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Tissue-specific Expressed Sequence Tags from the Olive flounder, *Paralichthys olivaceus*

Young-Ok Kim, Jeong-Ho Lee, Kyung-Kil Kim, Jong-yun Lee
Biotechnology Research Center
National Fisheries Research and Development Institute, Pusan, 619-902, Korea

Introduction

Expressed sequence tags (ESTs) are generated by single-pass DNA sequencing of clones obtained from cDNA libraries and are powerful tool in the genetic characterization of organisms, owing in large part to the speed and affordability of generating these sequences. Comparison of sequences obtained with those available in public sequence databases allows putative identification of many genes. ESTs have applications in the discovery of new genes, mapping of genome, and identification of coding regions in genome sequencing. In addition, ESTs are a rapid and efficient way to establish a detailed profile of genes expressed in a tissue or cell type. Olive flounder is one of the most important food fish of the both the fishing and aquaculture industries in Korea. In this study, we have constructed cDNA libraries from four different tissues of oliver flounder, and determined DNA sequences of a large number of the clones.

Materiasl and methods

Olive flounder, *Paralichthys olivaceus,* was obtained at the Koje Marine Hatchery. mRNAs were isolated from intestine, liver, spleen, and brain using a polyATtract mRNA purification kit (Promega). cDNA synthesis was performed using a cDNA synthesis kit (Stratagene) with an oligo(dT) primer and the cDNA library was constructed in λ ZAPII vectors according to the manufacturer's instructions (Stratagene). Conversion of the recombinant λ ZAPII into pBluescript plasmids was carried out by in vivo excision. Recombinant plasmid DNA was isolated by the alkaline lysis method. cDNA clones were sequenced using ABI PRISM genetic analyzer (Perkin-Elmer Corp., U.S.A.). Sequence data were compared with nucleotide and protein sequence databases of GenBank using the BLAST algorithm.

Results and Discussion

Expressed sequence tag datas were generated from complementary DNA libraries created from four different tissues (intestine, liver, spleen, and brain) of the Japanese Flounder *Paralichthys olivaceus*. The single-pass sequencing of ESTs from 1,396 clones was done and sequences of the cDNA clones were compared with sequences in the GeneBank databases. 71 clones(5%) appeared to be completely unknown and may represent newly described genes, whereas 1,325 clones (95%) were putatively identified on the basis of matches to sequences in databases and among them, 238 clones(18%) showed homology to a hypothetical protein of unknown function. The putative identities of these genes reflected the expected tissue specificity. Several sequences are the first isolated from fish, and may be of interest for gene mapping and studies of developmental biology. So, full scale EST collection is a valuable approach for the discovery of new genes of potential significance in various tissues.

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