa</i></b>	<b>AAQ72575.1</b>	<b>2.7</b>	<b>25</b>	<b>34.3</b>	<b>237</b>
BRF-B-55	Resistant to dieldrin CG10537-PB, isoform B	<i>Drosophila melanogaster</i>	NP_729462.1	2.4	47	35.0	554
BRF-B-58	Rps3a protein	<i>Xenopus tropicalis</i>	NP_001008075.1	1e-125	98	365	850
<b>BRF-B-66</b>	<b>RAB1A, member RAS oncogene family, isoform CRA_f</b>	<b><i>Homo sapiens</i></b>	<b>EAW99923.1</b>	<b>2e-102</b>	<b>95</b>	<b>375</b>	<b>924</b>
BRF-D-12	RPA interacting protein	<i>Mus musculus</i>	NP_081462.1	1e-04	41	48.9	283
BRF-E-71	Rbx1 protein	<i>Danio rerio</i>	AAT28894.1	2e-57	100	224	460
BRF-B-77	Ribosomal protein S7	<i>Solea senegalensis</i>	BAF45895.1	2e-100	97	367	634
BRF-B-38	Ribosomal protein S3a	<i>Solea senegalensis</i>	BAF45891.1	6e-127	96	446	923
BRF-D-9	Ribosomal protein L17	<i>Solea senegalensis</i>	BAF98664.1	3e-48	100	193	346
BRF-D-28	Ribosomal protein S23	<i>Danio rerio</i>	NP_001103591.1	3e-77	100	290	463
BRF-D-31	Ribosomal protein L36	<i>Solea senegalensis</i>	BAF98687.1	9e-46	97	187	930
BRF-D-67	Ribosomal protein large P0-like protein	<i>Sparus aurata</i>	AAT44430.1	6e-103	94	297	928
BRF-D-49	Ribosomal protein S4	<i>Solea senegalensis</i>	BAF45892.1	6e-146	96	520	860
BRF-E-85	Ribosomal protein L32	<i>Epinephelus coioides</i>	AAL48255.1	5e-64	98	248	930
BRF-E-95	Ribosomal protein L8	<i>Lateolabrax japonicus</i>	ABQ57487.1	1e-139	98	498	815
BRF-A-75	40s ribosomal protein S7	<i>Perca flavescens</i>	ABU54857.1	4e-42	96	429	323
BRF-D-51	40S ribosomal protein S14	<i>Scophthalmus maximus</i>	ABJ98607.1	6e-74	97	279	511

**Table 2.** (Continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	s <sup>b</sup>	bp
BRF-D-19	40S ribosomal protein Sa-like protein	<i>Sparus aurata</i>	AAT44424.1	2e-146	97	521	898
BRF-E-13	40S ribosomal protein S30	<i>Hippocampus comes</i>	AAQ63318.1	1e-55	95	218	540
BRF-C-13	Similar to ribosomal protein L36a	<i>Equus caballus</i>	XP_001492679	9e-17	97	89.0	240
BRF-A-29	Similar to Kelch-like protein 3	<i>Danio rerio</i>	XP_696980	4e-09	57	65.9	921
BRF-A-39	SEC-independent protein translocase protein	<i>Agrobacterium tumefaciens</i>	NP354692.1	4.6	27	33.5	432
BRF-A-45	Solute carrier family	<i>Fundulus heteroclitus</i>	BAF94332	6e-81	68	304	921
BRF-B-4	Serine carboxypeptidase 1	<i>Danio rerio</i>	AAH66718.2	0.55	78	37.4	581
<b>BRF-C-10</b>	<b>Serine protease inhibitor Kazal-type 4 precursor</b>	<b><i>Sus scrofa</i></b>	<b>P37109</b>	<b>6e-05</b>	<b>37</b>	<b>50.1</b>	<b>504</b>
<b>BRF-E-80</b>	<b>Serine peptidase inhibitor, Kazal type 3</b>	<b><i>Mus musculus</i></b>	<b>NP_0033284.1</b>	<b>2e-05</b>	<b>42</b>	<b>51.2</b>	<b>492</b>
<b>BRF-C-14</b>	<b>Surface antigen (D15)</b>	<b><i>Petrotoga mobilis</i></b>	<b>YP_001568267</b>	<b>2.0</b>	<b>32</b>	<b>35.4</b>	<b>584</b>
BRF-C-20	Src-like-adaptor	<i>Gallus gallus</i>	NP_989722	3e-75	53	285	928
BRF-B-19	Slc5a8 protein	<i>Xenopus tropicalis</i>	AAH61598.1	1e-76	68	290	1000
BRF-B-76	ST3 beta-galactoside alpha-2,3-sialyltransferase 4	<i>Danio rerio</i>	NP_001003854.1	9e-53	48	210	917
BRF-D-77	Similar to Centaurin, alpha 2	<i>Danio rerio</i>	XP_689160.1	6e-65	75	251	929
BRF-D-89	Similar to Uridine phosphorylase 1	<i>Ornithorhynchus anatinus</i>	XP_001506899.1	7e-07	73	57.8	760
BRF-D-2	Similar to olfactory receptor Olfr305	<i>Gallus gallus</i>	XP_424001.2	5.3	51	35.4	931
BRF-E-1	Sorting and assembly machinery component 50 homolog	<i>Danio rerio</i>	NP_956436.1	1e-124	81	449	932
BRF-E-16	Similar to Pancreatic secretory trypsin inhibitor precursor	<i>Canis familiaris</i>	XP_850557.1	2e-05	43	51.2	472
<b>BRF-E-51</b>	<b>Similar to v-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3</b>	<b><i>Danio rerio</i></b>	<b>XP_001334480.1</b>	<b>3e-45</b>	<b>71</b>	<b>159</b>	<b>1000</b>
BRF-E-68	Similar to SH2-B homolog	<i>Monodelphis domestica</i>	XP_001372483.1	3e-05	66	52.8	931
BRF-E-126	Similar to nonmuscle myosin heavy chain	<i>Danio rerio</i>	XP_683046.2	8.2	36	32.7	382
BRF-E-101	Similar to 19.9kD myosin light chain isoform 1	<i>Danio rerio</i>	XP_685183.1	3e-93	97	345	1000
BRF-D-55	Similar to HS1	<i>Equus caballus</i>	XP_001503158.1	3e-04	86	48.5	652
<b>BRF-A-73</b>	<b>Transmembrane 4 superfamily member 3</b>	<b><i>Xenopus laevis</i></b>	<b>NP_001080859.1</b>	<b>1e-24</b>	<b>33</b>	<b>117</b>	<b>888</b>
BRF-B-9	T-complex polypeptide 1	<i>Danio rerio</i>	AF143493_1	2e-150	89	535	926

