Genetic Diversity Testing for Standard Poodles

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers that will determine genetic diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions. This test panel will be useful to Standard Poodle breeders who wish to track and increase genetic diversity of the Standard Poodle breed as a long term goal.

For **other breeds**, please see Enrolling a Breed

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across the genome were used to gauge genetic diversity within an individual and across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity, and breed-wide allele frequency is provided.

DLA haplotypes: STR loci linked to the DLA class I and II genes were used to identify genetic differences in regions regulating immune responses and self/non-self recognition. Problems with self/non-self recognition, along with non-genetic factors in the environment, are responsible for autoimmune disease.

Internal Relatedness: The IR value is a measure of genetic diversity within an individual that takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog and cannot be compared between dogs. Two dogs may have identical IR values but with very different genetic makeups.

Introduction

The genetic information used to formulate the enclosed tables and graphs came from DNA samples of 782 dogs from North America, the UK, and Continental Europe. Therefore, we believe that these dogs represent almost all of the genetic diversity that still exists within the breed. This data will be updated as more dogs are tested, so allele and DLA haplotype frequencies may change to a limited extent over time. The breed appears to have reasonable breed-wide diversity, but this diversity is very unbalanced. As a result of genetic bottlenecks traced back to the mid-twentieth century and certain lines, a majority of Standard Poodles are relatively inbred and contain a minority of the existing genetic diversity. This has resulted in an

increased incidence of heritable traits, including characterized simple recessive disorders such as PRA, Von Willebrand's disease, and neonatal encephalopathy; possible recessive disorders such as juvenile renal disease, juvenile cataracts, and enamel dysplasia; and more complex genetic disorders such as autoimmune disease (e.g., SA, AD, IMHA, ITP, thyroiditis, chronic active hepatitis, masticatory myositis), allergies, hip dysplasia, elbow dysplasia, atrial septal defect, patent ductus arteriosus, degenerative myelopathy, and bloat. These various disorders appear to have resulted from both ancient and relatively new mutations that have been concentrated in certain lines as a result of inbreeding. The hope is that breeders will use genetic diversity testing, along with pedigrees, to re-establish genetic diversity across the breed by careful mate selection, while continuing to investigate diseases that appear to have a genetic basis.

The Canine Genetic Diversity Test

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The test panel contains 33 genomic STRs, 20 of which are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG), and additional markers developed by the VGL. The power of this panel to distinguish relatedness was confirmed by testing dogs that had been found to be unrelated using an Illumina 170K SNP and known sibling pairs (Fig. 1).

The diversity of alleles at each of the genomic STR loci and their frequency in the population were used to calculate the internal relatedness (IR) of each dog and for the Standard Poodle population as a whole (Fig. 1). IR is a measure of heterozygosity contributed by each parent. The lower the IR score, the more outbred the individual, and the higher the score, the more inbred. Internal relatedness calculated for over 782 Standard Poodles from North America, UK and Continental Europe ranged from <-0.2 (most outbred) to >0.4 (most inbred) (Fig. 2).

