Development of 5'-Nuclease Reactions for High-Throughput SNP Genotyping in Salmon

Christian T. Smith, James E. Seeb, and **Lisa W. Seeb**
Alaska Department of Fish and Game,
Commercial Fisheries Division, Gene Conservation Laboratory,
333 Raspberry Road, Anchorage, AK 99518, USA

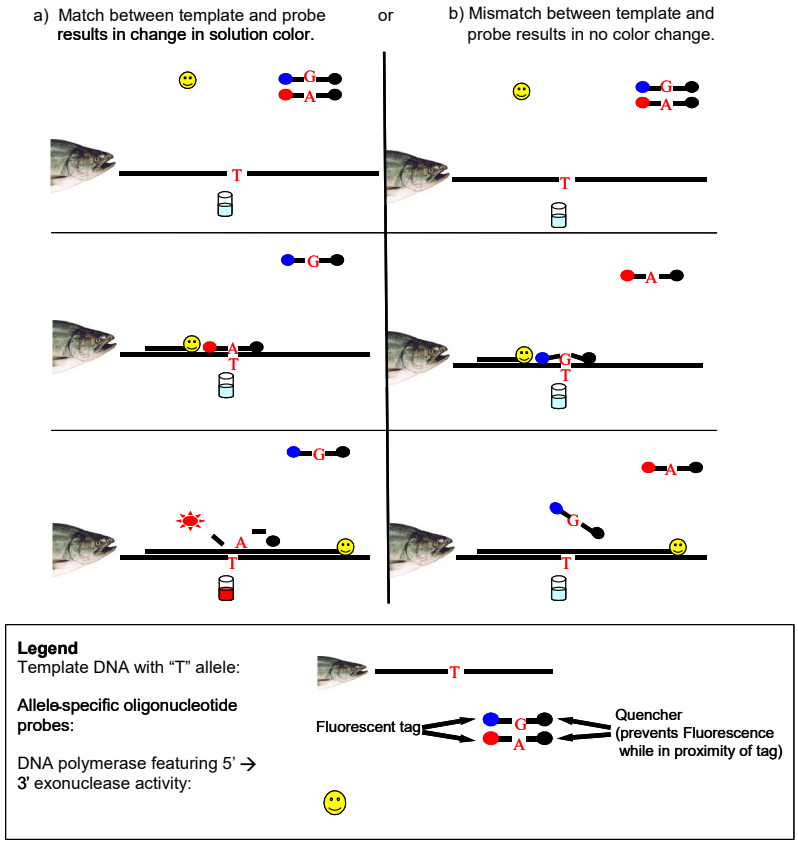
Keywords: 5'-nuclease, SNP, salmon

Information on the oceanic migration patterns and relative marine survival of individual stocks is critical to our understanding of fluctuations of salmonid populations under changing climatic conditions. Migrations following stock-specific corridors may lead to differing marine survival and varying rates of return among stocks during periods of changing marine conditions. By comparing genetic attributes of collections of fish taken in high-seas and near-shore areas with those characteristic of potentially contributing stocks one can infer the origin of the collection and thus a point on the migratory route of that stock.

Single nucleotide polymorphisms (SNPs) are a class of genetic markers consisting of differences in DNA bases between individuals or individual chromosomes. These polymorphisms have been assayed in salmonids and other taxa using a wide variety of technologies over the past couple of decades. Although many of these SNPs provided powerful information for fisheries management, the technologies used to collect genotype data for them were slow and expensive relative to those for alternative marker classes (e.g. allozymes and microsatellites). In recent years several high-throughput, low-cost SNP genotyping techniques have been developed (several are described in Kwok 2003). We are developing markers based on one of these techniques known as the 5'-nuclease reaction (Fig. 1) and are using these markers to genotype SNPs in large numbers of chinook, sockeye and chum salmon. These SNP genotyping assays are being developed in order to utilize the wealth of previously described polymorphisms that have not been widely applied to migration or mixture studies due to throughput constraints of older technologies.

