High-Throughput SNP Genotyping in Salmon and Steelhead

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Abstract

The Columbia River Inter-Tribal Fish Commission (CRITFC) is involved in conservation genetics research of salmon and is utilizing Fluidigm technology to characterize genetic variation in fish populations in the Columbia River. SNP genotypes of salmon from the Fluidigm EP1 system and 96.96 Dynamic Arrays provide a genetic signature for specific populations that may also be used to identify unknown origin fish during migration or in fisheries harvest. This may provide information to facilitate fisheries management in the Columbia River basin that includes several salmon stocks listed under the Endangered Species Act.

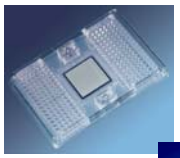
Columbia River Inter-Tribal Fish Commission

CRITFC was established in 1977 by four member tribes, the Yakama, Umatilla, Nez Perce, and Warm Springs with a mission to ensure a unified voice in the overall management of the fishery resources, and as managers, to protect reserved treaty rights through the exercise of the inherent sovereign powers of the tribes. Genetics research focuses on the conservation and recovery of salmon and other fish species in the Columbia River.

Salmon and Steelhead Trout Research

- Fluidigm technology is being used to characterize genetic variation in Columbia River populations to better understand diversity, adaptation, and dispersal in natural populations of salmon.
- This characterization creates a genetic signature for specific populations that may also be used to identify unknown origin fish during migration or in fisheries.
- This information may facilitate fisheries management in the Columbia River basin that includes several salmon stocks listed under the Endangered Species Act.

96.96 Dynamic Arrays and the Fluidigm EP1 System



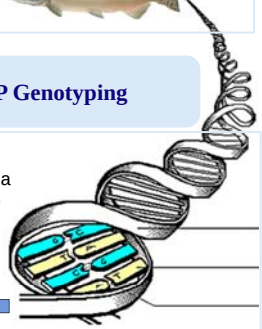
The 96.96 Dynamic Array (left) is capable of running 96 samples against 96 SNP genotyping assays for a total of 9,216 genotypes on a single chip. The 96.96 Dynamic Arrays are run on the Fluidigm EP1 system (right) for high-throughput, mid-multiplex SNP genotyping. The system offers a fast and easy workflow which provides results in only a few hours using gold standard Taqman® assays.

Why SNP Genotyping on Salmon?

- Chinook salmon in the Columbia River are closely related and remain difficult to distinguish even with a powerful set of 13 microsatellite markers.
- SNP loci are good candidates for standardization among laboratories and expanding existing baselines because SNP assays involve direct interrogation of genetic sequence variation.
- Also since SNP assays amplify a much shorter segment of DNA than microsatellites, SNPs have a greater probability of producing results in degraded samples (Campbell and Narum 2008b).
- In some cases (i.e., mainstem Columbia River Chinook fisheries), a finer level of stock discrimination is necessary for management of fisheries.
- Therefore, additional SNP loci will increase stock assignment reliability where greater resolution is required.
- Existing microsatellite baselines for steelhead and Chinook salmon will be expanded by genotyping up to 96 additional SNP markers for population samples.
- Approximately 7,500 samples will be genotyped for baseline expansion. This will include about 2,500 steelhead and 5,000 Chinook samples, each typed with 75-96 existing SNPs.

High Throughput SNP Genotyping

The EP1 system offers a fast and easy workflow



1. Pipette 96 Chinook salmon or steelhead samples and 96 SNP assays onto the 96.96 Dynamic Array

2. Place the 96.96 Dynamic Array onto the IFC Controller HX to automatically setup 9,216 genotyping experiments

3. Thermal cycle the 96.96 Dynamic Array using a standard program

4. Read the 96.96 Dynamic Array in the EP1 reader in a matter of minutes

SNP Genotyping Results

User optimized protocols and tests

- 98.9% call rates have been obtained in SNP genotyping runs.
- Results have demonstrated 99.8% genotype concordance with the ABI 7900 system

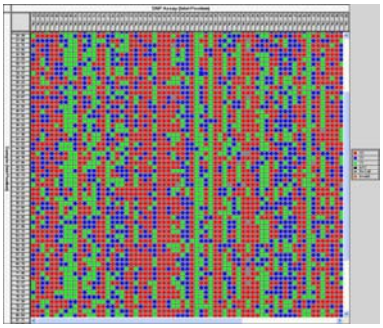


Figure 1. A call map view graphically displays the genotype results in a chip format. An entire chip showing 9,216 results is displayed at one time.

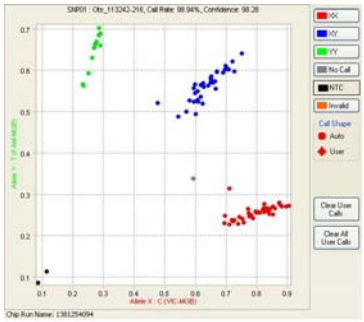


Figure 2. Typical SNP genotyping results for a single SNP assay on a 96.96 Dynamic Array. The results are displayed in a cluster plot.

Figure 3. Population distinction (STRUCTURE plot k=4) of Chinook salmon genotyped with 96 SNP assays.

Conclusions

- Laboratory tests of Fluidigm technology have been highly successful as demonstrated by high call rates and concordance with previous methods.
- High throughput capabilities provide significant time and cost savings over previous methods.
- SNP markers have strong potential to differentiate populations of Chinook salmon.

FLUIDIGM EP1 GENETIC ANALYSIS

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