MyDogDNA®
Technical Data Sheet

1. Introduction

Active international canine genetics research has resulted in the identification of more than 200 genetic variants implicated in disease risk or in the regulation of various conformational traits such as coat color, skull shape, and body size (1). Meanwhile, technological advances have enabled reliable, cost-efficient, high-throughput genotyping of such variants in any dog. This has fueled a transformation where genetic panel screening has replaced single gene variant testing as the state of the art within canine DNA testing.

MyDogDNA® is the first ever, original canine panel screening test service. Initially launched in 2013, the test is based on the solid experience gained by testing more than 300,000 dogs representing more than 350 different breeds. The test's fundamental philosophy is simple: to provide a comprehensive yet cost-efficient analysis of the dog genome to help dog owners, breeders, veterinary clinicians, and breed/kennel organizations better understand a dog's and breed's:

- genetic health risks
- appearance and morphology
- genetic diversity level (genetic "coefficient of inbreeding")
- population structure
- genetic relationship to other dogs and breeds

A further aim of the service is to provide the dog community with the hands-on tools needed for breed genetic management and sustainable breeding. This goal is achieved through the core of the **MyDogDNA**® service: a unique online database of canine DNA information featuring, among other things, real-time updated statistics on disease carrier frequencies and genetic diversity levels. We believe that a key to health improvement of pedigree dogs worldwide lies in the **MyDogDNA**® Breeder Tool concept: maintenance of genetic diversity with simultaneous informed management of known inherited disease risks.

2. Technology

The **MyDogDNA**® test panel uses a custom-designed beadchip microarray based on the robust, reliable and widely utilized Illumina Infinium® HD Ultra technology platform (2). All analyses are processed according to well-established routine protocols defined by the manufacturer (Illumina®, Inc., San Diego, USA).

All DNA identification and parentage verification profiling is separately processed using internationally approved standard ISAG (International Society for Animal Genetics) and AKC (American Kennel Club) short tandem repeat (STR) marker panels.

3. Panel test design

Disease and trait variant tests. All tests included in the **MyDogDNA**® panel are based on information retrieved from original scientific publications or work, with references indicated in the documentation accompanying each test. The test content is compiled based on extensive review of the publicly available scientific literature through Online Mendelian Inheritance in Animals (OMIA), and PubMed databases (1,3), with regular updates. For an up to date listing of featured content for a breed of interest, please refer to: https://www.mydogdna.com/crm/index.html#en/breeds. Assays directly targeting each mutation of interest were designed for the microarray with the aim to capture as many known canine point mutations, indels, and larger structural genomic rearrangements as possible. In a few rare but clearly stated exceptions, linked marker tests were included where no causal variants were indicated by the original publication, or where technical constraints prevented the use of a direct mutation test.

Genomic markers. The next generation version of the **MyDogDNA**® microarray will feature over 20,000 single nucleotide polymorphism (SNP) markers, carefully selected to cover each of the 39 chromosome pairs in the dog genome with a median intermarker distance ~110 kb. This content can be used for the assessment of genome-wide diversity/inbreeding level, genetic population analyses, genomic selection, and low resolution genome-wide association studies. Markers were selected based on public SNP databases generated as part of the dog genome project (4), offering partial overlap with data produced on the widely used Illumina® CanineHD 172K BeadChip array (5). A particular emphasis was placed on marker selection in the chromosome 12 DLA (dog leukocyte antigen; major histocompatibility complex [MHC]) region for improved coverage of this locus of known functional importance.

4. Test performance and validation

Microarray performance. The performance of the custom **MyDogDNA**® microarray was initially evaluated with a pilot study consisting of 2000 samples representing 50 breeds. A mixed representation of sample type (buccal swab/blood), and sample takers (dog owners, breeders and veterinarians) was sought for. Performance statistics from the pilot study are summarized in Table 1. The overall observed call rate of markers (an indicator of assay and sample quality) was high irrespective of sample type. No significant difference was observed in the call rates between blood and buccal cell samples. We used replicate concordance (the number of times copies of the same assay yield the same result) and test-retest concordance (the number of assays that give the same result in a rerun of the same sample) as additional indicators of test performance. Together, these measurements of within- and across array test result reproducibility indicated successful development of a highly reproducible and reliable genotyping method.

