illumina®

PorcineSNP60 v2 Genotyping BeadChip

More than 64,000 SNPs deliver superior coverage of the porcine genome.

Highlights

- **Comprehensive and Uniform Coverage**
  Evenly distributed polymorphic SNPs with a median < 28 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 24 samples can be interrogated in parallel

Introduction

The PorcineSNP60 v2 BeadChip (Figure 1) is the most comprehensive genome-wide genotyping array for the porcine genome, providing superior power to interrogate genetic variation across many porcine breeds, including Duroc, Landrace, Pietrain, and Large White. The PorcineSNP60 BeadChip was developed in collaboration with the International Porcine SNP Chip Consortium, comprising researchers from Wageningen University, Danish Institute of Agricultural Science, USDA-ARS, USMARC, Roslin Institute, University of Illinois, Iowa State University, INRA, University of Missouri, and Cambridge University.

Featuring more than 64,232 SNPs that uniformly span the porcine genome, the PorcineSNP60 v2 BeadChip enables a broad range of applications such as genome-wide selection, identification of quantitative trait loci, 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 highest call rates and reproducibility in the industry, along with precise detection and measurement of copy number variation (CNV) (Table 1). The assay uses PCR-free single-tube sample preparation, which 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 24 samples in parallel.

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

PorcineSNP60 v2 BeadChip Content

Illumina scientists and collaborators strategically selected informative markers across the porcine genome. After combining novel SNPs with several other existing databases and study results, the Consortium selected the highest-quality markers from over 510,000 potential SNPs. The selection was based on several criteria, including minor allele frequency (MAF), allele count, Infinium Assay quality scores, chromosomal spacing and location, and subsequent validation by genotyping 554 porcine samples representing diverse breeds. These stringent requirements resulted in the design of a high-density array featuring 64,232 SNPs that provide uniform genome-wide coverage with an average spacing of 43.4 kb (Figure 2).

The PorcineSNP60 v2 BeadChip has been validated in 7 economically important pig breeds. More than 64,232 SNPs were validated in these breeds, producing an average MAF of 0.29 across all loci (Table 2).

More than 47,000 of the SNPs on the panel were discovered using deep sequencing with Illumina next-generation sequencing (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 enzymatically digested 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, and an estimation of their MAFs. Finally, sequences were mapped back to a reference or draft genome to determine their location.

Of all the SNPs on the PorcineSNP60 v2 BeadChip, 90% map to the porcine reference genome (Build 9).[3] The remaining 10% were assigned a putative position on the porcine genome based on the porcine–human comparative map and their position on BAC-based fingerprinted contigs (FPC) map.

Figure 1: PorcineSNP60 v2 BeadChip—The PorcineSNP60 v2 BeadChip features more than 64,232 evenly spaced SNPs across the entire porcine genome.

PorcineSNP60 Probe Spacing (Chromosomes 1–18, X)

Legend: Gap Size
mean = 43.4 kb
median = 27.9 kb

Figure 2: PorcineSNP60 v2 BeadChip Probe Spacing—The PorcineSNP60 v2 BeadChip provides uniform coverage across the porcine genome.

High-Quality Data

The 64,232 SNPs on the PorcineSNP60 v2 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. Because 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 PorcineSNP60 v2 BeadChip offers > 99% average call rate across common breeds.

Table 1 shows the results from internal validation testing of the PorcineSNP60 v2 BeadChip content using samples provided by collaborators and the Porcine HapMap Consortium. Illumina scientists and collaborators identified and retained 2596 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 might provide biologically relevant information, especially between breed groups. These performance and content validation results clearly demonstrate the high data quality delivered by the product. With such high data quality, the PorcineSNP60 v2 BeadChip provides researchers the highest accuracy and reliability for interrogating porcine genotypes in numerous porcine breeds.

Table 1: Performance and Specifications

Parameter	Results	Product Specification
Average call rate[a]	99.8%	> 99%
Reproducibility	100%	> 99.9%
Mendelian inconsistencies	0.08%	< 0.1%

a. Based on genotyping 554 reference samples.

Illumina Solutions for Genotyping

The PorcineSNP60 v2 BeadChip is compatible with the iScan® and HiScan® Systems. These array scanners feature high-performance lasers and powerful optical systems that enable rapid scan times and precise assay detection.