**Table 2.** (Continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I <sup>a</sup>	s <sup>b</sup>	bp
BRF-B-18	Tcp1 protein	<i>Danio rerio</i>	AAH44397.1	6e-31	51	129	554
BRF-B-81	Tetraspanin 8	<i>Xenopus tropicalis</i>	AAH88067.1	1e-24	31	117	767
BRF-D-4	Transaldolase	<i>Ctenopharyngodon idella</i>	AAN62918.1	3e-57	83	225	928
BRF-D-58	Tubulin cofactor a	<i>Danio rerio</i>	NP_957348.1	6e-36	67	154	928
<b>BRF-E-9</b>	<b>Translationally-controlled tumor protein (TCTP)</b>	<i>Lateolabrax japonicus</i>	<b>AAP43627.1</b>	<b>6e-61</b>	<b>67</b>	<b>238</b>	<b>933</b>
BRF-E-89	Telomerase binding protein, p23	<i>Danio rerio</i>	NP_998335.1	2e-65	76	252	928
<b>BRF-D-50</b>	<b>Ubiquitin</b>	<i>Aedes aegypti</i>	<b>XP_001664266.1</b>	<b>2e-66</b>	<b>99</b>	<b>254</b>	<b>546</b>
<b>BRF-E-11</b>	<b>Ubiquitin-like domain containing CTD phosphatase1</b>	<i>Danio rerio</i>	<b>NP_001002319.1</b>	<b>3e-19</b>	<b>81</b>	<b>99.4</b>	<b>929</b>
<b>BRF-E-57</b>	<b>Utp15, U3 small nucleolar ribonucleoprotein, homolog</b>	<i>Danio rerio</i>	<b>NP_955998.1</b>	<b>4e-108</b>	<b>69</b>	<b>392</b>	<b>930</b>
<b>BRF-E-98</b>	<b>Ubiquitin-like protein</b>	<i>Oncorhynchus mykiss</i>	<b>AAO14689.1</b>	<b>1e-36</b>	<b>51</b>	<b>157</b>	<b>1000</b>
<b>BRF-E-116</b>	<b>Ubiquitin fusion degradation 1-like</b>	<i>Danio rerio</i>	<b>NP_001002451.1</b>	<b>1e-77</b>	<b>86</b>	<b>293</b>	<b>926</b>
BRF-E-52	V-ATPase subunit A	<i>Oreochromis mossambicus</i>	BAF94024.1	7e-165	95	583	928
<b>BRF-E-88</b>	<b>Zinc finger domain, LSD1 subclass family protein</b>	<i>Tetrahymena thermophila</i>	<b>XP_001017432.1</b>	<b>1.9</b>	<b>30</b>	<b>37.0</b>	<b>930</b>

<sup>a</sup> Identity (%).<sup>b</sup> Score (bite).

Bold are putative bio-defense and immune related genes.

phocytes, following stimulation, express numerous cytokine genes and immune response-related genes. However, it is difficult to detect the proliferation of normal lymphocytes in response to specific antigens, only a minute proportion of cells will be stimulated to divide. The teleost fish leukocyte immune system possesses B and T lymphocytes, similar to that of mammals, although they show different responses to band T-lymphocyte mitogens (Clem *et al.*, 1984; Manning, 1994). The concanavalin A (Con A) activation system has previously been used for the analysis of molecular determinants of fish leukocyte proliferation (Graham and Secombes, 1988; Yin *et al.*, 1999). Furthermore, phorbol myristate acetate (PMA) has also been used as a mitogen together with Con A or lipopolysaccharide (LPS) (Johnson *et al.*, 1987; Takeshita *et al.*, 1988;

Murphy and Norton, 1993).

In conclusion, this study reports an expressed sequence tag (EST) based gene identification analysis of 386 ESTs derived from rockfish leukocyte cDNA library. These EST analysis will be useful for construction of cDNA microarray. In particular, the application of cDNA microarrays may facilitate research attempting to answer questions concerning immune responses and other protective responses of rockfish upon infection of pathogens.

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