

## Simultaneous detection of multiple fish pathogens using DNA microarrays

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**Background:** Fish pathogens are generally fastidious organisms making them difficult to detect in tissues and environmental samples. For example, it can take up to 19 weeks to culture the causative agent for bacterial kidney disease (*Renibacterium salmoninarum*). Molecular tools such as polymerase chain reaction (PCR) offer a highly sensitive alternative for detecting DNA or RNA that is specific to a pathogen of interest. When coupled to DNA microarrays, PCR can be used to rapidly screen for a large number of pathogens in a single assay. The purpose of this project is to develop a DNA microarray suitable for simultaneously detecting and distinguishing between different fish pathogens based on 16S rDNA polymorphisms. **Methods:** Probes consist of 19 to 30-mer oligonucleotides that are deposited in a lattice pattern onto epoxy-silane derivatized glass using a robotic arrayer (Affymetrix, Santa Clara, CA). PCR products are generated (179 bp) using biotinylated universal primer sequences for the 16S rDNA gene. PCR products are then hybridized to the microarray (overnight at 58°C) and target:probe duplexes are detected using tyramide signal amplification (Perkin Elmer, Boston MA) with Alexa Fluor® 546 (Molecular Probes, Eugene, OR) and microarray results are imaged with an Applied Precision ArrayWoRx scanner (Issaquah, WA). **Results:** Our studies demonstrate excellent specificity for 18 probe sequences when tested against positive controls. We tested the array using PCR products generated from filtered hatchery water and detected *Aeromonas salmonicida* or *A. hydrophila* in 60% of water samples. Other pathogens (*Mycobacteria* spp. and *Yersinia ruckeri*) were detected less frequently. **Conclusions:** A 16S rDNA microarray detector appears suitable for testing water samples. We are currently comparing culture, microarray, enzyme-linked immunosorbent assay (ELISA) and fluorescent antibody (FA) techniques for the detection of *Renibacterium salmoninarum* in kidney tissue.

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