CanineSNP20 BeadChip

Developed in collaboration with the Broad Institute, the CanineSNP20 Panel features more than 22,000 evenly spaced and validated SNPs derived from the CanFam2.0 assembly. This 12-sample Infinium® BeadChip presents a cost-effective and high-quality genotyping solution for researchers conducting whole-genome association studies within domestic dog breeds.

INTRODUCTION

The unique breeding history of the domestic dog offers the research community a powerful opportunity for unraveling the genetic basis of disease, behavioral and morphological traits, and mammalian variation. The high frequency of certain phenotypes within breeds suggests that only a limited number of loci are responsible for each trait, making it potentially easier to identify the genetic basis of variation in dogs than in humans[1].

In 2005, researchers at the Broad Institute completed the assembly of the canine genome, CanFam2.0[2], and presented the scientific community with the first comprehensive and high-quality reference sequence for the domestic dog. Out of the individual dogs submitted as candidates, the Broad researchers selected a female boxer to serve as the reference sequence because this inbred dog displayed the least variation in its genome[3]. The Broad team sequenced more than 99% of the boxer's euchromatic genome and compiled a list of more than 2.5 million SNPs by comparing the boxer reference sequence to ten other breeds[3].

Illumina has developed the CanineSNP20 Genotyping BeadChip in collaboration with the Broad researchers who assembled the CanFam2.0 genome. This array contains more than 22,000 validated SNP probes derived from the CanFam2.0 assembly. Featuring highly polymorphic SNP content and providing uniform coverage, the CanineSNP20 BeadChip enables the interrogation of genetic variation in any domestic dog breed. Importantly, this BeadChip presents an average of eight SNPs per megabase (Mb), providing ample SNP density for robust within-breed association studies.

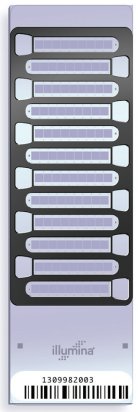
The CanineSNP20 BeadChip is Illumina's first non-human standard genotyping panel powered by the Infinium Assay. This revolutionary assay provides the industry's highest call rates, allows for flexible content deployment, and enables the detection and measurement of copy number variation. In addition, the assay's single-tube sample preparation without PCR or ligation steps[4,5] significantly reduces labor and potential sample handling errors. The multi-sample CanineSNP20 BeadChip further reduces experimental variability by allowing researchers to interrogate up to 12 samples in parallel.

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

CANINESNP20 BEADCHIP HIGHLIGHTS

- **Unrivaled Call Rates and Accuracy:** > 99% average call rates and 99.99% reproducibility
- **Comprehensive and Uniform Coverage:** Genome-wide coverage using over 22,300 evenly spaced SNPs derived from the CanFam2.0 reference sequence
- **Simple Workflow:** PCR-free protocol with the powerful Infinium Assay
- **High-Throughput Format:** Up to 12 samples can be interrogated in parallel

FIGURE 1: CANINESNP20 BEADCHIP



The CanineSNP20 Genotyping BeadChip features more than 22,000 evenly-spaced SNPs across the entire dog genome.

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SNP SELECTION STRATEGY

The SNP content featured on the CanineSNP20 BeadChip was selected from a diverse population of dog breeds. Single-direction reads from these breeds were aligned to the boxer reference sequence to identify polymorphic loci. Breed-specific SNPs were preferentially selected over the reference genome SNPs to provide researchers with a set of informative loci that are likely to segregate differently among breeds. Loci from the reference sequence were secondarily chosen when other breed SNPs were not available to maintain an average SNP density of eight SNPs per Mb. By employing this content selection strategy, Illumina has constructed a whole-genome genotyping microarray that empowers confident disease mapping in all dog breeds (*Table 2*). Additional CanineSNP20 BeadChip content includes 37 SNPs in 12 well-characterized cytokine genes for investigators interested in immunological studies.

COMPLETE COVERAGE WITH ~22,000 SNPS

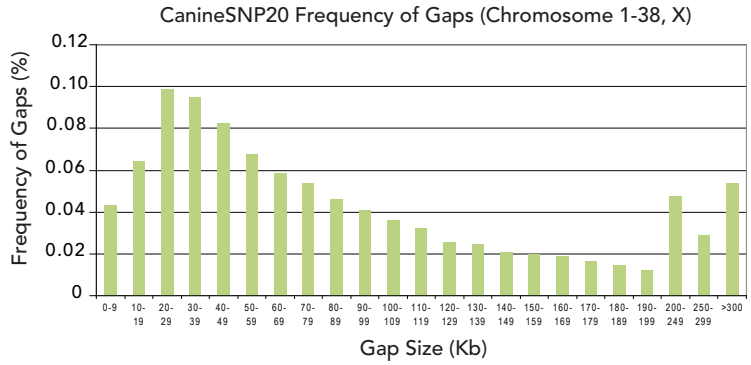
The haplotype structure of the modern domestic dog clearly reflects the two population bottleneck events that occurred during its evolution. The first bottleneck can be traced to the domestication of the wolf, approximately 7,000–9,000 generations ago. The recent bottleneck began less than 100 generations ago and is the result of intensive inbreeding to create modern domestic dog breeds. Consequently, short-range LD exists across dog breeds while long-range LD is present within breeds.