Test validation. The vast majority (72.2%) of all disease tests were validated using samples with known control genotypes. Only tests reaching a sensitivity and specificity of 100% in testing of samples with known genotypes were included in the final testing panel. The **MyDogDNA**® microarray was also successfully subjected to an external blind evaluation by an independent commercial DNA testing laboratory submitting samples with known genotypes for evaluation. Tests for disorders for which control samples were not available (i.e., disorders likely eradicated from the natural dog population subsequently to their discovery, or disorders limited in spread to specific families or lines of dogs) were validated with synthetic oligonucleotides prior to inclusion in the test panel.

Trait tests (e.g., coat color and coat type) were validated through extensive correlation of obtained genotypes with known phenotype information from the same dogs. The current experience in evaluation of trait genotypes and phenotypes of the **MyDogDNA**® team has its foundation in analysis of more than 20,000 dogs from around 350 different breeds.

Table 1. Summary of MyDogDNA® microarray performance data

Median call rate (blood samples)	98.1%
Median call rate (buccal swab samples)	98.2%
Reproducibility (within array)	99.8%
Reproducibility (between arrays)	99.8%
Sensitivity and specificity*	100%

*based on known validation genotypes for 127 different disorders.

5. Quality assurance schemes

The **MyDogDNA**® team is committed to providing only high-quality, reliable test results. Our analyses are run in a certified laboratory facility accredited according to ISO17025/ISO9001 quality management systems, and each set of animal data produced is subjected to stringent routine quality control measures. These actions are summarized in Table 2.

Table 2. MyDogDNA® quality assurance methods

All testing performed according to ISO17025/ISO9001 quality management systems
Certified parentage testing according to ISAG (International Society of Animal Genetics) and AKC (American Kennel Club) standards
Individual tests based on original scientific publications and critically evaluated prior to inclusion in panel
Each test performed in up to eight technical replicates for highest fidelity
Low quality data (<95% sample call rate) fully discarded, sample reanalyzed free of charge
Manual curation and review of all genotype data
Monitoring of sample quality (visual inspection, DNA concentration and purity measurement)
Barcoded sample and data flow, alerts for sample gender inconsistency and breed genetic outliers

6. Key test and reporting features

General. MyDogDNA® test results are reported in a secure online database, where the user has the option to manage ownership and sharing of a dog's result with others. All results are private and confidential unless the owner choses to make them public. Breeder networking is encouraged through additional system features. Key service features in comparison to other test providers are summarized in Table 3.

Disorders. Results for disorders known to be relevant for the breed are separately highlighted to keep reporting evidence-based and with a solid focus in canine genetics research. In addition, results for all tested disease variants are accessible, with their testing enabling novel research discoveries for the breed.

Traits. The main categories of traits included in the **MyDogDNA®** panel are tests for coat color, coat type, body size, and other morphological characteristics (e.g., bobtail and skull shape). Trait results can be explored through interpretation aids such as coat color determination flowcharts and body size graphs enabling comparison to other dogs.

Genetic diversity. The reporting features an indicator of the genome-wide measured diversity level of the tested dog, expressed in the form of heterozygosity (the ratio of measured genetic sites at which the dog has inherited a different allele [gene form] from its dam and sire). Graphical reporting illustrates whether the tested dog has a genetic diversity level higher or lower than the breed median (Figure 1). Moreover, the diversity status of the breed can be compared to other breeds, and all other dogs. A minimum of 30 tested dogs from a breed are required before breed-specific statistics and graphs can be displayed.

