illumina®

OvineSNP50 Genotyping BeadChip

More than 54,000 SNPs that deliver the densest coverage available for the ovine genome

Highlights

- **Comprehensive and Uniform Coverage**
  Evenly distributed polymorphic SNPs with a median < 43 kb gap spacing
- **Unrivaled Call Rates and Accuracy**
  > 99% average call rates and > 99.9% reproducibility
- **Simple Workflow**
  PCR- and ligation-free protocol
- **High-Throughput Format**
  Up to 12 samples can be interrogated in parallel

Introduction

The OvineSNP50 BeadChip (Figure 1) is the most comprehensive genome-wide genotyping array for the ovine genome, providing superior power to interrogate genetic variation across many breeds. The BeadChip was developed by Illumina in collaboration with the International Sheep Genomics Consortium (ISGC), comprising leading researchers from AgResearch, Baylor, UCSC, and Australia's Commonwealth Scientific and Industrial Research Organization (CSIRO).

Featuring more than 54,000 SNPs that uniformly span the entire ovine genome, the OvineSNP50 BeadChip enables a broad range of applications such as genome-wide selection, identification of quantitative trait loci (QTL), evaluation of genetic merit, cross-breed mapping, linkage disequilibrium studies, comparative genetic studies, and breed characterization for evaluating biodiversity.

This multi-sample genotyping panel is powered by the Infinium® HD Assay, delivering the industry's highest call rates and reproducibility, along with precise detection and measurement of copy number variation (CNV) (Table 1). The assay's PCR-free single-tube sample preparation significantly reduces labor and potential sample handling errors[1,2]. A multi-sample format further reduces experimental variability and overall project cost by allowing researchers to interrogate up to twelve samples in parallel.

The combination of Illumina's proprietary assay technology, unconstrained locus selection, and high-throughput format presents the most comprehensive solution for whole-genome studies of the ovine genome.

OvineSNP50 BeadChip Content

Illumina scientists and collaborators strategically selected informative markers across the ovine genome. SNP selection involved the application of several criteria, including minor allele frequency (MAF), allele count, Infinium Assay quality scores, chromosomal spacing and location, and subsequent validation by genotyping more than 3,000 samples representing diverse *Ovis aries* breeds and outgroup species. These stringent requirements resulted in the design of a high-density

Figure 1: OvineSNP50 BeadChip

The OvineSNP50 BeadChip features more than 54,000 evenly spaced SNPs across the entire ovine genome.

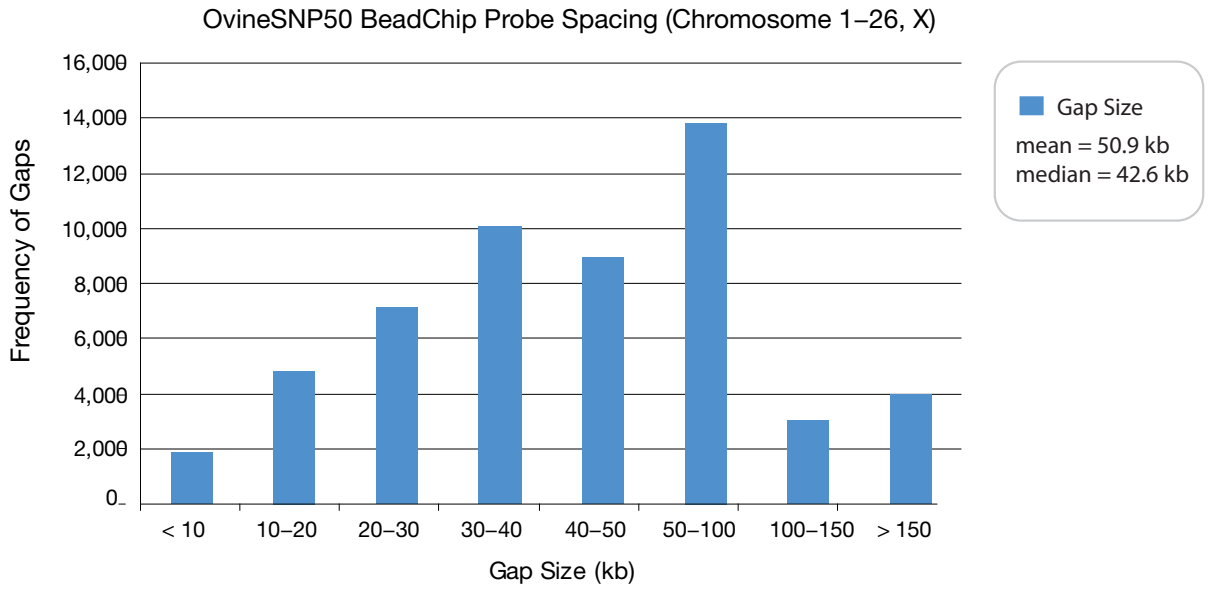
array featuring 54,241 SNPs with an average gap size of 50.9 kb and a median gap size of 42.5 kb (Figure 2). This level of coverage provides excellent SNP density to power robust genome-association studies and CNV detection in sheep.