As a part of their comprehensive analysis of the dog genome, Broad researchers compared the entire genome of the canine reference sequence with 6% of the genomes from ten additional breeds. They demonstrated that linkage disequilibrium (LD) within breeds stretches over distances of several megabases, while LD across breeds, on average, is limited to tens of kilobases[2]. They also discovered that discrete haplotype blocks within breeds contain approximately three to five haplotypes at each locus[3].

The long-range LD and reduced haplotype diversity within breeds suggest that a much lower density of SNPs is required to map genes of interest in the domestic dog. Furthermore, it indicates that additional SNP density offers diminishing returns. As few as 10,000 evenly spaced SNPs could be sufficient for dog association studies, depending on the breed and trait of interest[3].

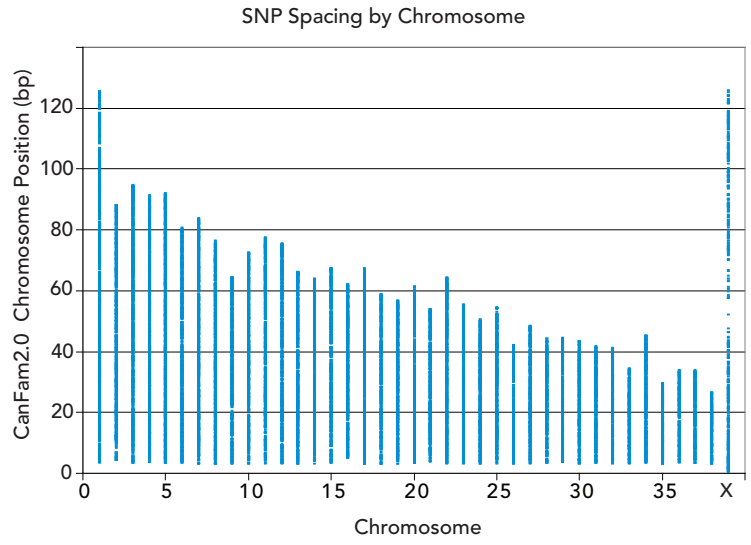
With more than 22,000 uniformly

FIGURE 2: CANINESNP20 BEADCHIP PROBE SPACING



The CanineSNP20 BeadChip provides uniform coverage across the entire genome.

FIGURE 3: PHYSICAL MAP OF CANINESNP20 BEADCHIP PROBES



The CanineSNP20 BeadChip offers comprehensive coverage that correlates well with the available SNPs per chromosome identified in the CanFam2.0 assembly.

distributed SNPs, the CanineSNP20 BeadChip offers more than sufficient coverage for confident identification and mapping of qualitative loci of interest. The average SNP density of eight per Mb enables researchers to differentiate up to five haplotypes per Mb within breeds.

TABLE 1: CANINESNP20 BEADCHIP PERFORMANCE DATA AND SPECIFICATIONS

PARAMETER	PERCENT	PRODUCT SPECIFICATION
Average Call Rate	99.81%	> 99%
Reproducibility	> 99.9%	> 99.9%
Mendelian Inconsistencies	0.09%	< 0.1%

Results based on 316 DNA samples including four replicates and 39 trios from nine breeds, and an F2 intercross between a Border Collie and Newfoundland[6].

TABLE 2: DIVERSITY OF BREEDS USED TO EVALUATE CANINESNP20 BEADCHIP

BREED	# OF DNA SAMPLES	CALL RATE*	POLYMORPHIC LOCI (MAF > 0.05)	AVERAGE MAF
Beagle	32	99.80	16,190	0.20
Brittany	33	99.81	17,866	0.21
Dachshund	32	99.82	18,146	0.23
German Shepherd	32	99.78	15,337	0.18
Greyhound	32	99.82	16,791	0.20
Jack Russell Terrier	32	99.81	19,975	0.25
Labrador Retriever	32	99.84	18,489	0.22
Shar Pei	32	99.82	17,739	0.21
Standard Poodle	32	99.75	18,368	0.22
F2 Intercross[6]	27	99.82	15,375	0.20
Total	316	99.81	21,074	0.27

*Call rates across sample set include 396 loci that appear to have deletion or third polymorphism.

