

EST-based Identification of Genes Expressed in the Brain of the Olive Flounder *Paralichthys olivaceus*

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We have constructed a cDNA library using brain samples of olive flounder *Paralichthys olivaceus*. Here, we described the study on gene identification by screening 356 clones from the brain cDNA library of olive flounder. Here, we screened 356 clones from the library to identify genes. Of these, 176 (49.5%) were identified as orthologs of known genes from olive flounder and other organisms. Among the 176 EST clones, 33 (18.7%) represented 11 unique genes that are identical to expressed sequence tags (ESTs) reported for olive flounder, and 120 (68.2%) represented 102 unique genes known from other organisms. The percentage of unknown genes (50.5%) is higher than in other olive flounder cDNA libraries (Lee et al., 2003, 2006, 2007), reflecting the high complexity of brain tissue. Further studies of expression characterization and developmental behavior related to these genes should provide useful insight into the physiological functions of the brain in olive flounder.

Key words: Olive flounder, Brain, Expressed sequence tags (ESTs), Expression profile

Introduction

Expressed sequence tags (ESTs) are partial 5' or 3' end sequences, generated by single-pass sequencing of randomly selected cDNA clones. These sequences have a number of practical benefits in that they can be generated rapidly and relatively inexpensively (Adams et al., 1991). To date, the GenBank EST database (dbEST) contains more than 46,050,546 entries from various organisms (release 092107). In teleosts, EST studies have been reported for model fishes such as the zebrafish *Danio rerio* (Ton et al., 2000) and medaka *Oryzias latipes* (Kimura et al., 2004).

However, only a few specific tissues have been used in EST projects for some aquaculture species, such as the winter flounder (Douglas et al., 1999), olive flounder (Aoki et al., 1999; Nam et al., 2000, 2003; Lee et al., 2003, 2006, 2007), salmon (Davey et al., 2001; Martin et al., 2002), channel catfish (Karsi et al., 1998, 2002; Ju et al., 2000; Kim et al., 2000;

Kocabas et al., 2002), red sea bream (Chen et al., 2004), tilapia (Shiue et al., 2004), common carp (Kono et al., 2004), and gilthead sea bream (Sarropoulou et al., 2005). The lack of EST resources for commercial fish species prohibits the use of modern functional genomics approaches for studying their growth, development, reproduction, stress biology, and molecular breeding (Chen et al., 2004).

The olive flounder *Paralichthys olivaceus* is one of the most widely cultured fish species in Korea and is considered an important source of protein. Investigations into molecular mechanisms are required to establish new methods for determining its performance traits, such as growth, feed conversion, and reproductive behavior. The screening and identification of genes expressed in the brain will provide useful insight into the molecular mechanisms of growth, reproduction, and sex differentiation. Furthermore, knowledge of gene expression patterns can provide important clues on the functional interactions of genes within the brain (Gawantka et al., 1998; Wen et al., 1998; Matoba et al., 2000).

Recently, we constructed a cDNA library using

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brain samples of olive flounder. In the present study, we identified genes, screened 356 clones from the library, and analyzed the expression profiles of the identified clones to understand the molecular composition of the olive flounder brain.

Materials and Methods

Fish and tissue preparation

Olive flounders were raised at the Genetics and Breeding Research Center, National Fisheries Research and Development Institute (NFRDI) and maintained in a 10 ton flow-through tank at $15 \pm 1^\circ\text{C}$ under a natural photoperiod.

Tissues were collected and cut into pieces as small as possible. Pooled tissues were frozen rapidly with liquid nitrogen, 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).