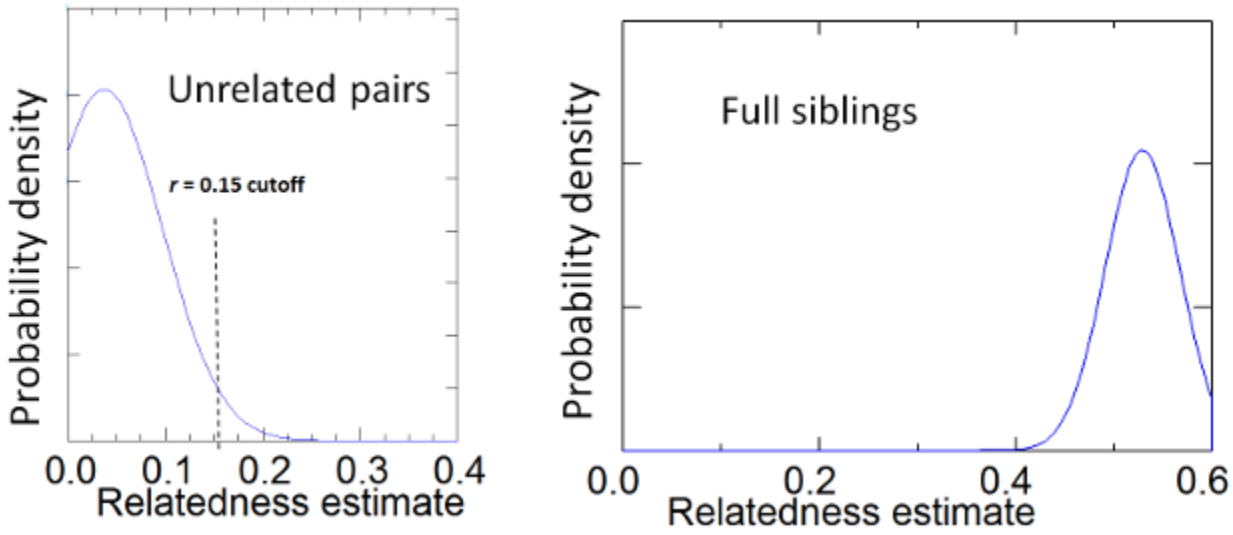


Figure 1. Pairs of unrelated dogs ($r \leq 0.15$) were identified using 170K SNP arrays. Known full-sibling pairs were also included for comparison. All of the dogs were then tested with 33 genomic STR loci. The STR panel was able to accurately identify 95% of unrelated dogs with a conservative relatedness value of $r < 0.15$. Full siblings were also accurately identified.

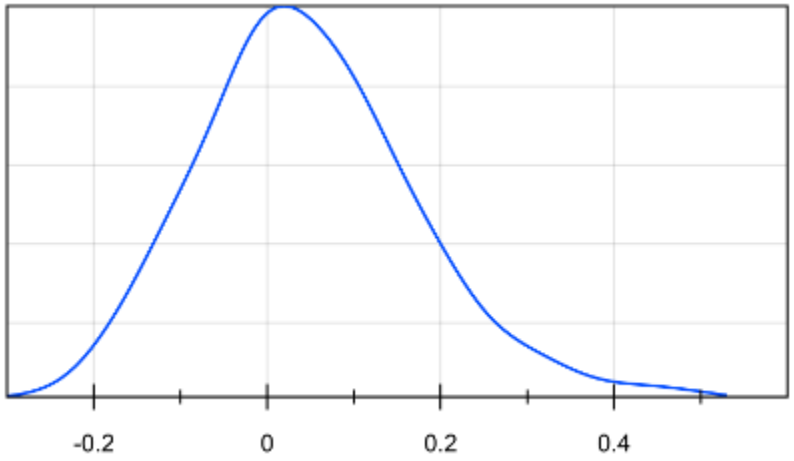


Figure 2. Distribution of internal relatedness scores for 782 Standard Poodles. There is a wide range of diversity across the breed and the goal for breeders should be to produce a greater and greater proportion of puppies with IR scores less than 0.

The DLA Haplotype

In addition to the markers used to estimate relatedness, which reflect genome-wide diversity, a set of STRs associated with specific genes in the DLA region, which contains the canine Major Histocompatibility Complex, can be used as proxy to represent gene diversity associated with immune function (see sidebar). We have identified 45 distinct DLA Class I, 29 distinct DLA Class II haplotypes (combinations of alleles from STRs near each other on a chromosome) in Standard Poodles (Tables 1 and 2). These STR-based haplotypes are strongly associated with known functional haplotypes that have been determined by sequencing of DLA-88, DRB1, DQB1, and DQA1 genes. DLA-Class I and Class II STR-based haplotype frequencies in Standard Poodles are provided in the Tables 1 and 2.

Dog DLA and STR haplotype diversity.

The DLA consists of four gene rich regions making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases. The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with the DLA88 are linked together in various combinations, forming specific haplotypes (Table 1). Groups of genes and their alleles that are inherited as a block, rather than singly, are called haplotypes. The class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at STR loci associated with each of the three Class II genes are strongly linked and also inherited as a single block or haplotype (Table 2). One haplotype comes

from each of the parents. The linkages between alleles within Class I or II regions are very strong; while linkages between regions of the DLA that are more distant from each other, such as Class I and II, are weaker. There are almost two million base pairs separating the class I and II regions, thus allowing for some genetic recombination to occur. This recombination is most apparent between the common DLA class I and II haplotypes, forming unique "extended DLA class I-II haplotypes. Extended class I-II haplotypes are inherited as a single block of genes.