Using two thermal cycler blocks four times per day, we observed that a single technician could genotype over 3,000 individuals. Since thermal cycling was the limiting step, the use of additional cycler units could multiply this throughput rate several times. The relative simplicity of the raw data analysis (Fig. 2) and the lack of an electrophoresis component rendered genotyping based on the 5'-nuclease reaction faster and thus less expensive than genotyping using the techniques originally published for each SNP. This greatly increases the potential utility of SNPs for fishery management applications.

Fig. 1. Genotyping via the 5'-nuclease reaction involves adding allele-specific fluorescent oligonucleotide probes to a typical polymerase chain reaction. During the reaction the probes anneal to the template DNA either (1a) perfectly or (1b) imperfectly. In the former case, the probe is cleaved causing fluorescence. In the latter case, the probe is simply knocked free and no color change is observed.

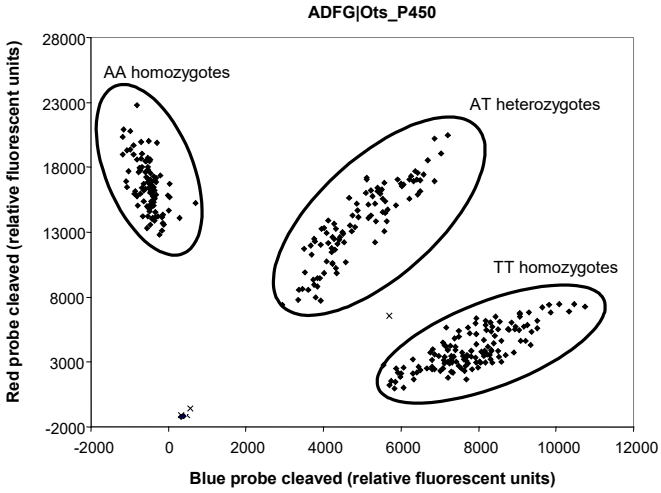


All correspondence should be addressed to C. Smith.
e-mail: christian_smith@fishgame.state.ak.us

The number of SNP loci required for migration and mixture studies will depend on the questions being addressed, but will likely be larger than the number of markers with greater allele numbers (such as AFLPs or microsatellites). This will impact the relative economy of applying SNPs. In the absence of compelling evidence for any simple relationship between number of alleles and utility of a marker for migratory studies, we agree with Banks et al. (2003) that an empirical approach is an appropriate way to find the optimal combination of markers with which to address each question.

By facilitating the detection of only one amplification product per reaction and by eliminating the multitude of potential inconsistencies associated with electrophoresis, SNP genotyping assays should be much easier to transfer among laboratories than other markers. Furthermore, SNP data collected using the 5'-nuclease reaction are readily combined with SNP data collected using any other method. This portability, in combination with the relatively low time and monetary requirements for running SNP genotyping assays and the wealth of previously described polymorphisms that may be accessed using these new technologies, suggest that SNPs will become an increasingly important tool for migration and mixture studies of salmonids on the high seas where large sample sizes and exhaustive baselines are the norm.

Fig. 2. Analysis of data produced by genotyping 380 fish using the 5'-nuclease reaction. Sequence Detection Software V. 2.0 (Applied Biosystems Inc.) was used to generate the chart below in which the two axes represent the amounts of signal from each of the allele-specific probes. Each dot on the chart below represents an individual fish, whose genotype is determined by where its dot falls on the axis. The circles indicate clusters of a single genotype each. Mitochondrial DNA assays produce two clusters while nuclear DNA assays (such as the example below) produce three clusters. The "x"s represent unreadable samples (air-bubbles, failed PCRs etc.) and the dots in the bottom left corner are the negative controls (no template DNA added).



REFERENCES

Banks, M.A., W. Eichert, and J.B. Olsen. 2003. Which genetic loci have greater population assignment power? Bioinformatics 19: 1436–1438.

Kwok, P-Y (*editor*). 2003. Single Nucleotide Polymorphisms - Methods and Protocols. Humana Press Inc. Totowa, New Jersey. pp. 269.