Construction of the olive flounder cDNA library

Olive flounder brain cDNA library was constructed from 10 individual fish using the Uni-ZAP XR cDNA synthesis/Gigapack cloning kit (Stratagene Cloning Systems). Total RNA was extracted using TRIzol reagent (Gibco BRL Life Technologies, Renfrewshire, UK), and mRNA was enriched using oligo-(dT) cellulose chromatography with the PolyA Tract mRNA isolation kit (Promega, WI, USA). cDNA synthesis was carried out using an oligo-(dT)₁₈ primer for the reverse transcription of approximately 5 μg mRNA and the libraries were constructed by directional cloning based on the manufacturer's instruction manual. All primary libraries were amplified and aliquots of each amplified library were stored at both 4 and -70°C . Mass excision was performed and the cDNA inserts from the amplified Uni-ZAP XR libraries were rescued as pBluescript phagemids in SOLR *Escherichia coli*.

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 QIAGEN Spin Column Miniplasmid kits. For the sequencing reactions, 3 μL plasmid DNA (0.5-1.0 μg) were used. Single-pass sequencing of the 5'-termini of selected cDNA clones

in phagemid form was performed using an ABI 3100 automatic DNA sequencer (PE Applied Biosystems, CA, USA) and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

Bioinformatics analysis

Bioinformatics analysis was conducted to determine gene identities using GeneMaster software (Ensoltek, Korea). The procedures used to establish orthologs are shown in Fig. 1. Briefly, vector sequences were removed and the database search was limited to ESTs >100 bp in length. Then, the ESTs were assembled in clusters of contiguous sequences (contig) using the ICAtools program with the default parameters (Parsons, 1995). Gene annotation procedures and homology searches of the sequenced ESTs were done locally using blastx for comparing amino acid similarity (Altschul et al., 1997). Matches with an Expect value (E) less than 1.0×10^{-4} was considered to be significant. After the blastx searches, a visual inspection was made to determine if significant similarities were caused by simple sequences. ESTs with significant similarities in the 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.

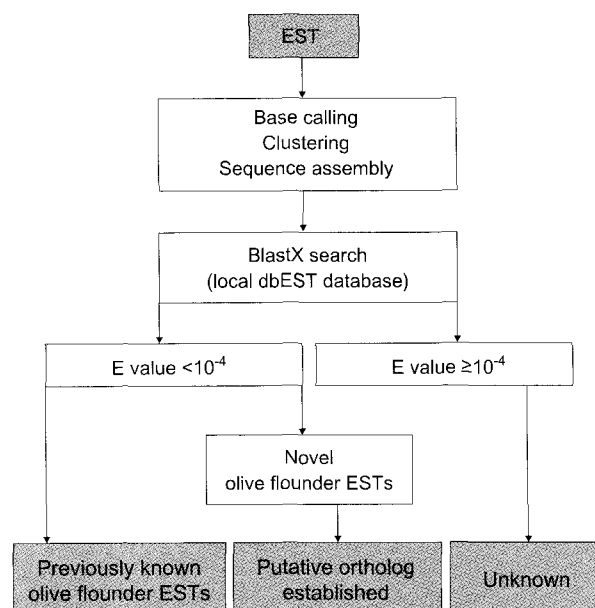


Fig. 1. Schematic presentation of sequence analysis and gene annotation using BlastX searches.

Results and Discussion

Sequencing, clustering and similarity search of brain ESTs

A total of 356 randomly selected clones were single-pass sequenced from the 5' end, resulting in the characterization of cDNA clones that were longer than 100 bp after eliminating the vector sequence. The number of clones sequenced from the cDNA library, average size of inserts, and redundancy of the obtained sequences are given in Table 1. The estimated average insert size was 1.7 ± 0.3 kb based on PCR amplification of inserts from 20 randomly selected clones. The assembly program ICAtools (Parsons, 1995) was used to organize the redundant ESTs into overlapping contigs. The results showed that the 356 brain ESTs consisted of 10 clusters and 299 singletons, suggesting that the overall redundancy of the library was 13.2%.

