Axiom Salmon Genotyping Array

The world's first-of-its-kind, expert-designed Atlantic salmon (*Salmo salar)* genotyping array for high-resolution genetics research and aquaculture breeding

The Applied Biosystems™ Axiom™ Salmon Genotyping Array for Atlantic salmon (*Salmo salar*) was designed using our Expert Design Program in collaboration with The Roslin Institute (University of Edinburgh), Edinburgh Genomics, Landcatch Natural Selection Ltd., University of Stirling, and University of Glasgow in the United Kingdom.

Benefits of high-density genotyping of salmon

The Axiom Salmon Genotyping Array provides extensive information on polymorphisms across the salmon genome. Such dense SNP information is required to capture population-wide linkage disequilibrium for effective salmon genome-wide association studies (GWAS) [1]. Further, dense marker maps are required for effective development of breeding programs that will have sufficient accuracy for genomic selection [2].

The high marker density on the Axiom Salmon Genotyping Array enables a broad coverage of the salmon genome and avoids underrepresentation of important regions of the salmon genome. This is a significant improvement over existing Atlantic salmon genotyping tools.

Salmonids experienced a recent whole-genome duplication event, which caused Atlantic salmon to have a mosaic genome. Although most of the genome segregates in a diploid fashion, it can be difficult to distinguish genuine segregating SNPs from paralogous variation. Axiom Salmon Genotyping Array and corresponding analysis tools partially overcome the challenges arising from this complexity, including false variant calls, cluster compression, and species-specific polymorphic site and structural diversity.

Highlights

Diverse content:

- >130,000 SNPs evenly spaced across the genome
- Preference given to transcribed regions
- Polymorphisms discovered using three different sequencing methodologies
- 4,714 markers from public sources
- 87 Y chromosome–specific probes for accurate gender determination

Multiple breeds represented:

- Farmed Scottish and Norwegian, and wild Scottish, Irish, Norwegian, and Spanish populations

Demonstrated use:

- Detection of population structure
- Determining gender of salmon
- GWAS for resistance to sea lice [3]

Applications

Complex trait research:

- GWAS
- Fine mapping of quantitative trait loci (QTL) affecting economically important traits in salmonids

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Molecular breeding:

- Association mapping and genomic selection
- Improved accuracy in aquaculture breeding programs
- Gender determination via Y chromosome–specific probes

Population and evolutionary genetics:

- Distinguish between fish of different origins
- Differentiate farmed and wild populations

SNP discovery and marker selection

SNP discovery was facilitated by sequencing both farmed and wild fish. Sequences were aligned to the draft Atlantic salmon reference genome (NCBI Assembly GCA_000233375.1).

Due to genome duplication and mosaicism in the salmon genome, paralogous sequence variants (PSVs) [4] may interfere with allelic SNPs and complicate SNP discovery. Therefore, three distinct approaches for genome complexity reduction were applied: reduced representation sequencing (RR-Seq), restriction site–associated DNA sequencing (RAD-Seq), and mRNA sequencing (RNA-Seq). The high sequence coverage and use of both pedigreed samples and a haploid individual were adopted in order to identify and remove PSVs and include informative polymorphisms. See Table 1 for details of these experiments.

RR-Seq:

- One haploid individual was included to identify heterozygous PSVs, which were then excluded from the final array design
- SNPs with minor allele frequency (MAF) ≤0.1 were removed
- SNPs with known repeat elements were removed

RAD-Seq:

- SNPs with >1 Mendelian errors across families were removed
- Repeat-masked SNPs were removed

RNA-Seq:

- Repeat-masked SNPs were removed

Putative SNPs were used to calculate *in silico* design scores, which predict the likelihood of success in the Axiom assay. SNPs with high scores were included for the final design.

Y chromosome–specific SNPs were included by conducting a homology search of the *sdY* gene, which was recently discovered in rainbow trout [5]. Partial sequences of the gene were verified with PCR, and two contigs were produced. Repetitive elements were removed, and 87 probes were designed for the array.

Table 1. Techniques of DNA and RNA sequencing experiments used for SNP discovery.

	RR-Seq	RAD-Seq	RNA-Seq
Samples	Farmed* (40), wild (16), haploid (1)	Farmed* (160)	Farmed* (72)
Initial discovered SNPs	472,072	467,268	816,570
SNPs selected for array**	99,097	83,151	229,754
Final SNPs for array design†	73,800	54,197	156,979

* Farmed fish were representative samples of the Atlantic salmon breeding company Landcatch Natural Selection Ltd.
** SNPs selected after filtering for PSVs, MAF, and repeat elements.
† SNPs selected after calculation of *in silico* design scores.

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Results

The Axiom Salmon Genotyping Array has been successfully used to genotype salmon samples of diverse origin from three main categories: farmed Scottish, farmed Norwegian, and wild fish from Scotland, Ireland, Norway, and Spain.

The Axiom Salmon Array has been used in studies that confirmed the sex locus in the Atlantic salmon with 100% concordance and showed that the array can be used to distinguish between different populations of both farmed and wild salmon. It is currently being applied in a GWAS for resistance to sea lice.

Samples were assayed on the Axiom Salmon Genotyping Array using the standard Applied Biosystems™ Axiom™ 2.0 assay and GeneTitan™ Multi-Channel (MC) Instrument running GeneChip™ Command Console™ Software (AGCC) 3.2.4 or higher.

The data analysis and clustering were automated using Applied Biosystems™ Genotyping Console™ Software 4.1.4 or higher and SNPolisher™ package 1.4. Sample QC was performed as per the Best Practice Supplement to Axiom Genotyping Solution Data Analysis (P/N 703083). SNP QC was performed as per the SNPolisher User Guide.

References

1. Houston R, et al. (2014) Development and validation of a high-density SNP genotyping array for Atlantic salmon *(Salmo salar)*. PAG XXII Conference.
2. Meuwissen TH, et al. (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157(4):1819–1829.
3. Parasite lost as science tackles sea lice menace (2013) *International Aquafeed* 16(1):3. http://issuu.com/international_aquafeed/docs/iaf1304_w1
4. Fredman D, et al. (2004) Complex SNP-related sequence variation in segmental genome duplications. *Nature Genetics* 36(8):861–866.
5. Yano A, et al. (2012) An immune-related gene evolved into the master sex-determining gene in rainbow trout, *Oncorhynchus mykiss*. *Current Biology* 22(15):1423–1428.

Ordering information

Product	Description	Cat. No.
Axiom Salmon Genotyping Array (ssalar01)	Contains one 96-array plate; reagents and GeneTitan Multi-Channel Instrument consumables sold separately	550540
Axiom GeneTitan Consumables Kit	Contains all GeneTitan Multi-Channel Instrument consumables required to process one Axiom array plate	901606
Axiom 2.0 Reagent Kit	Includes all reagents (except isopropanol) for processing 96 DNA samples	901758

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