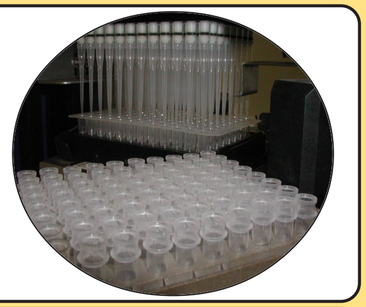
Single nucleotide polymorphisms (SNPs) provide high-throughput, high-resolution DNA data for BASIS studies

Cooperating investigators: Alaska Department of Fish & Game, Anchorage; NOAA Fisheries, Auke Bay; National Salmon Resources Center, Sapporo; Hokkaido University, Sapporo & Hakodate

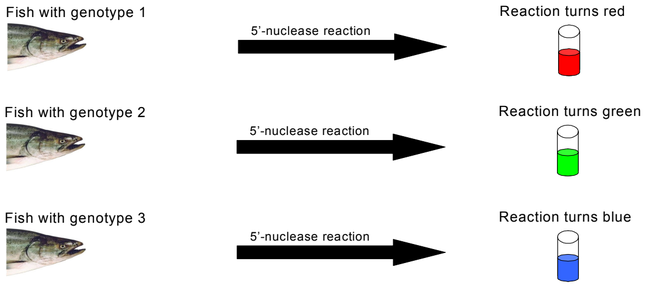


Abstract

Migratory studies require markers for which a large number of individuals can be processed in a relatively short time. Genetic markers, especially allozymes, have provided substantial insight into key questions asked by BASIS investigators. However, issues of sample collection and preservation as well as a desire for increased resolution have driven efforts to develop DNA markers to describe discrete aggregations of salmon stocks. Given the multi-jurisdictional range of these species, it is desirable that genetic markers and the corresponding data be transportable across laboratories. Allozymes meet these criteria while most DNA markers do not. To solve this dilemma, we are developing single nucleotide polymorphism genotyping assays (SNPs) based upon the 5'-nuclease reaction. We have approximately 20 SNP assays each in Chinook, chum, and sockeye salmon. Using these assays, a single technician with one thermal cycler can generate 3840 genotypes in a 7.5 hr day. These assays are easy to standardize across laboratories, and the resulting genotype data are readily combined with those collected using any other sequence detection platform.

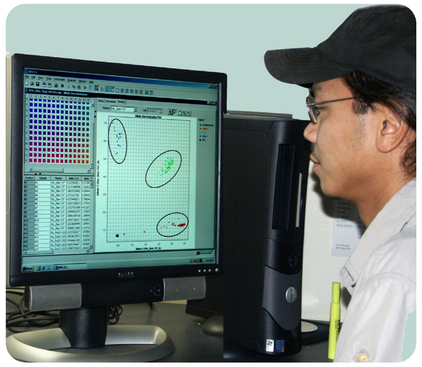
1. Genotyping Without Gels

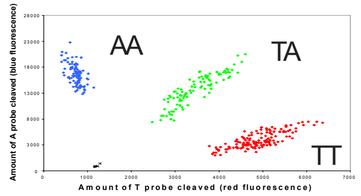
SNP genotyping involves simply amplifying target DNA in the presence of allele-specific dyes. The genotype of each fish is determined by the color of the resulting reaction.



2. Rapid Scoring

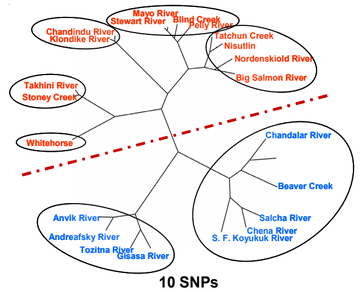
The color of each well in a 384 well plate is read by a scanner and the results are displayed as a scatter plot. The simplicity of interpreting such a scatter plot allows 384 genotypes to be scored in under 5 minutes.

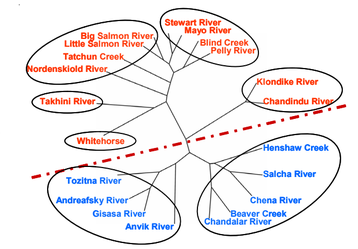




3. Resolution

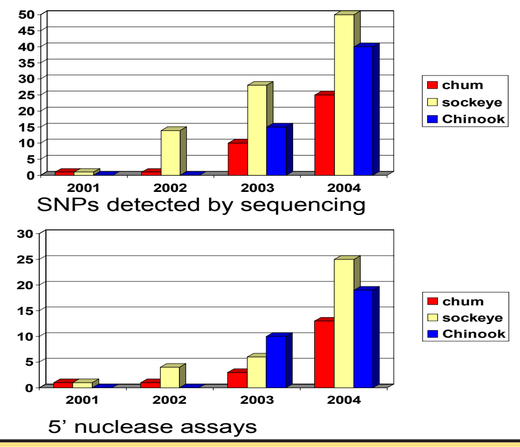
As an example, SNPs and microsatellites show similar resolution for US (blue) and Canadian (red) populations of Chinook salmon. An advantage of SNP assays is that both mtDNA and nuclear DNA as well as neutral and selected genes may all be interrogated. Addition of more such informative SNP loci will soon show superior resolution for studies of sockeye, Chinook, and chum salmon.





4. SNP Availability

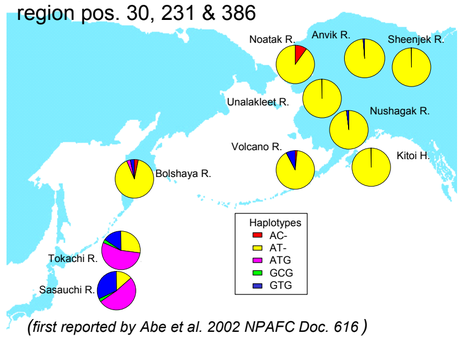
Number of assays for SNPs is rapidly increasing; 20-30 needed in each species for population studies.



5. Automatic Standardization

We compared SNP scores obtained using DNA microarray at Hokkaido University with those obtained using the 5'-nuclease reaction at Alaska Department of Fish and Game. We examined three SNPs that discriminate Asian and Alaskan stocks of chum salmon; 1142 of 1149 bases were scored identically (99.4% accuracy).

Haplotypes for chum salmon mtDNA control region pos. 30, 231 & 386



(first reported by Abe et al. 2002 NPAFC Doc. 616)

Summary: SNP assays are high throughput and data are easily standardized across laboratories and instruments. We anticipate very high resolution because both selectively neutral and rapidly evolving DNA markers are detected in both mtDNA and nuclear DNA. SNPs will become an increasingly important tool for mixture and migration studies as more high-throughput assays are developed. North Pacific Research Board grant R0205 and R0303 funded this research.

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