A single platform for accurate genomic evaluation across a diverse set of breeds

Recent advances in genetic analysis tools have resulted in increased animal performance through genetic gain. The Applied Biosystems™ Axiom™ Ovine Genotyping Array with 11,196 markers in a 384-array plate format offers the power and resolution for a wide range of applications in sheep breeding. This includes breed assignment, parentage verification, discovery, and traceability and monitoring of polymorphisms of known large effect. The array contains 88 single-nucleotide polymorphisms (SNPs) that were proposed by the International Sheep Genomics Consortium (ISGC) [1] for parentage verification. The 11K markers on the array facilitate highly accurate imputation in higher-density panels (e.g., up to 50K).

The Axiom Ovine Genotyping Array contains five scrapie markers that are of importance to the sheep community for identifying sheep that are susceptible to scrapie. Scrapie is a fatal degenerative disease that affects the nervous system of sheep, caused by infectious agents known as prions. Detection of the susceptible prion genotype can enable selective breeding, thereby helping to eradicate the disease in a flock.

Highlights

- Expert design developed in collaboration with Donagh Berry at Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Ireland

- Exceptionally high-quality SNPs with excellent call and concordance rates

- Includes SNPs for parentage as well as other traits, including fecundity and susceptibility to scrapie

- Compatible with the 15K array commonly used by the sheep breeding community and thus with previous studies

Applications

- Parentage verification and discovery

- Accurate genomic evaluation

- Genome-wide associations following accurate imputation to higher-density panels

- Breed assignment

- Monitoring of major genes

- Detection of aneuploidy to help enable identification of animals that are not suitable to breed for the next generation

- Measurement of genetic diversity, including calculation of genomic inbreeding statistics

- Mating designs

- Traceability

- Precision management for different genotypes

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Array SNP selection

The exceptionally high-quality markers for the Axiom Ovine Genotyping Array were selected to be compatible with the 15K array commonly used by the sheep breeding community, and to maximize imputation accuracy across multiple breeds. Table 1 shows the within-breed minor allele frequency (MAF) for a selection of breeds, along with the number of monomorphic SNPs on the array for each breed.

The following criteria were used for selection of SNPs on the Axiom Ovine Genotyping Array:

- Parentage SNPs as well as SNPs proposed by ISGC and also represented on many international parentage panels

- SNPs well distributed across the genome (Figure 1)

- High-quality–scoring SNPs that fell into the PolyHighResolution, NoMinorHomozygous, and MonoHighResolution categories of markers

- SNPs with >99% concordance between Axiom platform and current in-market array [2]

- High MAF in all five breeds shown in Table 1

- Call rates >95% from genotype data of approximately 10,000 samples

Automated data analysis and seamless integration with existing bioinformatics pipelines

The Axiom Ovine Genotyping Array was evaluated with samples from multiple diverse breeds to demonstrate array performance. A total of 384 samples were processed and analyzed using Applied Biosystems™ Axiom™ Analysis Suite software. The results from the data analysis are shown in Table 2. The data were automatically clustered, assigned genotypes, and classified into six categories for easy visualization. Average sample call rate for the PolyHighResolution SNPs was 99.73%.

Table 1. Breeds used in the selection of markers.

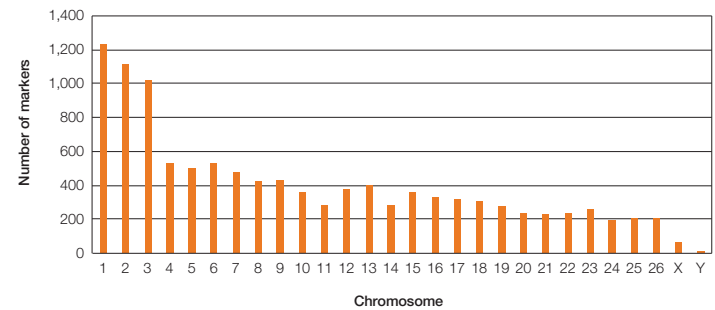| Breeds	Median MAF	Number of monomorphic SNPs
Belclare	0.33	43
Charollais	0.33	17
Suffolk	0.29	50
Texel	0.31	34
Vendéen	0.31	54

Figure 1. Distribution of markers across the sheep genome.

Table 2. Results from the Axiom Ovine Genotyping Array, assigned to six different categories. The markers in the recommended categories include **1)** PolyHighResolution markers: markers demonstrating three clusters with good cluster resolution and at least two examples of the minor allele; **2)** NoMinorHomozygous markers: markers exhibiting two clusters with no examples of the minor allele; **3)** MonoHighResolution markers: markers demonstrating a single cluster; **4)** OffTargetVariant markers: includes reproducible yet uncharacterized variants caused by double deletion, sequence nonhomology, or DNA secondary structure; and two other categories as shown below.