Tables 1 & 2: DLA Class I & II Haplotype Frequencies in Standard Poodles

DLA Class I Haplotype Frequencies (Updated Oct 10, 2019)

DLA1 #	STR types	Poodle (n=2822)
1001	380 373 281 182	0.2675
1002	380 365 281 181	0.1763
1003	387 375 277 186	0.1696
1004	393 379 277 183	0.0852
1005	389 371 277 181	0.0609
1006	387 375 293 180	0.0462
1007	380 372 281 182	0.0324
1008	386 373 289 182	0.0172
1009	382 377 277 184	0.0161
1010	384 371 277 186	0.0120
1011	376 365 281 180	0.0188
1012	388 369 289 188	0.0094
1013	392 373 289 186	0.0113
1014	375 373 287 178	0.0096
1015	380 373 291 186	0.0025
1016	382 371 277 178	0.0188
1017	386 373 289 178	0.0034
1018	375 373 287 186	0.0082
1019	380 373 287 185	0.0028
1020	388 369 289 184	0.0051
1021	380 373 289 186	0.0028
1022	380 375 281 181	0.0002
1023	380 379 281 181	0.0002
1024	387 373 281 182	0.0002
1025	380 365 281 186	0.0004
1026	390 369 289 186	0.0009

1027	391 371 277 181	0.0009
1028	376 369 291 186	0.0012
1029	380 365 281 182	0.0023
1030	380 373 293 178	0.0027
1031	382 371 277 186	0.0016
1032	382 377 277 178	0.0002
1033	382 379 277 181	0.0016
1034	382 379 277 182	0.0002
1035	386 373 277 184	0.0002
1036	389 365 289 180	0.0025
1040	380 371 277 186	0.0005
1043	393 381 277 183	0.0019
1045	376 371 277 186	0.0011
1046	376 379 291 180	0.0004
1053	382 377 277 186	0.0004
1092	376 379 277 181	0.0002
1102	389 375 293 180	0.0002
1103	389 375 293 181	0.0004
1105	382 379 277 178	0.0018
1141	380 365 281 180	0.0004
1169	380 365 277 180	0.0009
1220	387 375 277 178	0.0002
1225	387 374 287 186	0.0002
null		0.0004

DLA Class II Haplotype Frequencies (Updated Oct 10, 2019)

DLA2 #	STR types	Poodle (n=2822)
2001	343 324 284	0.6091
2002	343 327 280	0.0870
2003	343 324 282	0.0921
2004	351 327 268	0.0294
2005	339 322 280	0.0206
2006	339 325 280	0.0324
2007	351 327 280	0.0159
2008	339 327 276	0.0161
2009	351 324 280	0.0103

2010	345 329 280	0.0120
2011	345 322 284	0.0190
2012	345 322 280	0.0050
2013	345 327 284	0.0096
2014	339 322 284	0.0177
2015	339 327 280	0.0064
2016	339 323 284	0.0028
2017	343 322 280	0.0028
2019	345 324 284	0.0002
2020	349 324 284	0.0004
2021	339 324 268	0.0021
2022	339 327 282	0.0002
2023	341 323 282	0.0027
2024	343 323 280	0.0002
2025	351 321 280	0.0025
2026	351 324 284	0.0012
2028	345 327 288	0.0005
2035	341 323 280	0.0002
2039	345 327 276	0.0011
2101	341 324 280	0.0004
2115	343 327 284	0.0002

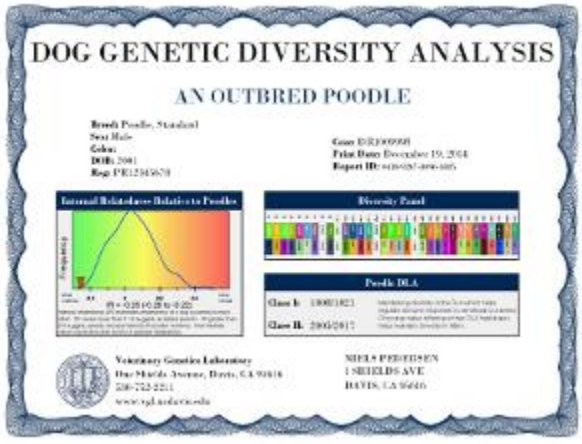
Table 2b: Associated DRB1/DQA1/DQB1 haplotypes

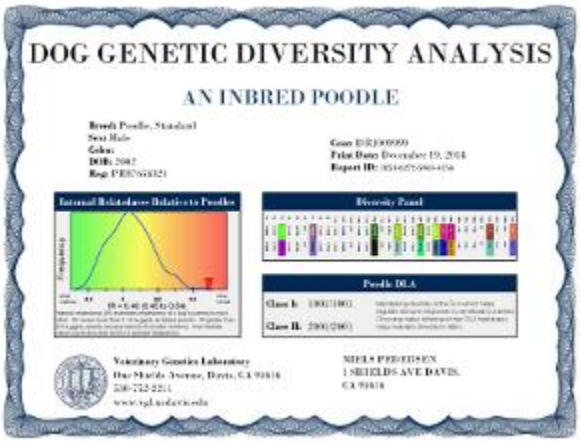
VGL #	Associated DRB1/DQA1/DQB1 haplotypes
2001	01501/00601/02301
2002	01501/00901/00101
2003	01503/00601/02301
2004	02001/00401/01303
2005	00101/00101/00201 00901/00101/008011
2006	01502/00601/02301
2007	02001/00401/01303
2008	00101/00101/03601
2010	01201/00401/013017

