

## Axiom® Trout Genotyping Array

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

Axiom® Trout Genotyping Array for studying rainbow trout (*Oncorhynchus mykiss*) was designed through Affymetrix' Expert Design Program in collaboration with the National Center for Cool and Cold Water Aquaculture, USDA-ARS, USA, and AquaGen, Trondheim, Norway. The array includes 57,501 markers and is available in both 96 format and 384 format.

### 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<sup>1</sup> and requires a large number of fragments for association analysis. Sequencing-based genotyping techniques are cost-effective but can be ineffective in genotyping trout because of missing data, inadequate resolution, complicated analysis pipelines, and false SNPs from low coverage and sequencing errors.

Axiom Trout Genotyping Array offers a standard high-throughput, cost-effective, and robust genotyping technology to conduct genome-wide association studies (GWAS), to study genetic architecture, to perform marker-trait association, and to increase accuracy of breeding programs. The high marker density on the array ensures a broad coverage of the trout genome to provide representation of all regions in the genome.

### Highlights

#### Comprehensive content and multiple SNP discovery projects represented

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

- 17,000 markers that are unique to SNPs discovered in a previous USDA study<sup>2</sup>
- 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)<sup>3</sup>

### Applications

#### Complex trait research and molecular breeding

- GWAS and quantitative trait locus 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 chromosome-specific probes
- Differentiation of farmed and wild populations

#### SNP discovery and marker selection

The markers were selected from the following sequencing discovery studies:

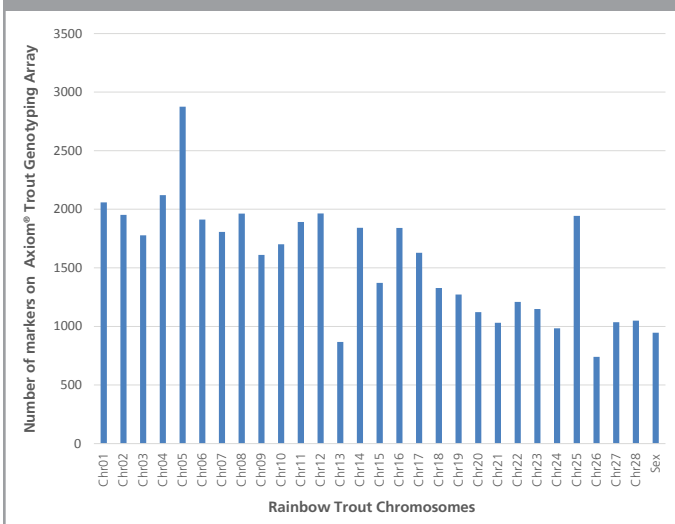
- USDA population: A majority of the putative high-quality markers were discovered in a panel of 19 rainbow trout doubled haploid (DH) lines using restriction site-associated DNA (RAD) tags.<sup>2</sup> The use of DH lines helped differentiate between PSVs and simple allelic markers.
- AquaGen population: 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.<sup>3</sup>
- Past projects: Markers from previous SNP discovery projects published in literature.<sup>4,5,6</sup>

Putative SNPs were submitted to Affymetrix 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 to 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.<sup>4,5,6</sup>

## Results

Axiom® Trout Genotyping Array was successfully validated in 960 samples representing various trout populations.<sup>3</sup> The validation study included samples from the 19 DH fish that were used in previous SNP discovery<sup>2</sup> and samples from other *Oncorhynchus* species, including cutthroat trout, Chinook salmon, and Coho salmon. A total of 924 (96%) samples 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,<sup>6</sup> demonstrating the high quality, reliability, and integrity of the genotype data from the Axiom® 2.0 assay.

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



A full annotation file for the array is available from the product page on Affymetrix' website. Figure 1 shows the distribution of markers on Axiom Trout Genotyping 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. To identify these markers when reviewing the genotype results from the two different array formats, the Affy\_SNP\_ID can be used in lieu of the probe set ID. The data analysis and clustering were automated using Affymetrix' Genotyping Console™ Software and Affymetrix' SNPolisher™ package. SNPs were filtered as per the *Best Practice Supplement to Axiom® Genotyping Solution Data Analysis User Guide* (P/N 703083).

## References

- Rexroad C. E., *et al.* Estimates of linkage disequilibrium and effective population size in rainbow trout. *BMC Genetics* **10**:83 (2009). doi:10.1186/1471-2156-10-83
- Palti Y., *et al.* A resource of single-nucleotide polymorphisms for rainbow trout generated by restriction-site associated DNA sequencing of doubled haploids. *Molecular Ecology Resources* **14**:588–596 (2014). doi: 10.1111/1755-0998.122042
- Palti Y., *et al.* The development and characterization of a 57K single nucleotide polymorphism array for rainbow trout. *Molecular Ecology Resources* (2014). doi:10.1111/1755-0998.12337
- Sanchez C. C., *et al.* Single nucleotide polymorphism discovery in rainbow trout by deep sequencing of a reduced representation library. *BMC Genomics* **10**:559 (2009). doi:10.1186/1471-2164-10-559
- Boussaha M., *et al.* Development and characterization of an expressed sequence tags (EST)-derived single nucleotide polymorphisms (SNPs) resource in rainbow trout. *BMC Genomics* **13**:238 (2012). doi:10.1186/1471-2164-13-238
- Salem M., *et al.* RNA-Seq identifies SNP markers for growth traits in rainbow trout. *PLoS ONE* **7**:e36264 (2012). doi:10.1371/journal.pone.0036264

## Ordering information\*

Part number	Description	Details
550468	Axiom® Trout Genotyping Array (96-format)	Contains one plate with 96 arrays
550571	Axiom® Trout Genotyping Array (384-format)	Contains one plate with 384 arrays

\*Axiom® 2.0 reagents and GeneTitan® MC Instrument consumables must be quoted separately.

Affymetrix, Inc. Tel: +1-888-362-2447 ■ Affymetrix UK Ltd. Tel: +44-(0)-1628-552550 ■ Affymetrix Japan K.K. Tel: +81-(0)3-6430-4020  
Panomics Solutions Tel: +1-877-726-6642 panomics.affymetrix.com ■ USB Products Tel: +1-800-321-9322 usb.affymetrix.com

[www.affymetrix.com](http://www.affymetrix.com) Please visit our website for international distributor contact information.

**For Research Use Only. Not for use in diagnostic procedures.**

P/N GGN00482 Rev. 1

©Affymetrix, Inc. All rights reserved. Affymetrix®, Axiom®, Command Console®, CytoScan®, DMET™, GeneAtlas®, GeneChip®, GeneChip-compatible™, GeneTitan®, Genotyping Console™, myDesign™, NetAffx®, OncoScan®, Powered by Affymetrix™, PrimeView™, Procarta®, and QuantiGene® are trademarks or registered trademarks of Affymetrix, Inc. All other trademarks are the property of their respective owners.

Products may be covered by one or more of the following patents: U.S. Patent Nos. 5,445,934; 5,744,305; 5,945,334; 6,140,044; 6,399,365; 6,420,169; 6,551,817; 6,733,977; 7,629,164; 7,790,389 and D430,024 and other U.S. or foreign patents. Products are manufactured and sold under license from OGT under 5,700,637 and 6,054,270.