Genetic relationships. The population structure of any breed of interest is visualized in an interactive multi-dimensional scaling (MDS) plot in three dimensions (Figure 2). This illustrates the similarity of the tested dog to other individuals of the same breed. The graphs also reveal potential geographical or lineage differentiation (e.g., show vs. hunting line separation) within the breed. The average genetic distance between breeds is also computed, allowing comparisons between breeds of putative shared ancestry.

Breeder tool. The **MyDogDNA®** database features a unique canine match-making and breeder networking tool. Although DNA-based information is no replacement for other selection criteria (e.g., clinical health examinations and information, field trials, behavior and character, conformation), it can be used as an additional supportive tool to preserve breed genetic diversity levels. The **MyDogDNA®** Breeder Tool ranks potential mates for a given dog in order of preference based on two main criteria: expected genome-wide diversity level of offspring from the mating, and compatibility in terms of tested disease variants. Taken together, this allows for the maintenance of breed genetic diversity while safely keeping carriers of recessive disorders in the gene pool.

Table 3. MyDogDNA® testing service key features

	MyDogDNA® (Optimal Selection in the US)	Other panel test/ combination product services
Disease/trait data		
Total genetic markers	5,500 carefully selected markers for optimal cost-benefit ratio	~2 - 200,000 markers
Genetic disease tests	150+ carefully validated and critically evaluated tests	~2 - 160 tests; test validation information not disclosed
Trait tests	30+ variants with a single analysis	<15 variants
Coat color	16 alleles	5 - 15 alleles
Coat length	5 variants	single FGF5 gene variant only
Body size	7 variants	5 variants
Results accepted by	Yes; e.g. The Kennel Club (UK) and other European kennel organizations, OFA (Orthopedic Foundation for Animals), Finnish National Kennel Club. Contact us for requests to establish result transfer cooperation agreement.	Yes/No. Refer to service provider to request test license and patent information.
Upgrades to test results	Yes; some tests possible to report on free-of-charge in retrospect after completed validation. Upgrades to newest panel test version offered at pricing less than a single gene test to ensure affordability of up to date results.	Yes/No. Dependent on marker inclusion in originally performed analysis.
Diversity and population data		
DNA database	Yes; interactive, real-time updated graphs and disease/trait carrier frequency statistics	No.
Diversity statistics	Yes; baseline data based on more than 20,000 dogs from 350 breeds	Yes; information on baseline data not disclosed
Population structure	Interactive visualization of breed lineage and genetic relationships	No.
Breeder tool	Yes; updated global breeder networking interface	Custom trial mating
Other		

	MyDogDNA® (Optimal Selection in the US)	Other panel test/ combination product services
Scientific contributions	Yes; peer-reviewed scientific publications based on panel testing. Research protocol for all novel findings.	No.
DNA profiling and parentage	Yes; ISAG (International Society of Animal Genetics) and AKC (American Kennel Club) standards as add-on service	No
Turnaround time	3 - 4 weeks to results	6 - 8 weeks to results
Pricing	99 €	~150 - 200€

