Suppression subtractive hybridization (SSH) cDNA library construction from fuel oil treated winter flounder (*Pseudoplueronectes americanus)*

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Polycyclic aromatic hydrocarbons (PAH) are among the most prevalent pollutants associated with urbanized aquatic ecosystems. Recent studies by Straub et al.[1] have detected transcript differences in the livers of marine bottom dwelling fish that appear to be related to the degree of anthropogenic contamination in the natural system. The purpose of this study was to isolate transcripts that were related directly to PAH exposure and compare these transcripts with those found in wild caught fish.

Young of the year winter flounder were caught in late summer and randomly assigned to 40 l filtered seawater aquaria, kept at 18° C and fed each day with blue mussels and chopped baitfish. After 14 days, the flounder were exposed to 0.8% w/v fuel oil #6 based on 1 kg of clean dry sand per aquaria. The fuel oil was mixed with 10 g of calcined clay, to adsorb the oil, and then added to aquaria. Control tanks received only calcined clay. After a 10 day exposure, the fish were sacrificed and their livers removed for RNA extraction. Two SSH cDNA libraries, forward and reverse, were constructed from treated and control mRNA used alternatively as tester and driver with a PCR Select (SSH) cDNA kit (BD-Clontech). The forward, or up-library, was enriched for transcripts predominating in the fuel oil treated fish and the reverse, or down-library, was enriched for transcripts predominating in the untreated control. The cDNA were cloned using a pGEM-T cloning kit (Promega) and individual clones prepared for plasmid sequencing. Clones were sequenced using a DCTS sequence kit and a CEQ 8000 genetic analyzer (Beckman-Coulter). Sequence was trimmed and submitted for BLAST search at the NCBI website.

A total of 157 up- and 123 down-regulated transcripts were sequenced. These sequences are entered into GenBank as accession numbers CO57806- CO576085 inclusive. Of the 280 sequenced transcripts, 68% were presumptively identified by BLAST search using a cutoff E-value of $1x10^{-5}$. Some transcripts common to the wild-caught pollution-impacted fish[1] and up-regulated with oil were: cytochrome P450 1A, complement component C-3, C-type lysozyme, fibrinogen, antifreeze protein, gastrulation specific protein and ceruloplasmin. Specific to the up-regulated with oil treatment were: defender against death cell protein, retinoid-x receptor-alpha, ribophorin1, GABA receptor associated protein, complement components C-5 and C-8, Cytochrome P450 24A, hepatic glucose transporter, antithrombin and cardiac morphogenesis protein ES/130. Unique down-regulated with oil treatment transcripts found were: cytochrome P450 2D, mRNA for spermatogonial stem-cell renewal factor, alpha-2-HS glycoprotein, saxitoxin-tetrodotoxin binding protein1, hemopexin-like protein, chemotaxin, acidic chitinase and matrix metalloproteinase. These transcripts can be used to further dissect the effects of specific environmental pollutants on the liver of model aquatic vertebrates.

1. **Straub, P.F, M.L. Higham, A. Tanguy, B.J. Landau, W.C. Phoel, L.S. Hales, T.K.M. Thwing.** Suppression Subtractive Hybridization cDNA Libraries to Identify Differentially Expressed Genes from Contrasting Fish Habitats. *Marine Biotechnology* 6:386-399.

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