Table 1. General characteristics of olive flounder brain ESTs

Total cDNA sequenced ^a	356
Average insert size ^b	1.7 ± 0.3 kb
Average EST length	533 bp
EST clusters ^c	10
Singletons ^d	299
Redundancy ^e	13.2 %
ESTs with E value $< 1 \times e^{-4}$ (matched)	176 (49.5%)
ESTs with E value $\geq 1 \times e^{-4}$ (unknown)	180 (50.5%)

^aLength of sequence used for comparison after editing (inserts < 100 base pairs were excluded).

^bThe average insert size was calculated for 20 randomly selected cDNA clones.

^cESTs with 90% or greater identity over a 100 bp region were clustered together forming 10 EST clusters.

^d299 sequences did not sufficiently match any sequence in the data set to allow assembly.

^eRedundancy = The number of genes / total ESTs.

The ESTs with significant similarity ($E < 1 \times e^{-4}$) to known proteins were evaluated to determine if the significant similarities were caused by simple amino acid matches (Table 2). Of the 356 clones, 176 (49.5%) were identified as orthologs of known genes from olive flounder and other organisms. The remaining 180 (50.5%) could not be identified by similarity comparisons ($E \geq 1 \times e^{-4}$); most of them are expected to be derived from hitherto uncharacterized or novel genes, although for some of them, the homology may be too low to detect in the region that was sequenced. Among the 176 EST clones, 33 (18.7%) represented 11 unique genes that are identical to reported olive flounder ESTs and 120

(68.2%) represented 102 unique genes known from other organisms (Fig. 2). Twenty-three (13.1%) clones representing 23 unique genes showed significant similarities to known sequences with unknown functions from model systems such as *Homo sapiens*, *Macaca fascicularis*, and *Mus musculus*. Although their functions are not yet known, their conservation in fish adds to the evidence demonstrating the long evolutionary history of these gene families. Once a gene is characterized in any one of these species, comparative functional genomics allows the annotation of these orthologous genes.

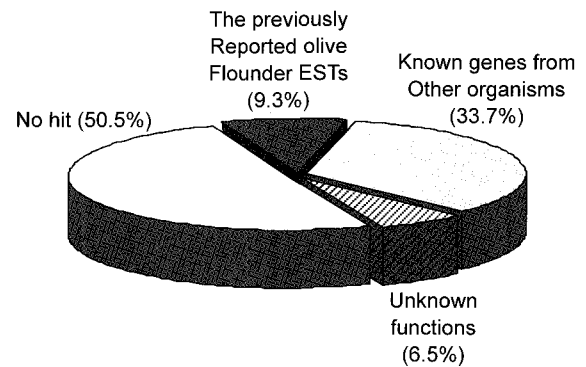


Fig. 2. Classification of 356 ESTs from the olive flounder brain cDNA library. ESTs with E value $< 1 \times e^{-4}$ were classified: the previously reported olive flounder ESTs, known genes from other organisms and known sequences of unknown functions. ESTs with E value $\geq 1 \times e^{-4}$ were classified as novel.

Expression profile and gene identification of brain ESTs

The expression profiles of the EST clones identified from the olive flounder brain are shown in Fig. 3. Of the 356 EST clones identified using blastx, 299 (84.0%) were singletons. Although redundancy will increase with the number of clones sequenced, the high percentage of singletons indicates that this olive flounder brain cDNA library has high complexity and coverage. Of the 356 brain ESTs, the redundancy factors of 299 (84.0%) clones, 13 (3.7%) clones, and 44 (12.3%) clones were 1.0, 2-2.5, and > 2.5 , respectively. These results suggest that this library has a relatively even distribution of cDNA clones with relatively few clones that tend to contribute to redundancy; consequently, this library can supply more unique, novel cDNA sequences in an olive flounder EST project.