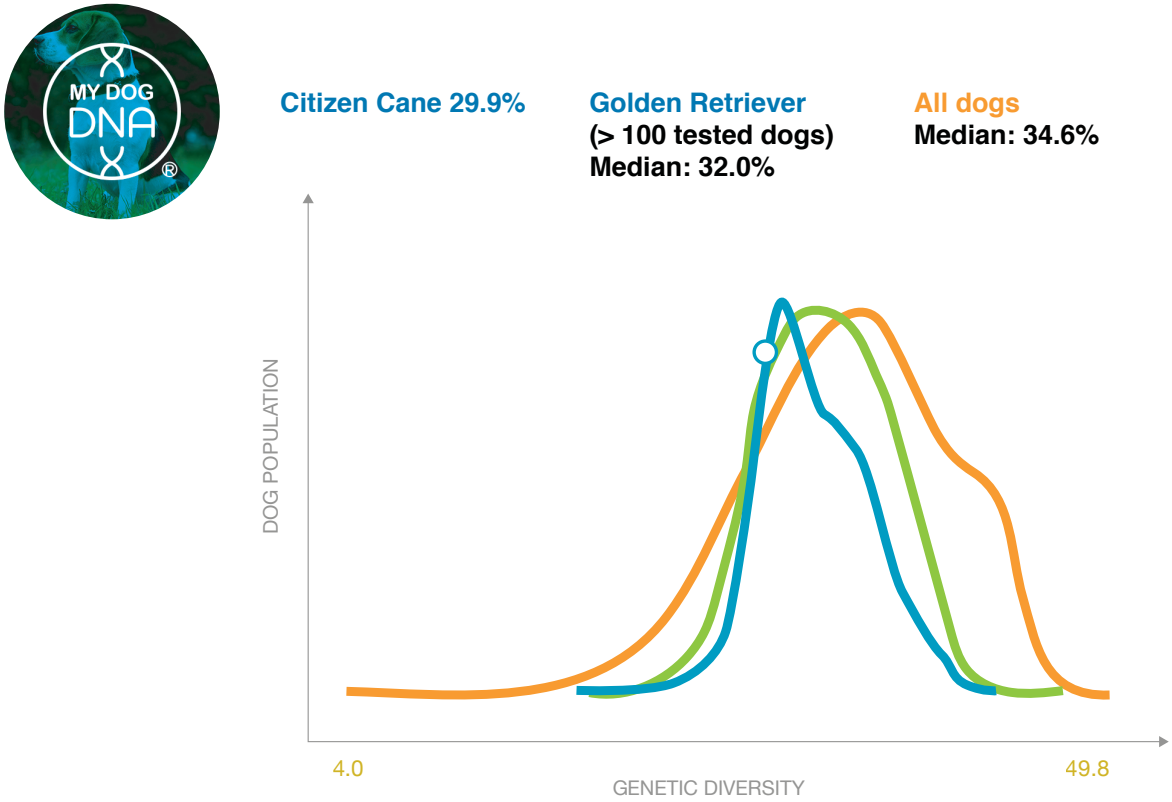

Figure 1. Diversity reporting indicating the genetic inbreeding level of the tested dog and its breed.

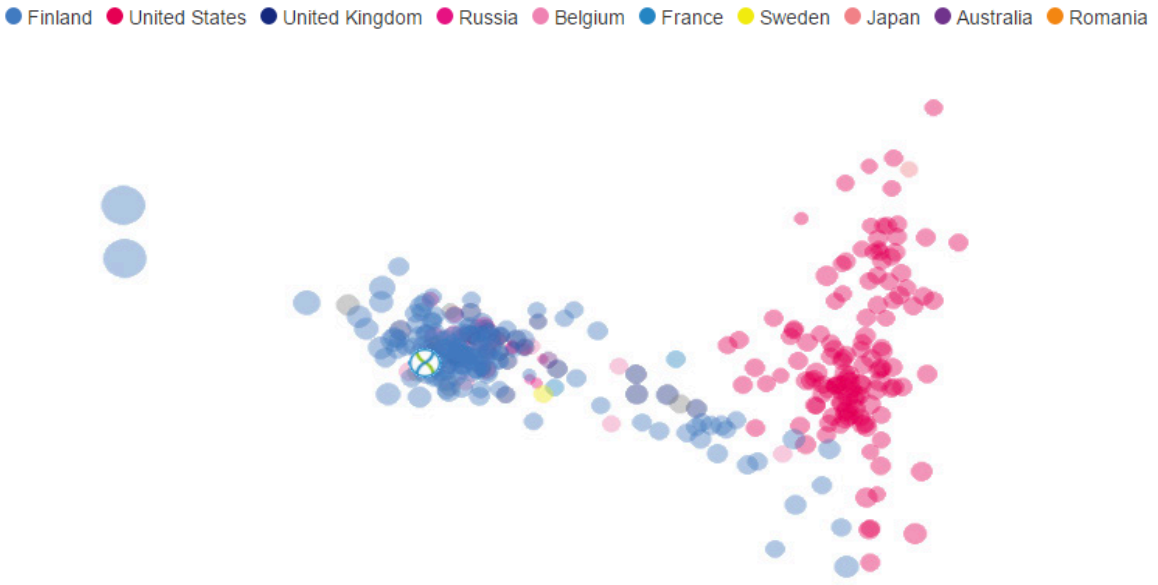

Figure 2. Interactive real-time updated visualization of breed population structure.

