

BovineSNP50 Genotyping BeadChip

Developed in collaboration with leading bovine researchers, the BovineSNP50 BeadChip features more than 54,000 evenly spaced SNP probes that span the bovine genome. This 12-sample BeadChip presents a cost-effective and high-quality solution for cattle researchers interested in genome-wide genotyping applications.

INTRODUCTION

Illumina, in collaboration with the USDA ARS, University of Missouri, and the University of Alberta, has developed the first high-density, genome-wide genotyping array for the interrogation of genetic variation in cattle. The BovineSNP50 BeadChip features more than 54,000 highly informative SNP probes that uniformly span the entire bovine genome, empowering applications such as genome-wide enabled selection, identification of quantitative trait loci, evaluation of genetic merit of individuals, and comparative genetic studies.

More than half of the probes on this BeadChip are designed to target novel SNPs discovered using Illumina's

Genome Analyzer, a next-generation sequencing system. Additional high-value content is derived from publicly available sources such as the bovine reference genome, Btau¹, and the Bovine HapMap Consortium data set².

The BovineSNP50 BeadChip covers common SNPs validated in economically important beef and dairy cattle breed types and presents an average minor allele frequency (MAF) of 0.25 across all loci. Importantly, this BeadChip offers uniform coverage with an average probe spacing of 51.5kb to provide more than sufficient SNP density for robust genome-association studies in cattle.

The BovineSNP50 BeadChip is a multi-sample genotyping panel powered by Illumina's Infinium® II Assay. This revolutionary assay provides the industry's highest call rates, allows for flexible content deployment, and enables the detection and measurement of copy number variation. In addition, the assay's PCR-free single-tube sample preparation^{3,4} significantly reduces labor and potential sample handling errors. Illumina's multi-sample format further reduces experimental variability and overall project cost by allowing researchers to interrogate up to 12 samples in parallel.

The combination of Illumina's proprietary assay technologies,

FIGURE 1: BOVINESNP50 BEADCHIP



The BovineSNP50 BeadChip features more than 54,000 evenly-spaced SNPs across the entire bovine genome.

unconstrained locus selection, and high-throughput format presents the most comprehensive solution for whole-genome studies in beef and dairy cattle.

BOVINESNP50 BEADCHIP CONTENT

Illumina scientists and collaborators set out to develop an informative and high-density SNP genotyping microarray that could be used to investigate genetic variation in any cattle breed. More than 12,000 probes were designed to target validated common SNPs (MAF \geq 0.05) described by the Bovine HapMap Consortium, which, to date, has genotyped approximately 500 cattle across 19

BOVINESNP50 BEADCHIP HIGHLIGHTS

- **Unrivaled call rates and accuracy:** > 99% average call rates and 99.9% reproducibility
- **Comprehensive and uniform coverage:** > 54,000 evenly distributed polymorphic SNPs with a mean gap of 51.5kb
- **Simple Workflow:** PCR- and ligation-free protocol
- **High-throughput format:** Up to 12 samples can be interrogated in parallel

popular beef and dairy breeds. BeadChip developers also mined publicly available resources for common SNPs including the bovine reference genome¹ and whole-genome shotgun reads assembled by researchers at Baylor College of Medicine⁵. Additional content sources include parentage markers identified by researchers from the U.S. Meat Animal Research Center, Clay Center⁶, and SNPs identified by researchers at the USDA ARS through the comparison of Holstein bacterial artificial chromosomes (BAC) sequence data⁷ to the bovine genome assembly (Table 1).

More than 24,000 SNP probes featured on BovineSNP50 BeadChip target novel SNP loci that were discovered by sequencing three pooled populations of economically important beef and dairy cattle. Using Illumina's Genome Analyzer, researchers at the USDA ARS discovered more than 62,000 putative SNPs by deeply sequencing approximately 2% of the *Bos taurus* genome⁸. More than 23,800 SNPs derived from this novel data source were chosen based upon their spacing, expected MAF, and Infinium II Assay performance. In addition, BeadChip developers selected loci that target the largest unmapped contigs to ensure comprehensive coverage.

