

**DEVELOPMENT OF A GENE MICROARRAY FOR THE STUDY
OF ENVIRONMENTAL CONTAMINANT EFFECTS ON EARLY LIFE-STAGES**

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ABSTRACT: Although several DNA microarrays are now commercially available for evaluating simultaneous expression of thousands of genes in different animal models, these have been for the most part targeted towards adult life stages. Since there is good evidence showing differential gene expression across life stages, currently available microarrays are likely to overlook genes important for early developmental processes. This limitation is of great biological importance, since early life-stages are known to be several orders of magnitude more sensitive to environmental pollutants compared to adults. The objective of our work is to develop and test an “early life-stage” DNA microarray for use as a high-throughput screening method for emerging contaminants. Close to four hundred known and putative fathead minnow (*Pimephales promelas*) developmental genes have been identified for use in this targeted DNA microarray. These gene targets have been identified through computational cDNA subtraction where sequenced cDNA libraries from developing minnows (less than 14 days old) were screened for sequence similarity against adult minnow cDNAs, which identified nearly 400 unique cDNA sequences in the developing minnow (i.e. no sequence similarity in 250k adult minnow cDNAs). Three different, non-overlapping long oligo-probes were designed for each target gene (from the developing minnow) and DNA microarray production was outsourced to Agilent using their E-array system. The E-array system involves eight separate (but identical) arrays each up to 15K features within an array (8 x 15k = 120k total features on the slide) that are separated using a specialized gasket. Here we will present data on the use of this microarray after exposure of fathead minnow embryos to a potent estrogen, ethinyl estradiol. Successful completion of this work will result in the development of tools that are broadly applicable, offer robust and biologically relevant results, and can be developed into highly marketable products with a wide range of potential uses. In addition, since there is a great degree of gene homology between fishes and humans, we expect to unravel new mechanisms of toxic action that could be applied to epidemiological human studies.

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