The OvineSNP50 BeadChip covers SNPs validated in many economically important breeds, including more than 75 *Ovis aries* breeds, generating > 99.9% average call rates with a mean MAF of 0.28 (Table 2). More than 49,000 SNPs were validated across all breeds, with a MAF > 0.05.

More than 18,000 SNPs were discovered by sequencing 60 animals across 15 breeds using the Illumina sequencing platform (Table 3). Using a method called restricted representation sequencing (RRS), researchers selectively sequenced a subset of the genome from multiple individuals across many breeds. Pooled samples were first digested with an enzyme to generate an ideal number of fragments representing a random subset of the genome. These restricted representation libraries (RRLs) were then deeply sequenced on the Genome Analyzer$_{II}$, enabling the discovery of hundreds of thousands of true polymorphisms as well as the estimation of their MAFs. The sequences were then mapped back to a reference or draft genome to determine their location. In addition, over 37,000 SNPs were derived from the ovine draft genome (Version 1), which was assembled from whole-genome shotgun reads of six female sheep, and from resequencing data from nine additional individuals.

The BeadChip content includes coverage of autosomal, mitochondrial, and sex-linked (X/Y) SNPs. Other high-value content includes the current set of 138 candidate parentage SNPs and 600 SNPs identified by

Figure 2: OvineSNP50 BeadChip Probe Spacing



The OvineSNP50 BeadChip provides uniform coverage across the entire ovine genome.

BAC-end sequencing that were subsequently validated using Illumina GoldenGate® Genotyping Assays in over 403 animals from 23 breeds.

High-Quality Data

The 54,241 SNPs on the OvineSNP50 BeadChip were subjected to rigorous functional testing on multiple breeds to ensure strong performance using the Infinium HD assay. High call rates and accurate genotype calls are required for successful whole-genome association studies. Since complex traits often have relatively small gene effects, potential associations can be missed if the assayed SNP in linkage disequilibrium with the SNP of interest has a low call rate or incorrect genotype call. Illumina ensures that every OvineSNP50 BeadChip offers > 99% average call rate across common breeds.

Table 1 shows the results from internal validation testing of the OvineSNP50 BeadChip content using samples provided by collaborators and the Ovine HapMap Consortium[3]. Illumina scientists and collaborators identified and retained 3,079 loci that appear to have an adjacent polymorphism or deletion among the breeds sampled. Although these loci yielded lower call rates when compared to most loci on the panel, they were retained because they may provide biologically relevant information for traits of interest and future improvements in the genome build. These performance and content validation results clearly demonstrate the robust data quality delivered by the product. With such high data quality, the OvineSNP50 BeadChip provides researchers the highest accuracy and reliability for interrogating ovine genotypes in *Ovis aries* breeds.

Table 1: Performance and Specifications

Parameter	Results*	Product Specification
Average Call Rate	> 99.9%	> 99%
Reproducibility	> 99.9%	> 99.9%
Mendelian Inconsistencies	< 0.01%	< 0.1%

* Based on genotypes from reference samples

Illumina Solutions for Genotyping

The OvineSNP50 BeadChip is compatible with the BeadArray™ Reader, iScan, and HiScan™SQ systems. These array scanners feature high-performance lasers and powerful optical systems that enable rapid scan times and precise assay detection. The HiScanSQ system can also perform Illumina sequencing by synthesis chemistry, the world's most widely adopted next-generation sequencing platform.

The convenient modular design enables researchers to easily build out the system for evolving research needs. An optional Laboratory Information Management System (LIMS) is available to accurately and efficiently track samples. Robotic automation capabilities can be added to improve throughput for labs processing large numbers of samples. With the Infinium Assay workflow, data are processed directly into Illumina's GenomeStudio® software to provide streamlined genotype calling, analysis, and reporting. Researchers can also choose to use Illumina's convenient FastTrack Genotyping service to have samples genotyped and data delivered in a format suitable for GWAS or QTL analysis.