All 54,001 SNP probes on the BovineSNP50 BeadChip have been validated in 19 common beef and dairy breeds (Table 3). This product targets evenly distributed SNPs that are polymorphic across the breeds tested and provides an average probe spacing of 51.5kb and a median spacing of 37.3kb (Figure 2).

Current research on linkage disequilibrium (LD) in multiple breeds of cattle suggests haplotype blocks of approximately 70kb on

average⁹, indicating that the resolution offered by the BovineSNP50 chip is well within the resolution of LD in cattle.

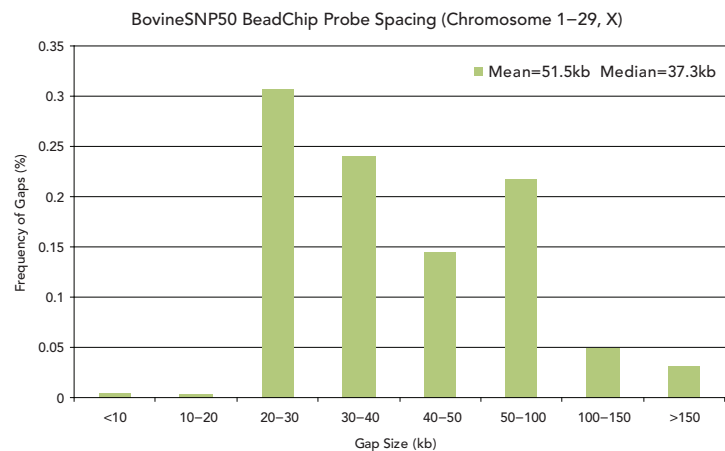
More than 52,255 SNP probes (97%) on the BovineSNP50 BeadChip map to Btau 4.0, the most current bovine reference genome assembly.

HIGH-QUALITY DATA

The 54,001 SNP assays on the

BovineSNP50 BeadChip were subjected to rigorous functional testing to ensure strong performance using Illumina's Infinium II Assay. Whole-genome association studies are successful, in part, due to high call rates and accurately called genotypes. Since complex traits often have relatively small gene effects, potential associations may be missed if the assayed SNP is in linkage disequilibrium with

FIGURE 2: BOVINESNP50 BEADCHIP PROBE SPACING



The BovineSNP50 BeadChip provides uniform coverage across the entire genome. Fewer than 5% of all gaps are larger than 100kb.

TABLE 1: BOVINESNP50 BEADCHIP CONTENT SOURCES

SOURCE	NUMBER OF PROBES
Novel SNPs derived from sequencing common cattle breeds using Illumina's Genome Analyzer	23,840
Bovine HapMap Data Set	12,298
Btau	9,361
Whole-Genome Shotgun Reads [†]	5,808
Holstein BAC Sequence Data	1,409
Other [‡]	1,285

[†]Reads derived from six cows (Norwegian Red, Holstein, Brahman, Angus, Jersey, and Limousin)⁶ compared against Btau 2.0⁵.

[‡]Includes 116 highly curated parentage markers⁶, plus common SNPs validated by the Institute for Food and Agricultural Sciences Alberta and INRA, the French International Institute of Agriculture.

the SNP of interest has a low call rate or incorrect genotype call. Illumina ensures that every BovineSNP50 BeadChip offers > 99% call rate.

Table 2 shows the outstanding results from Illumina's internal validation of the BovineSNP50 BeadChip content using samples provided by the Bovine HapMap Consortium. Product developers identified and retained 1,429 loci that appear to have an adjacent or third polymorphism (e.g., deletion or third allele) among the breeds sampled. Although these loci yield lower call rates when compared to the other loci on the panel, they were retained because they may provide biologically relevant information for traits of interest and for future improvements in the genome build.

The performance and content validation results clearly show the reliability and outstanding data quality the BovineSNP50 BeadChip delivers. With this BeadChip, researchers can predict the genetic merit for phenotypes of interest and investigate the genetic basis of variation in virtually all cattle breed types.