In conclusion, this study described preliminary work using an EST approach to identify genes in the olive flounder brain. From the 356 sequenced clones, 176 (49.5%) were identified and assembled into 113

Table 2. List of identified ESTs from cDNA library of olive flounder brain

Clone no.	Putative identification	Closest species	Accession no.	E-value	I ^a	F ^b
brain-2-F2	gelatinase	<i>Paralichthys olivaceus</i>	BAB68365	0.00E+00	100	1
brain-2-E8	glutamine-binding protein	<i>Escherichia coli</i>	1WDNA	0.00E+00	100	1
brain-2-B8	cytochrome b	<i>Paralichthys olivaceus</i>	NP_037594	0.00E+00	95	2
brain-2-C2	Na ⁺ /K ⁺ ATPase alpha1B1 subunit	<i>Danio rerio</i>	NP_571761	0.00E+00	95	1
brain-3-E11	receptor for activated protein kinase C	<i>Oreochromis niloticus</i>	O42249	0.00E+00	95	1
brain-2-D3	cAMP-dependent protein kinase type I-beta	<i>Homo sapiens</i>	P31321	0.00E+00	95	1
brain-3-E10	eukaryotic translation elongation factor 2	<i>Homo sapiens</i>	XP_009189	0.00E+00	92	1
brain-2-A10	hypothetical protein Sand	<i>Takifugu rubripes</i>	T30808	0.00E+00	89	1
brain-2-E10	tyrosylprotein sulfotransferase 1	<i>Danio rerio</i>	Q9PTE6	0.00E+00	88	1
BRAI02E07	transferrin	<i>Paralichthys olivaceus</i>	AAF33234	3.00E-99	98	1
brain-1-E10	ubiquitin fusion degradation 1-like protein	<i>Xenopus laevis</i>	AAG25922	4.00E-97	91	1
brain-3-F7	somatolactin precursor	<i>Paralichthys olivaceus</i>	P20362	1.00E-95	93	19
brain-2-C5	Ras-related protein ORAB-1	<i>Discopyge ommata</i>	P22125	1.00E-91	98	1
brain-2-F9	apoptosis specific protein	<i>Homo sapiens</i>	NP_004840	1.00E-89	75	1
brain-3-C10	guanine nucleotide-binding protein, beta-1	<i>Homo sapiens</i>	NP_032168	4.00E-89	89	1
brain-2-A1	somatotropin precursor	<i>Paralichthys olivaceus</i>	P09537	3.00E-87	100	1
brain-3-B10	elongation factor 1-alpha chain	<i>Xenopus laevis</i>	P13549	9.00E-87	92	1
brain-2-C11	glycyl tRNA synthetase	<i>Homo sapiens</i>	AAC71652	3.00E-85	85	1
BRAI01D01	phosphoglycerate mutase 1	<i>Homo sapiens</i>	NP_002620	6.00E-83	89	1
brain-2-C9	ubiquitin-conjugating enzyme E2D 2	<i>Mus musculus</i>	NP_003330	7.00E-81	94	1
brain-2-B4	probable Bax inhibitor-1 (BI-1)	<i>Paralichthys olivaceus</i>	Q9IA79	3.00E-79	98	1
brain-3-H4	KIAA0895 protein	<i>Homo sapiens</i>	BAA74918	9.00E-78	78	1
brain-1-F4	taurine transporter	<i>Oreochromis mossambicus</i>	BAB18038	1.00E-77	96	1
brain-2-A7	membrane protein, palmitoylated 2	<i>Mus musculus</i>	NP_057904	1.00E-77	72	1
BRAI02B03	cytochrome c oxidase subunit I	<i>Paralichthys olivaceus</i>	NP_037584	4.00E-76	94	3
BRAI01G10	BTB (POZ) domain containing 2	<i>Mus musculus</i>	XP_030647	2.00E-75	90	1
BRAI01F10	myosin regulatory light chain, isoform L20-B1	<i>Gallus gallus</i>	P24032	4.00E-73	95	1
