Rapid, Cost-Effective SNP Genotyping Using Standard BioTools SNPtype™ Assays and X9™ Real-Time PCR System

Standard BioTools (Shanghai) Instrument Technology Co., Ltd
Standard BioTools Inc., South San Francisco, United States of America

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Abstract

The use of single nucleotide polymorphism (SNP) genotyping with non-model organisms—those whose genomes are yet to be sequenced—has increased in importance as it provides robust, comparative data sets that can be easily shared across organism communities for a variety of purposes. Non-model organisms have the added burden of low available genomic sequence information requiring custom SNP assay development. Until recently, SNP genotyping technologies have been prohibitive to these communities due to the high cost of developing and running quality SNP genotyping panels. Standard BioTools SNPtype Assays and the X9 Real-Time PCR System have addressed these barriers with a custom assay design service, cost-effective and high quality SNP assays, and a high-throughput workflow minimizing hands-on time. The salmon research community has been specifically hampered by the cost barriers, and would benefit from the technology for conservation and management purposes. Using chum salmon (Oncorhynchus keta) as an example, we describe a simple workflow using Standard BioTools SNPtype Assays, the X9 System, and 96.96 Dynamic Array™ Integrated Fluidic Circuits (IFCs) for Genotyping to achieve cost-effective and rapid development of a SNP genotyping panel. Moreover, SNPtype Assays provide significant cost savings for high-throughput, routine testing post panel development.

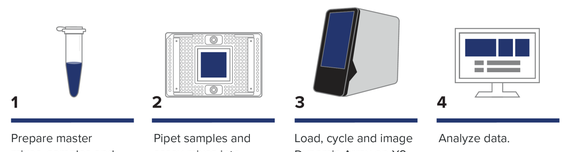
Introduction

SNPtype Assays and the X9 System provided a rapid and cost-effective way to develop and implement a SNP genotyping panel. For the chum salmon panel development, a candidate list of 143 SNPs was selected based on their ability to provide population structure and harvest composition information within the chum species of salmon. The sequences around these SNPs were submitted to the Standard BioTools Assay Design Group for design and manufacture of allele-specific PCR primers. TaqMan® assays were also ordered for the same SNPs for comparison. Assays were run with 95 samples from three different locations in Washington State (Hamma Hamma River – 24, Kalama River – 24, Skookum River – 23), and one from British Columbia (Squakum Creek – 24). The Standard BioTools SNP Genotyping User Guide (PN 68000098) was followed, including specific target amplification (STA), or preamplification.

Figure 1. Oncorhynchus keta, or Pacific Chum Salmon

Source: The Fishes of Alaska." Bulletin of the Bureau of Fisheries, Vol. XXVI, 1906. P. 360, Plate XXXI. which is at http://www.photolib.noaa.gov

High-Throughput, Automated Workflow

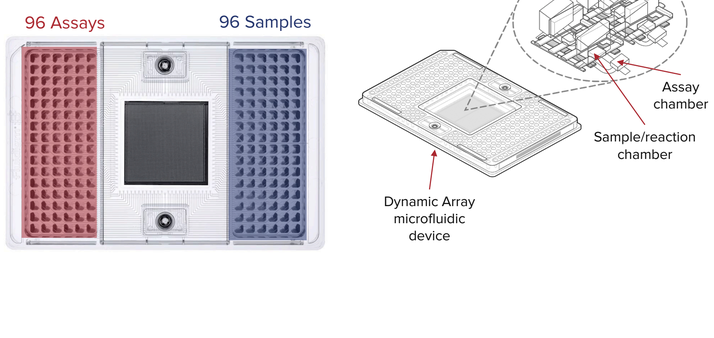


1. Sample mix is loaded in the sample inlets

   SNP Assays are loaded in the assay inlets

2. The 9,216 individual reactions are assembled automatically by the X9 system, dramatically reducing reagent and pipetting requirements

3. Allele-specific PCR products are generated and imaged in the X9 Real-Time PCR system



Panel Development Workflow

Step 1: Select Candidate SNPs
- Utilize databases and academic resources.
- Selection should be representative of the population in order to avoid ascertainment bias.
- Establish panel size.
- Number of candidate SNPs should be in excess of the final panel size. For example, 143 SNPtype Assays were designed and tested for a final panel selection of 96 for the chum salmon panel development.

Step 2: Build Assays
- SNPtype Assays are designed and manufactured for all candidate SNPs by Standard BioTools.

Step 3: Test Assays
- Test SNPtype Assays on Standard BioTools System (X9, Biomark or EP1)
- Decide if preamplification is needed for your samples.
- Recommend testing high sample number, representative of the population of interest.

Step 4: Assemble Panel
- Grade your assays: establish a grading scale and score each assay (Figure 3).
- Avoid scoring bias: have multiple parties individually score assays.
- Select your assays: choose high performing, consistent assays (Figure 2 and 4).

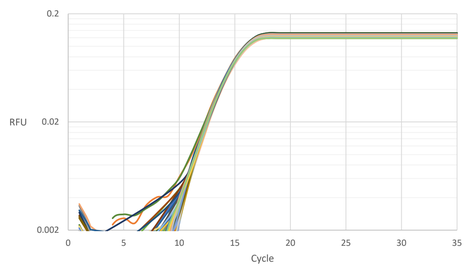


Figure 3. Example of uniform results. To demonstrate uniformity, the Ct values for 96 sample replicates against a single assay targeting GAPDH were assessed on a 96.96 Dynamic Array IFC.With a standard deviation of 0.06 Ct on a mean Ct of 12.4, these results demonstrate the excellent uniformity of microfluidic real-time PCR on the X9 System, indicating the ability to derive accurate results for all samples.