ILLUMINA SOLUTIONS FOR GENOTYPING

As with all of Illumina's standard products, an optional Laboratory Information Management System (LIMS) and robotic automation are available to accurately and efficiently track samples throughout analysis. Researchers can genotype their own samples using the Illumina BeadStation or Illumina's FastTrack Genotyping Service and can assess Illumina's genotyping products through the Illumina Customer Sample Evaluation (CSE) Program.

TABLE 2: BOVINESNP50 BEADCHIP PERFORMANCE DATA AND PRODUCT SPECIFICATIONS

PARAMETER	RESULTS	PRODUCT SPECIFICATION
Average Call Rate	99.50%† / 99.3%‡	> 99%
Reproducibility	100%*	> 99.9%
Mendelian Inconsistencies	0.04%**	< 0.1%

†Based on 565 individuals from 19 major cattle breeds, 46 trios, and two replicates.

‡Includes data from an outgroup consisting of 18 individuals from six different breeds: *Bos bison*, *Bos gaurus*, *Bos grunniens*, *Bos javanicus*, *Bubalus depressicornis*, and *Syncerus caffer*.

TABLE 3: BOVINESNP50 BEADCHIP CONTENT VALIDATION

BREED	SAMPLES	POLYMORPHIC LOCI*	MEAN MAF†	MEDIAN MAF‡
Angus	60	41,491	0.21	0.21
Beefmaster	24	42,925	0.22	0.21
Bos indicus Gir	24	23,971	0.11	0.02
Bos indicus Nelore	21	25,814	0.11	0.02
Brahman	25	30,284	0.13	0.08
Brown Swiss	24	36,347	0.19	0.17
Charolais	26	42,589	0.22	0.21
Guernsey	21	38,632	0.19	0.17
Hereford	32	42,992	0.20	0.23
Holstein	64	42,730	0.22	0.22
Jersey	28	35,976	0.18	0.14
Limousin	45	42,821	0.22	0.22
N'Dama	25	29,049	0.14	0.08
Norwegian Red	21	42,782	0.22	0.21
Piedmontese	24	42,185	0.22	0.21
Red Angus	15	40,188	0.21	0.20
Romagnola	24	38,830	0.20	0.19
Santa Gertrudis	24	42,064	0.22	0.21
Sheko	20	35,726	0.17	0.12
Outgroup‡	18	11,206	0.05	0.00
Overall	565	47,545	0.25	0.24

*MAF > 0.05

†Across all 54,001 loci

‡*Bos bison*, *Bos gaurus*, *Bos grunniens*, *Bos javanicus*, *Bubalus depressicornis*, and *Syncerus caffer*.

ORDERING INFORMATION

CATALOG NO.	PRODUCT	DESCRIPTION
WG-31-120	BovineSNP50 Whole Genome Genotyping Kit (48)	Kit contains four BeadChips and reagents for processing 48 samples.
WG-31-121	BovineSNP50 Whole Genome Genotyping Kit (288)	Kit contains 24 BeadChips and reagents for processing 288 samples.
WG-31-122	BovineSNP50 Whole Genome Genotyping Kit (1152)	Kit contains 96 BeadChips and reagents for processing 1152 samples.
FT-20-109	Infinium Bovine SNP50 FastTrack Service Project	Illumina's FastTrack Services require a minimum of 94 samples and 1.5 µg of DNA for each sample.

REFERENCES

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- (9) Khatkar MS, Zenger KR, Hobbs M, Hawken RJ, Cavanagh JAL, et al. (2007) A Primary Assembly of a Bovine Haplotype Block Map Based on a 15,036-Single-Nucleotide Polymorphism Panel Genotyped in Holstein-Friesian Cattle. *Genetics* 176(2):763-772.

ADDITIONAL INFORMATION

To learn more about Illumina's whole-genome genotyping solutions, please visit www.illumina.com.

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 Pub. No. 370-2007-029 Current as of January 07, 2008