BRAI02B02	catenin (cadherin-associated protein), alpha	<i>Danio rerio</i>	NP_571531	6.00E-73	90	1
brain-1-H10	leucine rich repeat (in FLII) interacting protein 2	<i>Homo sapiens</i>	NP_006300	8.00E-72	73	1
BRAI02D02	HMG-1	<i>Oncorhynchus mykiss</i>	AAA58771	4.00E-71	84	1
brain-2-H6	phospholipid hydroperoxide glutathione	<i>Gallus gallus</i>	AAM18080	2.00E-70	75	1
BRAI01G11	unnamed protein product	<i>Mus musculus</i>	BAB29242	7.00E-67	74	1
brain-2-G5	MAD homolog 5	<i>Danio rerio</i>	NP_571443	2.00E-66	100	1
brain-3-A7	prolyl 4-hydroxylase alpha subunit	<i>Gallus gallus</i>	P16924	6.00E-66	92	1
brain-2-F3	myelin proteolipid protein	<i>Oncorhynchus mykiss</i>	P79826	1.00E-65	86	1
BRAI01C03	creatine kinase B	<i>Oryctolagus cuniculus</i>	P00567	1.00E-65	80	1
brain-3-B3	cytochrome c oxidase subunit II	<i>Paralichthys olivaceus</i>	NP_037585	2.00E-65	81	1
brain-1-B9	basic leucine-zipper protein BZAP45	<i>Mus musculus</i>	NP_055485	3.00E-65	75	1
brain-1-B10	protein phosphatase 1, catalytic subunit	<i>Rattus norvegicus</i>	NP_002700	2.00E-64	95	1
BRAI01C12	ERD-2-like protein, ELP-1	<i>Homo sapiens</i>	A42286	1.00E-63	79	1
brain-3-A9	beta tubulin	<i>Notothenia coriiceps</i>	AAG15316	2.00E-62	100	1
brain-1-B2	cytochrome c oxidase subunit III	<i>Paralichthys olivaceus</i>	NP_037588	2.00E-62	83	1
brain-2-E4	annexin A13	<i>Danio rerio</i>	NP_571849	2.00E-62	82	1
brain-3-C3	cathepsin F	<i>Homo sapiens</i>	NP_003784	3.00E-62	61	1
brain-3-D1	cyclophilin 1	<i>Drosophila subobscura</i>	AAB87889	2.00E-59	84	1
brain-3-F11	14-3-3 protein	<i>Caenorhabditis elegans</i>	NP_502235	5.00E-59	68	1
BRAI02B11	LOC226591 protein	<i>Mus musculus</i>	AAH02098	2.00E-58	68	1
brain-1-H8	endothelial differentiation-related factor 1	<i>Homo sapiens</i>	NP_003783	4.00E-58	92	1
BRAI02B10	ISCU2	<i>Homo sapiens</i>	AAG37428	1.00E-57	93	1
BRAI02F01	casein kinase I alpha LS	<i>Gallus gallus</i>	AAB96334	7.00E-57	74	1
brain-2-F7	nuclear receptor-interacting factor	<i>Homo sapiens</i>	AAL55819	7.00E-56	54	1
brain-2-D12	cellular repressor of E1A-stimulated genes	<i>Homo sapiens</i>	NP_003842	2.00E-55	59	1
brain-3-D8	ras suppressor protein 1	<i>Homo sapiens</i>	NP_036557	8.00E-55	84	1
brain-1-B4	inositol-1(or 4)-monophosphatase (IMPase)	<i>Bos taurus</i>	P20456	8.00E-55	69	1
brain-1-A10	glycogen phosphorylase	<i>Homo sapiens</i>	1402394A	4.00E-54	73	1
brain-2-D7	glycine cleavage system H protein	<i>Gallus gallus</i>	P11183	6.00E-54	72	1
brain-1-C8	enterocyte differentiation promoting factor	<i>Homo sapiens</i>	G02766	2.00E-53	71	1
brain-3-C6	gamma-SNAP protein	<i>Bos taurus</i>	S32369	3.00E-53	73	1
brain-2-G2	calcyclin binding protein	<i>Mus musculus</i>	AAC16757	3.00E-52	55	2