2011	00901/00101/008011
2012	00901/00101/008011
2013	00201/00901/00101
2016	00601/05011/00701
2018	00601/05011/00701

Table 2 demonstrates the strong relationship between the STR-associated DLA class II haplotypes and the official international DLA class II designations for alleles within the DRB1, DQA1, and DQB1 genes. One (or more) STR haplotypes is associated with each of the official DRB1/DQA1/DQB1 haplotypes identified in Standard Poodle. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking with the first haplotypes being identified being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class II haplotypes. It is not unusual for various dog breeds to share common and even rare haplotypes, depending on common ancestry. Therefore, identical haplotypes in other breeds are assigned the same number. The numerical nomenclature used by VGL for DLA class I and II haplotypes does not correlate with numerical rankings used by others.

After a sample is submitted for genetic testing, the identity of the dog and owner will be replaced by a laboratory barcode identifier. This identifier will be used for all subsequent activities. After testing, each owner will be provided with a certificate that reports the internal relatedness, genomic STR genotypes and DLA class I and II haplotypes for the dog(s) tested. The internal relatedness value for the dog being tested is related to the population as a whole.





The goal for breeders should be to produce a greater and greater proportion of puppies with IR scores less than 0, and with time even lower scores. There appears to be ample genetic diversity in the breed to achieve this goal over a number of generations. This will require using different combinations of breeding stock, including even those from inbred lines with high IR values. IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically

different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

Potential sires and dams should be first screened for genetic differences in the genome and in the DLA regions by first comparing allele differences at each STR locus, and then at the DLA class I and II haplotypes. Some thought should be given to rare vs common alleles. This information is included on all certificates and on the website. This preliminary comparison will identify promising pairings and if desired, genetic information on the potential sires and dams can then be used to calculate actual IR expectations for their puppies. Puppies, once born, should be tested for their actual IR values, which will reflect the actual genetic impact of each parent on internal diversity. Considerations of mate choices for genetic diversity should be balanced with other breeding goals, but improving genetic diversity in puppies should be paramount.

What are the genetic relationships between Standard Poodles from Europe, USA and Canada, and with Miniature Poodles and Standard Poodle/Miniature Poodle crosses?

This study of genetic diversity in Standard Poodles attempted to identify new diversity from North America and Europe. One way to view this diversity is by PCA plots using allele frequencies from the 33 genomic STRs (Figs. 3, 4). Individual dogs from various regions and types are plotted in three dimensions (coordinates), but displayed in two dimensions. Each symbol represents a dog from the study. The closer two individuals position themselves on the plot the more related they are and the more distant, the less related. It is apparent that Standard Poodles, regardless of their geographic origins are interrelated, with the bulk of the population centered around and to the left of the central axis where coordinates 1 and 2 cross (Fig. 3). Genetic outliers are found at various points in the periphery of this central mass of closely related dogs, but more so to the right of center. The more distant the outliers are to the bulk of the population, the more genetically distinct they are from the majority of Standard Poodles. Outliers are equally present in USA, Canadian and European populations. Miniature Poodles comprise a separate population to the right of the axis and represent a related but yet distinct population from Standard Poodles (i.e., distantly related). The Standard Poodle/Miniature Poodles, as might be expected, form their own distinct population that overlaps and is just below the Miniature Poodles. The greatest difference in genetic diversity is between Miniature and Standard Poodles, and Standard Poodle/Miniature Poodle crosses and Standard Poodles. However, there are a small number of Standard Poodles that are equally distant outliers.