Table 2: BeadChip Content Validation

Species	Breed	Samples	Polymorphic Loci	Mean MAF	Median MAF
Ovis aries	Namaqua Afrikaner	17	33631	0.15	0.18
Ovis aries	Ronderib Afrikaner	17	40602	0.21	0.22
Ovis aries	Afshari	37	45593	0.27	0.26
Ovis aries	Altamurana	23	46656	0.28	0.27
Ovis aries	Awassi	2	30046	0.25	0.20
Ovis aries	Bangladeshi BGE	24	41429	0.23	0.23
Ovis aries	Barbados Blackbelly	24	43998	0.25	0.25
Ovis aries	Black Headed Mountain	24	44296	0.25	0.25
Ovis aries	Blackface	5	43365	0.30	0.25
Ovis aries	Blackface - Scottish	57	46713	0.28	0.27
Ovis aries	Boreray	19	37318	0.16	0.19
Ovis aries	Brazilian Creole	22	46600	0.30	0.27
Ovis aries	Bundner Oberlander	23	44476	0.26	0.26
Ovis aries	Castellana	22	46970	0.30	0.28
Ovis aries	Changthangi	29	46030	0.28	0.26
Ovis aries	Chios	23	43360	0.24	0.24
Ovis aries	Churra	118	47048	0.28	0.27
Ovis aries	Comisana	24	46747	0.29	0.27
Ovis aries	Composite	16	46316	0.28	0.27
Ovis aries	Cyprus Fat Tail	30	42322	0.23	0.23
Ovis aries	Deccani	24	43681	0.25	0.25
Ovis aries	Dorper	10	45584	0.25	0.24
Ovis aries	African Dorper	21	43151	0.26	0.25
Ovis aries	African White Dorper	6	37987	0.17	0.21
Ovis aries	Dorset	11	43531	0.27	0.26
Ovis aries	Poll Dorset	7	43664	0.29	0.24
Ovis aries	Dorset Horn	21	39029	0.19	0.21
Ovis aries	East Friesian Brown	39	41295	0.22	0.22
Ovis aries	East Friesian White	9	41800	0.22	0.22
Ovis aries	Engadine Red	24	46418	0.29	0.27
Ovis aries	Ethiopian Menz	34	42799	0.24	0.24
Ovis aries	Finnsheep	106	46566	0.28	0.27
Ovis aries	Galway	49	44443	0.26	0.25
Ovis aries	Garole - Bangladeshi	25	40591	0.22	0.23
Ovis aries	Garole - Indian	26	39012	0.21	0.21
Ovis aries	Garut	22	42529	0.25	0.24
Ovis aries	Gulf Coast Native	95	48143	0.30	0.29
Ovis aries	International Mapping Flock	27	45895	0.28	0.27
Ovis aries	Italian Sarda	5	43318	0.30	0.25

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Table 2: BeadChip Content Validation (continued)

Species	Breed	Samples	Polymorphic Loci	Mean MAF	Median MAF
Ovis aries	Karakas	18	45205	0.25	0.25
Ovis aries	Katahdin	9	44661	0.22	0.24
Ovis aries	Lacaune	177	47840	0.28	0.27
Ovis aries	Leccese	22	46852	0.30	0.28
Ovis aries	Merino	7	46016	0.29	0.26
Ovis aries	Australian Merino	46	47726	0.29	0.28
Ovis aries	Chinese Merino	23	45938	0.28	0.27
Ovis aries	Macarthur Merino	10	31054	0.10	0.16
Ovis aries	Merinolandschaf	21	45531	0.29	0.27
Ovis aries	Moghani	34	46571	0.28	0.27
Ovis aries	Morada Nova	21	41324	0.24	0.23
Ovis aries	Navaho	1	19302	0.00	0.19
Ovis aries	Norduz	20	44694	0.25	0.25
Ovis aries	Ojalada	24	47412	0.29	0.28
Ovis aries	Old Norwegian Saelsau	15	45479	0.27	0.26
Ovis aries	Qezel	35	47048	0.29	0.27
Ovis aries	Rambouillet	112	46670	0.28	0.27
Ovis aries	Rasa aragonesa	20	48676	0.30	0.29
Ovis aries	Red Maasai	46	43513	0.24	0.24
Ovis aries	Romanov	8	40228	0.19	0.22
Ovis aries	Romney	6	44278	0.25	0.25
Ovis aries	New Zealand Romney	21	44962	0.29	0.26
Ovis aries	Sakiz	22	40451	0.23	0.23
Ovis aries	Santa Ines	46	45914	0.27	0.26
Ovis aries	Sardinian Ancestral Black	20	45451	0.25	0.25
Ovis aries	Soay	432	36728	0.17	0.19
Ovis aries	Spael-coloured	3	35619	0.17	0.21
Ovis aries	Spael-white	29	44293	0.26	0.25
Ovis aries	St Elizabeth	10	47441	0.30	0.27
Ovis aries	Suffolk	9	45002	0.28	0.24
Ovis aries	Irish Suffolk	55	43627	0.25	0.25
Ovis aries	American Suffolk	5	42012	0.20	0.24
Ovis aries	Sumatra	24	41703	0.23	0.23
Ovis aries	Swiss Black-Brown Mountain	22	44961	0.27	0.26
Ovis aries	Swiss Mirror	20	46122	0.28	0.26
Ovis aries	Swiss White Alpine	21	44986	0.26	0.26
Ovis aries	Texel	17	45155	0.26	0.26
Ovis aries	German Texel	43	45987	0.28	0.27
Ovis aries	New Zealand Texel	22	44348	0.27	0.26
Ovis aries	Scottish Texel	80	44521	0.25	0.25