Table 2. (continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I ^a	F ^b
brain-1-A5	DEAQ RNA-dependent ATPase	<i>Mus musculus</i>	NP_291084	3.00E-51	54	1
brain-3-B9	nucleobindin 2 precursor	<i>Homo sapiens</i>	NP_005004	4.00E-51	56	1
brain-2-C8	cdk inhibitor p21 binding protein	<i>Homo sapiens</i>	AAH09771	6.00E-50	67	1
brain-2-D2	alpha-2-HS-glycoprotein	<i>Platichthys flesus</i>	CAC27520	1.00E-48	85	8
brain-3-A1	uracil-DNA glycosylase precursor	<i>Gadus morhua</i>	CAB85707	1.00E-48	84	1
BRAI02F02	stathmin	<i>Gallus gallus</i>	P31395	1.00E-47	74	1
BRAI01H12	tubulin alpha chain, neuron-specific isoform	<i>Torpedo marmorata</i>	P36220	5.00E-46	98	1
brain-2-G11	katanin p80 subunit B 1	<i>Homo sapiens</i>	NP_005877	9.00E-45	48	1
brain-2-D1	tyrosine 3-monooxygenase	<i>Rattus norvegicus</i>	NP_113791	5.00E-44	89	1
brain-3-G4	KIAA0939 protein	<i>Homo sapiens</i>	BAA76783	1.00E-41	72	1
brain-3-G9	POMC-II precursor protein	<i>Paralichthys olivaceus</i>	AAG28378	1.00E-40	71	2
BRAI02A02	ATP synthase, H ⁺ transporting	<i>Rattus norvegicus</i>	NP_001680	2.00E-40	64	1
brain-3-B5	fatty acid binding protein 7	<i>Homo sapiens</i>	NP_001437	4.00E-40	85	1
brain-3-H6	ASB-1 protein	<i>Homo sapiens</i>	NP_057198	6.00E-40	56	1
BRAI01D04	mitochondrial phosphate transporter precursor	<i>Rattus norvegicus</i>	NP_620800	3.00E-39	80	1
brain-1-D10	unnamed protein product	<i>Mus musculus</i>	BAB27541	5.00E-39	67	1
brain-1-G5	hypoxia-inducible gene 1	<i>Gillichthys mirabilis</i>	AAG13326	7.00E-36	76	1
brain-1-C5	Apolipoprotein A-I precursor (Apo-AI)	<i>Sparus aurata</i>	O42175	4.00E-38	42	1
brain-1-F9	25-hydroxyvitamin D-1 alpha hydroxylase	<i>Homo sapiens</i>	XP_103864	2.00E-36	63	1
brain-1-D4	hypothetical protein	<i>Homo sapiens</i>	CAD28466	6.00E-36	48	1
BRAI01H03	guanine nucleotide binding protein (G protein),	<i>Danio rerio</i>	NP_571916	5.00E-35	93	1
BRAI01B07	RIKEN cDNA 2010000G05	<i>Mus musculus</i>	NP_079904	2.00E-33	66	1
BRAI02A10	diazepam-binding inhibitor	<i>Rana ridibunda</i>	AAB60606	8.00E-32	70	1
brain-1-F10	GRB2-associated binding protein 1	<i>Homo sapiens</i>	NP_002030	1.00E-31	65	1
brain-1-D6	unnamed protein product	<i>Homo sapiens</i>	BAA91551	2.00E-31	53	1
BRAI02G10	schwannomin interacting protein 1	<i>Mus musculus</i>	NP_038956	3.00E-31	72	1
BRAI02F11	NADH dehydrogenase subunit 2	<i>Paralichthys olivaceus</i>	NP_037583	5.00E-31	77	1
brain-3-A4	Unknown (protein for IMAGE:3926450)	<i>Homo sapiens</i>	AAH04508	2.00E-30	85	1
brain-1-H4	hypothetical protein	<i>Macaca fascicularis</i>	BAB33051	4.00E-30	46	1
