Genomic tools for understanding chemical tolerance in a wild population of the estuarine fish, *Fundulus heteroclitus*.

Dina Proestou, John Martinson, Eric Waits, Denise Champlin, and Diane Nacci. US EPA Office of Research and Development.

Wild populations of the killifish *Fundulus heteroclitus* residing in heavily contaminated North American Atlantic coast estuaries have recently and independently evolved dramatic, heritable, and adaptive polychlorinated biphenyl (PCB) tolerance. However, currently available genomic tools limit our ability to characterize the genetic and biochemical mechanisms associated with PCB tolerance in this species. In order to enhance ongoing research designed to reveal the genetic basis for PCB tolerance, we focused on genes associated with the aryl hydrocarbon receptor (AHR) putative target pathway and developed a suite of Single Nucleotide Polymorphism (SNP) markers. Expressed Sequence Tag (EST) sequences derived from > 100 genes of interest were mined from the F. heteroclitus EST database and assembled into 155 contigs using the sequence assembly program CAP3. The QualitySNP pipeline, an algorithm designed to detect polymorphisms in EST data, was used to identify 'true' SNPs within contigs. Over 400 SNPs were detected and from those, 120 markers generated. Eight fish, which differed in PCB sensitivity and represent the parental generation of 4 mapping families, were genotyped at each SNP locus using a melting temperature (T_m) -shift method. Genotype data were used to determine the utility of each marker in genetic map construction and Quantitative Trait Locus (QTL) analysis. A subset of these markers is currently being evaluated for its application in population genetic studies. This research is a reflection of the need for developing genomic tools in wild, ecologically-relevant species to better understand stress response and evolved adaptive differences among wild populations.