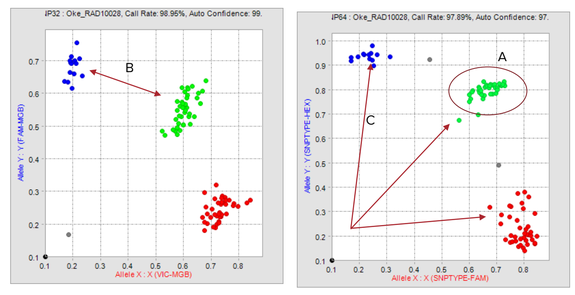


Figure 3. Example of Assay Grading Criteria

A	Spread of clusters	Measure of variation among individual samples within a particular genotype
B	Space between clusters	Measure of the distance between the edge of each genotype cluster
C	Alignment of clusters	Alignment relative to the origin accounting for both distance from the origin and between clusters

SNP Genotyping Results

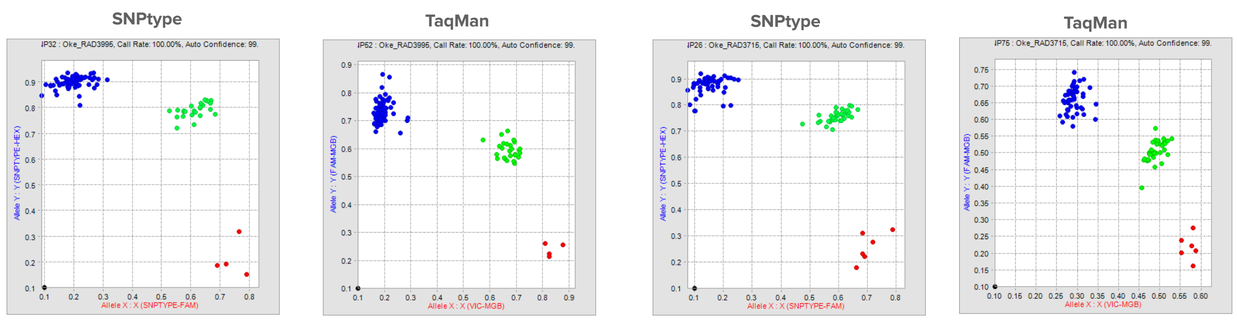


Figure 4. Typical cluster plots from Standard BioTools X9 Real-Time PCR System demonstrating high-quality genotyping data

As an example, 143 chum salmon sequences containing target SNPs were submitted to the Assay Design Group for design of the assays. 100% of the sequences were converted to SNPtype Assays using Standard BioTools proprietary software pipeline. All 143 SNPtype Assays were tested with the 95 samples mentioned in the Introduction, and 107 high-quality assays were selected, which was more than the final panel size of 96. Data in Figures 3 and 4 were from the chum salmon IFC runs.
Cost effectiveness of the SNPtype Assays greatly increased the opportunities for design and testing of assays targeting novel SNPs.

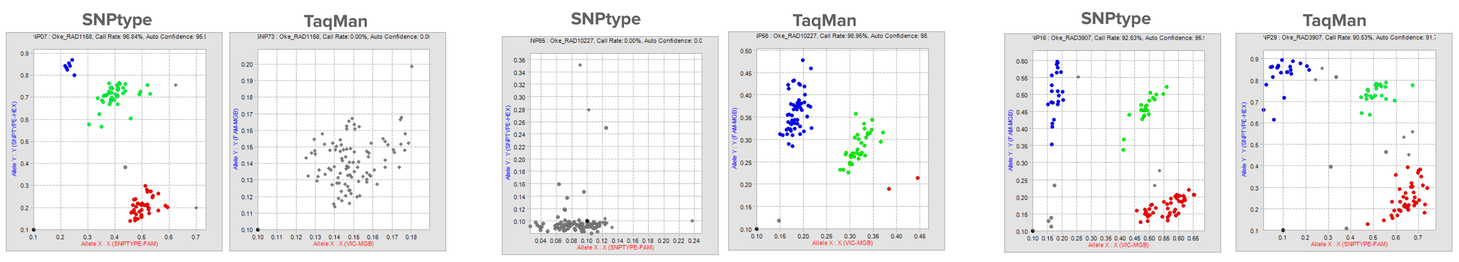


Figure 4.Minority of cluster plots demonstrating lower quality of genotyping data where SNPtype Assays occasionally out perform TaqMan assays (two plots from left), TaqMan assays occasionally out perform SNPtype Assays (middle two plots), and equal performance between the two assays (two plots from right)

Conclusions

- SNPtype Assays greatly reduced routine running costs of SNP genotyping panels for non-model organisms that previously were inhibited by the high cost of TaqMan assays.
- Recent developments in DNA sequencing technology greatly increased the numbers of SNPs available for non-model organisms. More SNPs can be tested using SNPtype Assays due to significant cost savings over TaqMan assays and researchers now have the option to develop multiple sets of high-quality 96 SNP panels for increased discrimination power.
- Additionally, Standard BioTools X9 System provided a rapid, high-throughput, low hands-on workflow that accelerated the panel development process and routine run time.

Standard BioTools (Shanghai) Instrument Technology Co., Ltd
A-1709, Hong Hui International Plaza, 1600 Zhong Shan West Road, Xu Hui District, Shanghai, China Tel: +86-21-32558368
standardbiotools.com.cn

Standard BioTools Inc.,
2 Tower Place, Suite 2000, South San Francisco, CA 94080 USA
+1 650 266 6000 • Toll-free in the US and Canada: 866 359 4354
standardbiotools.com

Learn more about the X9 Real-Time PCR System

Learn more about how the X9 is used in AgBio research