BRAI01B10	amphotropic murine leukemia virus receptor	<i>Cricetulus griseus</i>	AAG21945	2.00E-28	87	1
brain-2-A6	beta-2 microglobulin precursor	<i>Ictalurus lupus</i>	AAC64992	4.00E-28	59	1
brain-1-B1	hippocalcin-like protein 4	<i>Homo sapiens</i>	NP_057341	9.00E-28	89	2
brain-2-A11	homolog to HSPC233 (pyrimidine 5'-nucleotidase)	<i>Mus musculus</i>	BAB27677	1.00E-27	53	1
brain-2-G7	KIAA0722 protein	<i>Homo sapiens</i>	BAA34442	1.00E-27	40	1
BRAI01E09	human putative tumor suppressor	<i>Homo sapiens</i>	AAB42216	2.00E-27	46	1
BRAI02A04	FTZ-F1	<i>Oryzias latipes</i>	BAA32394	3.00E-25	40	1
brain-3-C2	HIF-prolyl hydroxylase 1	<i>Homo sapiens</i>	NP_444274	1.00E-24	91	1
brain-2-H11	cold-inducible RNA binding protein	<i>Xenopus laevis</i>	O93235	2.00E-24	60	1
brain-1-B11	tricarboxylate carrier protein	<i>Mus musculus</i>	BAB23794	6.00E-24	56	1
brain-1-C12	BASH	<i>Gallus gallus</i>	BAA36275	4.00E-23	68	1
brain-2-G10	S100 calcium-binding protein, beta (neural)	<i>Rattus norvegicus</i>	NP_037323	7.00E-23	60	10
BRAI01B03	intestinal fatty acid binding protein	<i>Danio rerio</i>	AAF00925	6.00E-22	69	1
brain-2-F11	heat shock protein 90 beta	<i>Platichthys flesus</i>	CAC27523	9.00E-22	90	1
BRAI01B09	swelling dependent chloride channel	<i>Danio rerio</i>	NP_571499	3.00E-21	60	1
BRAI01F07	HMG-box containing protein 1	<i>Rattus norvegicus</i>	NP_037353	7.00E-21	90	1
BRAI01E05	clathryn light chain (LCA2)	<i>Rattus norvegicus</i>	AAA40869	3.00E-20	79	1
brain-3-F2	small integral membrane protein of lysosome	<i>Gallus gallus</i>	BAB85678	1.00E-19	42	1
brain-2-E9	heterogeneous nuclear ribonucleoprotein H3	<i>Homo sapiens</i>	NP_067676	5.00E-18	73	1
BRAI01D02	KLP2 protein	<i>Xenopus laevis</i>	T30335	1.00E-16	40	1
BRAI01G06	unnamed protein product	<i>Homo sapiens</i>	BAB14308	3.00E-16	94	1
brain-1-A7	hypothetical protein	<i>Homo sapiens</i>	XP_069903	2.00E-15	43	2
brain-1-E11	CD36 antigen-like 2	<i>Rattus norvegicus</i>	NP_446453	5.00E-15	40	1
brain-2-E3	Unknown (protein for MGC:25689)	<i>Mus musculus</i>	AAH19430	1.00E-14	43	1
brain-1-A8	tail:host specificity	<i>Bacteriophage lambda</i>	NP_040600	2.00E-13	100	1
brain-1-B6	neuropilin-like protein	<i>Mus musculus</i>	AAL30179	2.00E-13	24	1
brain-2-C6	unnamed protein product	<i>Homo sapiens</i>	BAB55046	3.00E-13	64	1
brain-2-B3	sphingosine-1-phosphatase	<i>Homo sapiens</i>	NP_110418	3.00E-13	52	1
brain-1-C6	phospholipase D1	<i>Mus musculus</i>	Q9Z280	5.00E-13	83	1
brain-3-A11	PIUS protein	<i>Rattus norvegicus</i>	NP_067692	1.00E-12	70	1