SNP classification	Number	Percentage (%)
PolyHighResolution	10,259	91.63
NoMinorHomozygous	173	1.55
MonoHighResolution	152	1.36
OffTargetVariant	39	0.35
CallRateBelowThreshold (<97%)	192	1.71
Other	381	3.40
All markers	**11,196**	**100**

The data can also be transformed seamlessly into long format using the Applied Biosystems™ Axiom™ Long Format Export (AxLE) Tool. The tool formats Axiom genotype data using the top (TOP) and bottom (BOT) designations based on the polymorphism itself, or the surrounding sequence in the context. The tool also designates the AB allele. This enables users to easily correlate genotype calls made on the day of the study to prior data. An example of the tab-delimited file is shown in Figure 2.

[Header]										
Version	2.4.0									
Processing Date	10/26/2016 10:30									
Content	Axiom_OviCap.r3									
Num SNPs	62586									
Total SNPs	62586									
Num Samples	166									
Total Samples	166									
[Data]										
SNP Name	Sample ID	Allele1 - Forward	Allele2 - Forward	Allele1 - TOP	Allele2 - TOP	Allele1 - AB	Allele2 - AB	Confidence	SNP Classification	Log2Ratio BAF
AX-106745792	OV_0016327_F05.CEL	G	G	G	G	B	B	0.00002	PolyHighResolution	-0.2439 0.985
AX-106745792	OV_0016344_A11.CEL	G	G	G	G	B	B	0.00003	PolyHighResolution	0.0465 1
AX-106745792	OV_0016357_A09.CEL	G	G	G	G	B	B	0.00024	PolyHighResolution	0.088 0.955
AX-106745792	OV_0016358_C01.CEL	G	G	G	G	B	B	0.00002	PolyHighResolution	0.0951 1
AX-106745792	OV_0016365_E05.CEL	A	G	A	G	A	B	0.00001	PolyHighResolution	-0.1293 0.5145
AX-106745792	OV_0016369_G09.CEL	G	G	G	G	B	B	0.00004	PolyHighResolution	-0.3891 0.9639
AX-106745792	OV_0016374_E03.CEL	G	G	G	G	B	B	0.0001	PolyHighResolution	-0.1863 1
AX-106745792	OV_0016376_E04.CEL	A	G	A	G	A	B	0.00001	PolyHighResolution	-0.072 0.5107
AX-106745792	OV_0016378_F06.CEL	G	G	G	G	B	B	0.0001	PolyHighResolution	-0.3547 0.9445
AX-106745792	OV_0016381_A10.CEL	A	G	A	G	A	B	0.00001	PolyHighResolution	-0.1032 0.5526
AX-106745792	OV_0016407_B11.CEL	G	G	G	G	B	B	0.00001	PolyHighResolution	-0.1602 0.9927
AX-106745792	OV_0016408_B12.CEL	G	G	G	G	B	B	0.00002	PolyHighResolution	-0.1573 1
AX-106745792	OV_0016418_D01.CEL	A	G	A	G	A	B	0.00001	PolyHighResolution	-0.1624 0.4504

Figure 2. An example of a tab-delimited export file using the AxLE tool.

Good cluster separation for a scrapie marker, 15K_OAR13_46225765, is shown in Figure 3. This marker, located on codon 171, is a good example of a marker that has poor cluster resolution on other array platforms and can be genotyped on the Axiom platform with high accuracy.
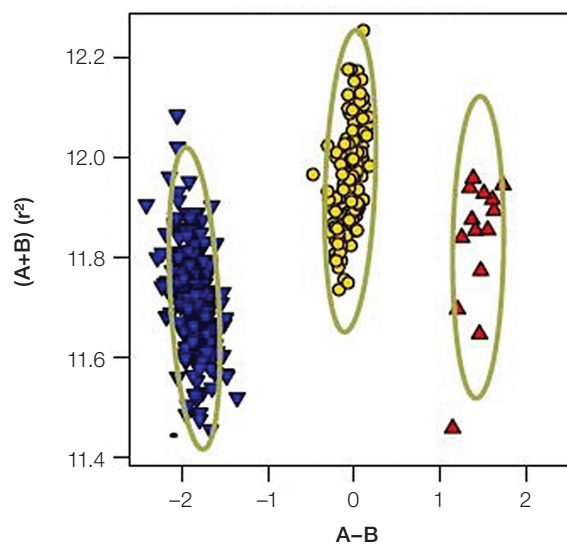

Figure 3. Good cluster resolution for scrapie marker 15K_OAR13_46225765.

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References

1. Kijas JW et al. (2012) Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biol* 10(2):e1001258.

2. Berry DP et al. (2016) Inter-and intra-reproducibility of genotypes from sheep technical replicates on Illumina and Affymetrix platforms. *Genet Sel Evol* 48(1):86.

Ordering information

Product	Description	Cat. No.
Axiom Ovine Genotyping Array	Contains one 384-array plate. Reagents and GeneTitan Multi-Channel Instrument consumables sold separately	550838
Axiom 2.0 384HT Reagent Kit	Includes all reagents (except isopropanol) for processing 384 DNA samples	902245
Axiom 2.0 384HT GeneTitan MC Consumables Kit	Contains all GeneTitan Multi-Channel Instrument consumables for processing 384 DNA samples	902234

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