The convenient modular design enables researchers to build out the system easily for evolving research needs. An optional Laboratory Information Management System (LIMS) is available to track samples accurately and efficiently. 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 GenomeStudio® software to provide streamlined genotype calling, analysis, and reporting. Researchers can also choose to use the convenient FastTrack™ Genotyping service to have samples genotyped and data delivered in a format suitable for GWAS or QTL analysis.

Summary

Developed through collaboration between Illumina scientists and leading thought leaders, the PorcineSNP60 v2 BeadChip features 64,232 evenly spaced SNPs that provide comprehensive coverage of the porcine genome, enabling a diverse range of genomic research applications. This 24-sample BeadChip, along with the proven Infinium HD assay, presents a powerful high-throughput solution for whole-genome studies for many porcine breeds.

Table 2: PorcineSNP60 v2 BeadChip Content Validation

Breed	Samples	Polymorphic Loci[a]	Median MAF	Mean MAF
Birkshire	58	38,573	0.13	0.21
Duroc	76	41,845	0.18	0.20
Hamshire	63	43,496	0.19	0.20
Landrace	76	49,946	0.25	0.24
Large White	132	51,447	0.27	0.26
Meishan	30	30,289	0.07	0.14
Pietrain	87	48,094	0.23	0.23
Synthetic (Large White and Pietrain)	6	39,806	0.17	0.19
Wild boar	20	45,942	0.22	0.22
Other	6	N/A	N/A	N/A
All	**554**	**55,210**	**0.28**	**0.29**

a. MAF > 0.05

Table 3: PorcineSNP60 v2 BeadChip Content Sources

Source	PorcineSNP60 Probes
Wageningen University; Illumina Genome Analyzer sequences[4]	43,582
Wageningen University; Illumina Genome Analyzer sequences—pilot experiment[5]	3422
DIAS	1202
INRA	2528
ISU	37
MARC[6]	12,121
Roslin/Sanger	324
UMB, Norway	35
Other	981
Total	**64,232**

References

1. Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS. A genome-wide scalable SNP genotyping assay using microarray technology. *Nat Genet.* 2005;37:549-554.
2. Steemers FJ, Chang W, Lee G, Barker DL, Shen R, Gunderson KL. Whole-genome genotyping with the single-base extension assay. *Nat Methods.* 2006;3:31-33.
3. www.ebi.ac.uk/embl
4. Ramos AM, Crooijmans RP, Affara NA, et al. Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. *PLoS One.* 2009;4:e6524.
5. Amaral AJ, Megens HJ, Kerstens HH, et al. Application of massive parallel sequencing to whole genome SNP discovery in the porcine genome. *BMC Genomics.* 2009;10:374.
6. Wiedmann RT, Smith TP, Nonneman DJ. SNP discovery in swine by reduced representation and high throughput pyrosequencing. *BMC Genet.* 2008; 9:81.

Ordering Information

Catalog No.	Product	Description
WG-410-2001	PorcineSNP60 v2 Whole-Genome Genotyping Kit (48 samples)	Kit contains 2 BeadChips and reagents for processing 48 samples.
WG-410-2002	PorcineSNP60 v2 Whole-Genome Genotyping Kit (288 samples)	Kit contains 12 BeadChips and reagents for processing 288 samples.
WG-410-2003	PorcineSNP60 v2 Whole-Genome Genotyping Kit (1152 samples)	Kit contains 48 BeadChips and reagents for processing 1152 samples.
WG-410-2005	PorcineSNP60+ DNA Analysis Kit v2 (48 samples)	Kit contains 2 BeadChips and allows researchers to include up to 25,000 additional custom probes per sample for targeted studies.
WG-410-2006	PorcineSNP60+ DNA Analysis Kit v2 (288 samples)	Kit contains 12 BeadChips and allows researchers to include up to 25,000 additional custom probes per sample for targeted studies.
WG-410-2007	PorcineSNP60+ DNA Analysis Kit v2 (1152 samples)	Kit contains 48 BeadChips and allows researchers to include up to 25,000 additional custom probes per sample for targeted studies.
FT-410-2001	Infinium PorcineSNP60 v2 FastTrack Service Project	Illumina scientists provide personalized service, industry-leading data quality, and guaranteed turnaround time for porcine genotyping studies.

Each PorcineSNP60 DNA Analysis BeadChip can process 24 samples and analyze 64,232 loci.

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