HIGH-QUALITY DATA

The 22,362 SNP assays on the CanineSNP20 BeadChip were subjected to rigorous functional testing to ensure strong performance using Illumina's Infinium Assay. Whole-genome association studies are successful, in part, due to high call rates and accurately called genotypes. Since complex disease traits often have relatively small gene effects, potential associations may be missed if the assayed SNP in linkage disequilibrium with the disease SNP has a low call rate or incorrect genotype call. Illumina ensures that every CanineSNP20 BeadChip offers > 99% call rate.

Table 1 shows the outstanding results from Illumina's internal validation of the CanineSNP20 BeadChip. During this product's development, 396 loci were identified and retained 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.

More than 21,000 SNP loci (94%) on the CanineSNP20 BeadChip are polymorphic across the samples that were interrogated for product quality testing (*Table 2*). The average minor allele frequency (MAF) across all the breeds and reference family is 0.27, while the breed-specific MAFs range from 0.18 to 0.25 (*Table 2*).

Sixty-five markers were identified as fixed for opposite alleles in breed-breed comparisons among the ten sample groups surveyed (*Table 3*), indicating that this panel is suitable for researchers interested in genotyping for breed confirmation among the breeds represented. These breeds were selected to represent diverse ancestries and encompass ancient gene lineages. Thus, the data from the nine evaluated breeds strongly predict that the CanineSNP20 BeadChip performance can be generally extrapolated to most, if not all, domestic dog breed populations.

ILLUMINA SOLUTIONS FOR GENOTYPING

The high-quality data and low per-sample cost of the CanineSNP20 BeadChip are part of the powerful Illumina Whole-Genome Genotyping Solution. In addition, optional automation and the Laboratory Information Management System (LIMS) lower costs by eliminating errors associated with manual processing. Illumina genotyping products can be accessed via Illumina Fast Track Genotyping Services, the Illumina Customer Sample Evaluation (CSE) Program, or one's own Illumina BeadStation. Illumina solutions provide industry-leading levels of accuracy, flexibility, and affordability.

TABLE 3: CANINESNP20 BEADCHIP BREED INFORMATIVE MARKERS

	BEAGLE	BRITTANY	DACHS-HUND	GERMAN SHEPHERD	GREY-HOUND	JACK RUSSELL	LABRADOR RETRIEVER	SHAR PEI	STANDARD POODLE	F2 INTER-CROSS
Beagle	X	0	0	1	2	0	0	0	1	7
Brittany	0	X	0	2	0	0	0	0	0	1
Dachshund	0	0	X	1	5	0	0	0	0	3
German Shepherd	1	2	1	X	6	1	1	3	1	15
Greyhound	2	0	5	6	X	0	2	1	3	13
Jack Russell Terrier	0	0	0	1	0	X	1	0	0	0
Labrador Retriever	0	0	0	1	2	1	X	1	0	2
Shar Pei	0	0	0	3	1	0	1	X	1	4
Standard Poodle	1	0	0	1	3	0	0	1	X	3
F2 Intercross	7	1	3	15	13	0	2	4	3	X

Loci fixed for opposite alleles can be used as breed informative markers for 17 of the 36 possible pairwise breed comparisons examined in the development of this panel.

ORDERING INFORMATION

CATALOG NO.	PRODUCT	DESCRIPTION
WG-31-123	CanineSNP20 Whole-Genome Genotyping Kit (for 48 Samples)	Each package contains four BeadChips and reagents for processing 48 samples.
WG-31-124	CanineSNP20 Whole-Genome Genotyping Kit (for 288 Samples)	Each package contains 24 BeadChips and reagents for processing 288 samples.
WG-31-125	CanineSNP20 Whole-Genome Genotyping Kit (for 1152 Samples)	Each package contains 96 BeadChips and reagents for processing 1152 samples.
FT-20-110	CanineSNP20 FastTrack Service Project	Illumina's FastTrack Services requires a minimum of 94 samples and 1.5 µg of DNA for each sample.

The CanineSNP20 Genotyping BeadChip can process twelve samples in parallel and genotype > 22,362 loci per sample.

REFERENCES

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(4) Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS (2005) A genome-wide scalable SNP genotyping assay using microarray technology. Nat Genet 37(5): 549-554.

(5) Steemers FJ, Weihua 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.

(6) Neff MW, Broman KW, Mellersh CS, Ray K, Acland GM, et al. (1999) A second-generation genetic linkage map of the domestic dog, Canis familiaris. Genetics 151 (2): 803–820

ADDITIONAL INFORMATION

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

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