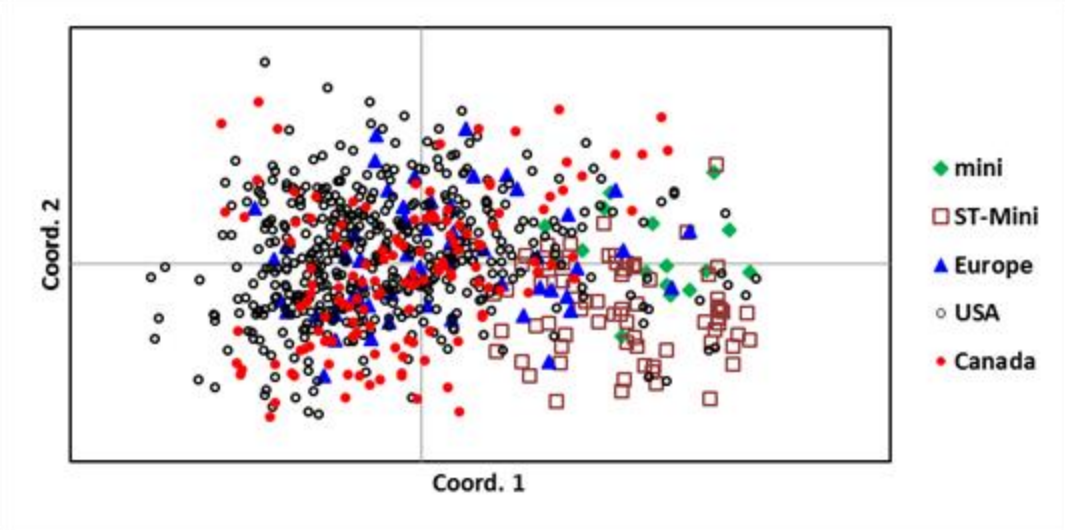


Figure 3. A principal component analysis (PCA) plot showing the genetic relationships of Standard Poodles from the USA (n=483), Europe (n=57), Canada (n=139) , Miniature Poodles (n=16) and Standard Poodle/Miniature Poodle crosses (n=72).

How do healthy Standard Poodles from the USA relate to dogs with Addison's disease (AD) or Sebaceous adenitis (SA).

A PCA plot was done with healthy and diseased Standard Poodles only from the USA. Miniature Poodles and crosses were removed. Once again, it is apparent that most Standard Poodles from the US form a cluster near the center of the plot, with outliers of less genetic relationship extending in various directions, but mainly to the right. Standard Poodles suffering from AD and SA cluster only with the closely related dogs in the center of the plot and to a much less extent with genetic outliers. This is one proof that inbreeding for desired conformational traits has inadvertently allowed for positive selection of the genetic traits responsible for AD and SA. It also indicates the need to increase genetic diversity in the breed.

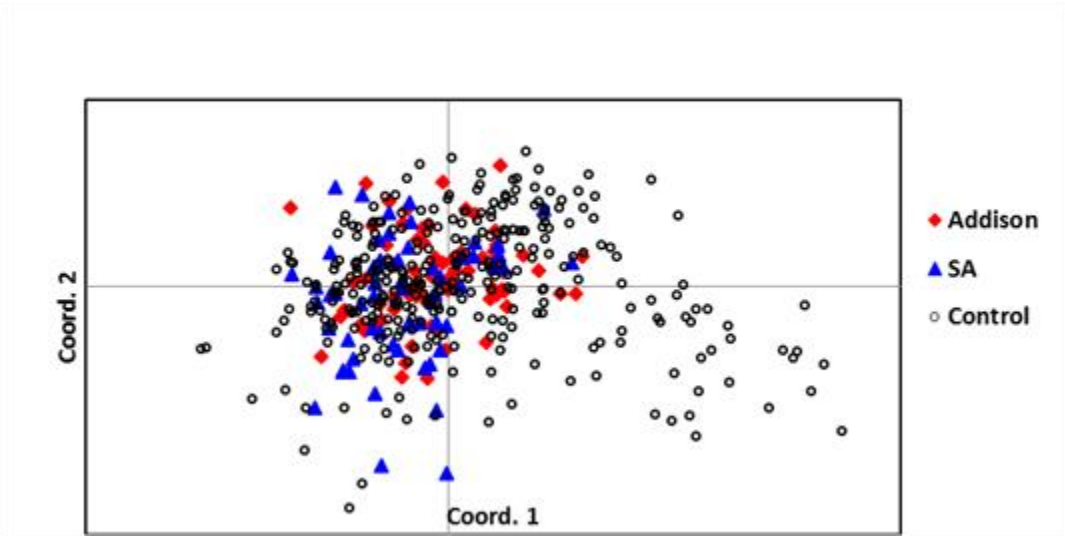


Figure 4. PCA of Standard Poodles from USA showing genetic similarities between healthy dogs, and dogs with Addison's disease (n=75) and SA (n=61). No Miniature Poodles are Standard Poodle/Miniature Poodle crosses were included in this study.