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Table 2: BeadChip Content Validation (continued)

Species	Breed	Samples	Polymorphic Loci	Mean MAF	Median MAF
Ovis aries	Indonesian Thin Tail	5	40026	0.20	0.22
Ovis aries	Sumatran Thin Tail	5	38363	0.20	0.21
Ovis aries	Tibetan	42	44578	0.26	0.25
Ovis aries	Valais Blacknose	23	41547	0.24	0.23
Ovis aries	Valais Red	21	40855	0.24	0.23
Ovis aries	Wiltshire	23	36460	0.17	0.19
Ovis aries	**All Domestic sheep**	**2812**	**49451**	**0.30**	**0.28**
Other Ovis	Wild sheep - All	116	41392	0.18	0.20
Ovis musimon	Mouflon - Sardinian	28	43053	0.23	0.23
Ovis musimon	Mouflon - European	24	29444	0.10	0.16
Ovis vignei	Wild Urial	4	25577	0.13	0.15
Ovis ammon	Wild Argali	6	17912	0.00	0.07
Ovis dalli	Thinhorn	2	503	0.00	0.00
Ovis canadensis	Bighorn	50	421	0.00	0.00
Ovis canadensis	Wyoming Bighorn	2	366	0.00	0.00
Outgroup	Outgroup	50	11994	0.00	0.06

Table 3: BeadChip Content Sources

Source	OvineSNP50 Probes
Validated SNPs (Sanger)*[4]	557
Illumina RRS	17,042
mtDNA	8
Other	36,634
Total	**54,241**

*Validated SNPs from a 1536-plex that the consortium did in advance of the SNP50.

Product Summary

Developed through a collaboration between Illumina scientists and leading ovine thought leaders, the OvineSNP50 BeadChip features more than 54,000 evenly spaced SNPs that provide comprehensive coverage of the ovine genome, enabling a diverse range of genetic research applications. As the first high-density whole-genome genotyping array for sheep, the 12-sample OvineSNP50 BeadChip presents a powerful, efficient, and cost-effective tool for a wide variety of genome-wide genetic analysis applications across the majority of sheep breeds worldwide

References

1. Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS (2005) A genome-wide scalable SNP genotyping assay using microarray technology. Genet 37(5): 549-554.
2. Steemers FJ, Chang W, Lee G, Barker DL,Shen R, et al. (2006) Whole-genome genotyping with the single-base extension assay. Nat Methods 3(1): 31-33.
3. http://www.sheephapmap.org/
4. Kijas JW, Townley D, Dalrymple BP, Heaton MP, Maddox JF, et al. (2009) A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PLoS One. 2009;4(3):e4668.

Ordering Information

Catalog No.	Product	Description
WG-410-1001	OvineSNP50 Whole-Genome Genotyping Kit (48 samples)	Kit contains four BeadChips and reagents for processing 48 samples.
WG-410-1002	OvineSNP50 Whole-Genome Genotyping Kit (288 samples)	Kit contains 24 BeadChips and reagents for processing 288 samples.
WG-410-1003	OvineSNP50 Whole-Genome Genotyping Kit (1152 samples)	Kit contains 96 BeadChips and reagents for processing 1152 samples.
FT-410-1001	Infinium OvineSNP50 FastTrack Service Project	Illumina's FastTrack Services require a minimum of 94 samples and 1.5 µg of DNA for each sample.

Each OvineSNP50 DNA Analysis BeadChip can process 8 samples and analyze 54,241 loci.

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