Table 2. (continued)

Clone no.	Putative identification	Closest species	Accession no.	E-value	I ^a	F ^b
brain-1-D9	organic anion transporting polypeptide Oatp	<i>Raja erinacea</i>	AAL66021	3.00E-12	54	1
brain-3-G6	hexosaminidase B preproprotein	<i>Homo sapiens</i>	NP_000512	4.00E-12	51	1
brain-3-H7	KIAA1617 protein	<i>Homo sapiens</i>	XP_166140	7.00E-12	33	1
brain-1-A3	Raichu404X	<i>Homo sapiens</i>	BAB61868	5.00E-11	75	1
BRAI01H11	Tetraspanin 39D	<i>Drosophila melanogaster</i>	NP_523612	1.00E-10	33	1
BRAI02C12	HSPC059 protein	<i>Homo sapiens</i>	NP_057620	3.00E-10	37	1
BRAI02E12	Ras-related GTP-binding protein	<i>Homo sapiens</i>	NP_006561	5.00E-10	86	1
brain-2-A8	KIAA0546 protein	<i>Homo sapiens</i>	XP_049055	5.00E-10	54	1
BRAI02D04	hypothetical protein	<i>Homo sapiens</i>	NP_112216	2.00E-09	52	1
brain-1-F5	Similar to HSPC156 protein	<i>Homo sapiens</i>	AAH09499	2.00E-09	39	1
brain-1-E9	hypothetical protein	<i>Homo sapiens</i>	CAB92850	4.00E-09	72	1
BRAI02B09	chromosome 3 open reading frame 4	<i>Homo sapiens</i>	NP_063948	4.00E-09	50	1
brain-2-A12	rectachrome 1	<i>Homo sapiens</i>	AAF76328	9.00E-09	55	1
brain-3-C11	apolipoprotein E	<i>Scophthalmus maximus</i>	CAB65356	1.00E-08	75	1
BRAI02F04	similar to FLJ00179 protein	<i>Mus musculus</i>	XP_130080	5.00E-08	70	1
brain-3-G7	Ig superfamily receptor LNIR; nectin 4	<i>Homo sapiens</i>	NP_112178	4.00E-06	40	1
brain-1-D5	hypothetical protein	<i>Homo sapiens</i>	XP_090095	2.00E-05	46	1
BRAI01A08	myosin heavy chain-like protein	<i>Arabidopsis thaliana</i>	NP_198994	5.20E-05	27	7
brain-3-H1	VW domain binding protein 2	<i>Mus musculus</i>	NP_058548	8.00E-05	60	1

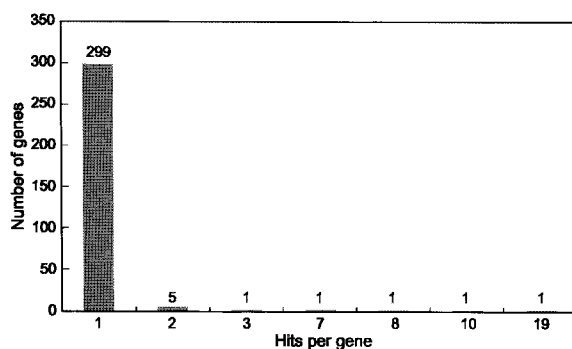
^aIdentity (%).^bFrequency of the clones in the sequenced pool.

Fig. 3. Expression profiles and sequencing redundancy in the analysis of ESTs from the olive flounder brain.

known genes. The percentage of unknown genes (50.5%) is higher than in other olive flounder cDNA libraries (Lee et al., 2003, 2006, 2007), reflecting the high complexity of brain tissue. Further studies of expression characterization and developmental behavior related to these genes should provide useful insight into the physiological functions of the brain in olive flounder.

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