

Washington Department of Fish and Wildlife (WDFW) Lab Overview – SNP Workshop

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Since 2000, WDFW has developed allele specific PCR species identification assays based on nucleotide substitutions in the cytochrome b and CO III / ND3 regions of the salmonid mitochondrial genome. The current protocol is a one-tube, one-PCR assay that includes a labeled forward primer and a positive-control reverse primer that produces same-length PCR fragments in all target species. Ten allele-specific reverse primers (the 3' nucleotide matches the target species but mismatches all others) that anneal between the forward primer and the positive-control reverse primers produce species-diagnostic length PCR fragments. We electrophorese the labeled PCR products in capillary-based automated genetic analyzers (ABI 3100 or ABI 3730). We manipulate relative fragment sizes by incorporating nonsense nucleotide sequence tags on the 5' end of selected primers in a fashion similar to the development of some commercial, multiplexed SNP assays.

Recently, we obtained four TaqMan assay plates from ADFG that we scored in our BioTek Synergy HT multi-function plate reader. We constructed an Excel spreadsheet-based template to receive our plate reader output and, with minimal user input, convert that output to genotype calls. Our genotype calls were concordant with the ADFG calls for 381 of the 384 genotypes assayed; we considered the other three genotypes to be ambiguous whereas ADFG called them. During the funding year beginning July 1, we will participate in a multi-lab SNP-scoring concordance test funded by the Pacific Salmon Commission – Chinook Technical Committee. In that effort we will explore assays based on non-proprietary chemistry and our existing instrumentation. Currently, the numbers of SNPs developed for Chinook, coho, and chum salmon probably will not support the broad-scale applications needed by fishery managers, so WDFW will devote a portion of recently acquired SNP-related funding to join other labs in SNP discovery. We hope to coordinate our discovery efforts with those in other labs.