Axiom Trout Genotyping Array

A high-density genotyping array for high-resolution genetics research and aquaculture breeding in rainbow trout

The Applied Biosystems™ Axiom™ Trout Genotyping Array for studying rainbow trout (*Oncorhynchus mykiss*) was designed through our Expert Design Program in collaboration with the National Center for Cool and Cold Water Aquaculture, USDA-ARS, USA, and AquaGen, Trondheim, Norway.

Benefits of genotyping trout

Fish species such as trout and salmon are particularly difficult to genotype because of their partially polyploid genomes, which result in paralogous sequence variants (PSVs). Rainbow trout also exhibits low linkage disequilibrium (LD) [1] and requires a large number of fragments for association analysis. Genotyping-by-sequencing solutions for trout can be ineffective because of missing data, inadequate resolution, complicated analysis pipelines, and false SNPs from low coverage and sequencing errors.

The Axiom Trout Genotyping Array offers a cost-effective and robust standard high-throughput genotyping solution that is ideal for genome-wide association studies (GWAS) to analyze genetic architecture and marker–trait association, and increase the accuracy of breeding programs. The high marker density on the array ensures comprehensive coverage to provide representation of all regions of the trout genome.

Highlights

Comprehensive content and multiple SNP discovery projects represented

The array includes 57,501 markers spaced across the genome:

- 17,000 markers unique to SNPs discovered in a previous USDA study [2]
- 20,000 markers unique to an outbred Norwegian commercial population
- Amino acid–shifting SNPs
- SNPs preferentially located within a gene and with minor allele frequency (MAF) >0.2
- Y chromosome–specific SNPs near the *sdY* gene (male-specific in rainbow trout) [3]

Applications

Complex trait research and molecular breeding

- GWAS and quantitative trait locus (QTL) mapping
- Identification of economically important traits
- Improved accuracy in aquaculture breeding programs through genomic selection

Population and evolutionary genetics

- Differentiation of fish that have different origins
- Gender determination via *sdY* gene–specific probes
- Differentiation of farmed and wild populations

ThermoFisher SCIENTIFIC

SNP discovery and marker selection

The markers were selected from the following sequencing discovery studies:

- **USDA population**—A majority of the putative high-quality markers were discovered in a panel of 19 rainbow trout doubled-haploid (DH) lines, using restriction site–associated DNA (RAD) tags [2]. The use of DH lines helped differentiate between PSVs and simple allelic markers.
- **AquaGen population**—SNP discovery from resequencing 12 fish in the AquaGen breeding nucleus. The *de novo* markers were aligned to the rainbow trout US draft genome assembly [3].
- **Past projects**—Markers from previous SNP discovery projects published in literature [4-6].

Putative SNPs were submitted to us to calculate *in silico* design scores. SNPs with high scores were included for the final design. The SNPs within each dataset were ranked based on (i) priority for sequences from transcribed regions, (ii) uniqueness of hit to the draft genome assembly, (iii) genetic map information from previous studies, and (iv) MAF information from previous studies [4-6].

Results

The Axiom Trout Genotyping Array was successfully validated in 960 samples representing various trout populations [3]. The validation study included samples from the 19 DH fish that were used in previous SNP discovery [2] and samples from other *Oncorhynchus* species, including cutthroat trout, Chinook salmon, and Coho salmon. A total of 924 samples (96%) passed the genotyping quality filtering and the call rate threshold set at 97%. A total of 49,468 SNPs (86%) were categorized as high-quality and polymorphic, and an additional 654 SNPs (1.1%) as high-quality but monomorphic, using the default quality-filtering thresholds. The markers on the array also passed quality criteria established through four separate tests—six demonstrating the high quality, reliability, and integrity of the genotype data from the Applied Biosystems™ Axiom™ 2.0 assay.

Figure 1 shows the distribution of markers on the array. The probe set ID for 48 markers on the 384-format array is different from the probe set ID on the 96-format array. Use the Affy_SNP_ID instead of the probe set ID to identify these markers when reviewing the results from the two different array formats. The data analysis and clustering were automated using the Applied Biosystems™ Genotyping Console™ Software and SNPolisher™ package.

SNPs were filtered according to the Best Practice Supplement to the Axiom Genotyping Solution Data Analysis User Guide (P/N 703083). Today, the Applied Biosystems™ Axiom™ Analysis Suite should be used for analysis following the Best Practices Workflow as described in the Axiom Genotyping Solution Data Analysis Guide (P/N 702961).