7. Partnerships and research contributions

Key partners. The **MyDogDNA**® team is committed to improving the health of dogs worldwide, and aspires to be an active member of the dog community. We are a proud sponsor and leadership partner of the Harmonization of Genetic Testing for Dogs Initiative launched by the International Partnership for Dogs (IPFD; 6). Our US customers are served through the **Optimal Selection™** product, the US equivalent of **MyDogDNA®**, in partnership with Mars Veterinary (7). Mars Veterinary is the holder of the exclusive worldwide license for genetic dog breed detection, and the only commercial US license for MDR1 drug sensitivity screening. We have further partnered with OptiGen (8) to provide our customers with certified test results for key tests such as prcd-PRA and collie eye anomaly (CEA). Our main academic research collaborator is the canine genetics research group at the University of Helsinki, Finland (9). Protocols for test result acceptance and transfer have been, and are being, established with organizations such as the Kennel Club (UK), OFA (Orthopedic Foundation for Animals), and Finnish National Kennel Club.

Original scientific publications. The **MyDogDNA**® team regularly makes novel research discoveries, and publishes its original work in peer-reviewed journals. Whenever a disease variant is found in a breed in which it was not previously known to exist, we follow up on the finding by confirming it with a second genetic technology and pursuing clinical studies to establish the relevance of the finding for the breed. We have published on the feasibility and applications of the panel testing approach (10) in a study honored as an OMIA landmark article. Moreover, we have explored canine disease variant prevalence and distribution in the largest canine sample ever directly examined for inherited disorders (152 genetic disease variants screened in 100,000 dogs; 11). Our data also enables focused studies on the population genetics of any breed, such as our study on genetic diversity in Finnish and Nordic Spitzes (12). Finally, we are actively involved in research projects aiming to elucidate the genetic background of complex disorders such as hip dysplasia, dilated cardiomyopathy, obsessive-compulsive disorder, and adverse drug effects.

8. References

(1) Online Mendelian Inheritance in Animals, OMIA. Faculty of Veterinary Science, University of Sydney (*http://omia.angis.org.au/home/*)

(2) Illumina, Inc. (*http://www.illumina.com/*)

(3) US National Library of Medicine, National Institutes of Health. PubMed. (*http://www.ncbi.nlm.nih.gov/pubmed/*)

(4) Lindblad-Toh K et al., Nature 2005; 438(7069):803-819. Genome sequence, comparative analysis and haplotype structure of the domestic dog.

(5) Illumina, Inc. Data Sheet DNA Genotyping/CanineHD BeadChip: (*https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/data-sheet_caninehd.pdf*)

(6) International Partnership for Dogs (*https://dogwellnet.com/*)

(7) Mars Veterinary (*http://www.wisdompanel.com/*)

(8) OptiGen (*http://optigen.com/index.html*)

(9) Canine genetics research at University of Helsinki / Folkhälsan Research Center (*https://www.koirangeenit.fi/english/*)

(10) Donner J et al., PLoS ONE 2016; 11(8): e0161005. Genetic Panel Screening of Nearly 100 Mutations Reveals New Insights into the Breed Distribution of Risk Variants for Canine Hereditary Disorders. (*http://dx.doi.org/10.1371/journal.pone.0161005*)

(11) Donner J et al. Frequency and Distribution of 152 Genetic Disease Variants in over 100,000 Mixed Breed and Purebred Dogs. Manuscript in preparation.

(12) Kumpulainen et al., J Anim Breed Genet 2017. Founder representation and effective population size in old versus young breeds - genetic diversity of Finnish and Nordic Spitz. DOI: 10.1111/jbg.12262.