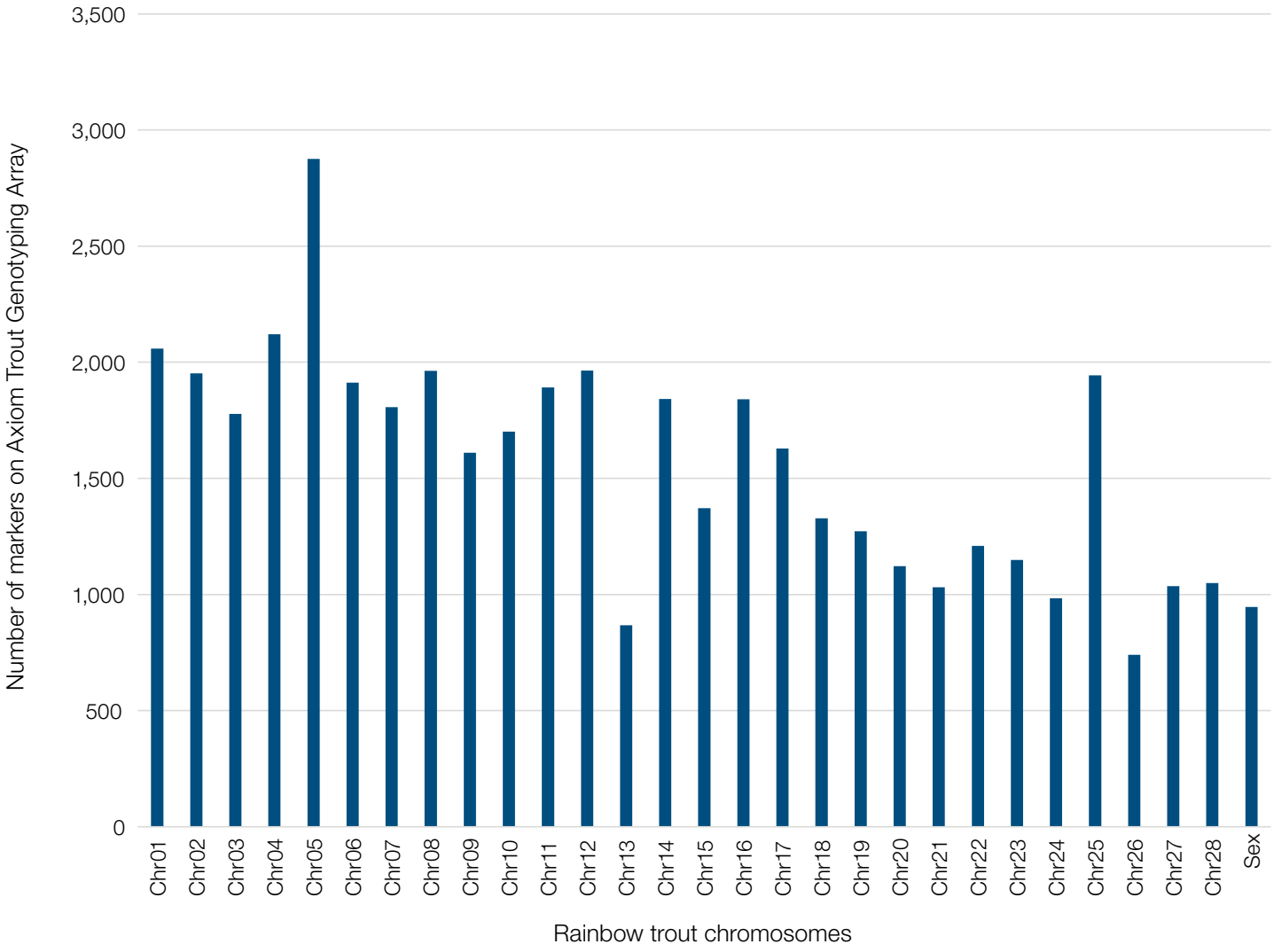

Figure 1. Distribution of SNPs on the Axiom Trout Genotyping Array across the rainbow trout chromosomes.

applied biosystems

Ordering information*

Product	Description	Cat. No.
Axiom Trout Genotyping Array (96 format)	Contains one 96-array plate; reagents and GeneTitan Multi-Channel Instrument consumables sold separately	550468
Axiom GeneTitan Consumables Kit	Contains all GeneTitan Multi-Channel Instrument Consumables required to process one 96-array plate	901606
Axiom 2.0 Reagent Kit	Includes all reagents (except isopropanol) for processing one 96-array plate	901758
Axiom Trout Genotyping Array (384 format)	Contains one 384-array plate; reagents and GeneTitan Multi-Channel Instrument consumables sold separately	550571
Axiom 2.0 384HT GeneTitan MC Consumables Kit	Contains all GeneTitan Multi-Channel Instrument consumables required to process one 384-array plate	902234
Axiom 2.0 384HT Reagent Kit	Includes all reagents (except isopropanol) for processing one 384-array plate	902245

References

1. Rexroad CE et al. (2009) Estimates of linkage disequilibrium and effective population size in rainbow trout. *BMC Genetics* 10:83.
2. Palti Y et al. (2014) A resource of single-nucleotide polymorphisms for rainbow trout generated by restriction-site associated DNA sequencing of doubled haploids. *Mol Ecol Resour* 14:588-596.
3. Palti Y et al. (2015) The development and characterization of a 57K single nucleotide polymorphism array for rainbow trout. *Mol Ecol Resour* 15:662-672.
4. Sanchez CC et al. (2009) Single nucleotide polymorphism discovery in rainbow trout by deep sequencing of a reduced representation library. *BMC Genomics* 10:559.
5. Boussaha M et al. (2012) Development and characterization of an expressed sequence tags (EST)-derived single nucleotide polymorphisms (SNPs) resource in rainbow trout. *BMC Genomics* 13:238.
6. Salem M et al. (2012) RNA-Seq identifies SNP markers for growth traits in rainbow trout. *PLoS ONE* 7:e36264.

Find out more at **thermofisher.com/microarrays**

ThermoFisher SCIENTIFIC