

Genetic Diversity Testing for Miniature 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 within the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions. This test panel will be useful to Miniature Poodle breeders who wish to determine the amount of genetic diversity that exists in the breed and to compare that diversity with that of Standard Poodles.

Breeders and owners have submitted enough DNA samples from as many as 242 Miniature Poodles to create an accurate and complete baseline for genetic diversity in the breed. However, given the high amount of genetic diversity that has been determined so far, it is possible that even more diversity still exists. However, the incidence of new autosomal STR alleles and DLA class I and II haplotypes will probably be very low, and if identified, will be added to the current tables and figures.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across 25 of 39 (panel 1) or 58 of 39 (panel 2) chromosomes 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: Seven additional 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. IR values can be adjusted using data from highly random bred village dogs from across the Middle East, SE Asia and Island Pacific nations. Adjusted IR values, known as IRVD, are estimates of the amount of genetic diversity that has been lost over the entire period of breed evolution from when breed ancestors left this region thousands of years ago, over the several hundred years during which the proto-breed (Poodles) evolved.

I. Introduction

A. Breed History

The history of the Miniature Poodle is intimately tied up with the history of the Standard Poodle, which goes back six centuries or more. Although it might be presumed that the Standard Poodle came first, miniaturization of dogs has gone on for thousands of years and it is just as likely that various sizes of Poodles evolved during the same time according to human needs. Standard Poodles would be used as hunting and working dogs, while miniature and toy varieties were used primarily for companionship.

The Standard Poodle breed probably originated as a water fowl retriever in Germany rather than France. The name “Poodle” is the same as the German “pudel”, which means refers to splashing in water (i.e., water retrieving). Others believe that the Poodle is a descendent of the French Barbet and has been proclaimed the national dog of France. Regardless of its origin, the Poodle rapidly became one of the most popular dogs in Continental Europe with paintings of dogs identical to Poodles originating in Germany as early as the 15th to 16th century. The Standard Poodle was the principal pet dog in Spain in the 18th century and Toy Poodles were found in many royal households in France during the same period.

The poodle has been bred in several sizes. Standard, Miniature, and Toy Poodles are recognized by the American Kennel Club. The British Kennel Club also recognizes three sizes, with the miniature and toy being merely smaller versions of the standard. The Fédération Cynologique Internationale (FCI) recognizes the three sizes but adds in the medium Poodle. The fact that the various sizes of Poodles are viewed as varieties and not breeds, crosses between the various sizes is allowed. However, genetic testing of Miniature Poodles from North America showed them to be a distinct breed compared to Standard Poodles. However, the genetic imprint of Standard Poodles is evident from shared genomic alleles and DLA class I and II haplotypes. This research can be openly accessed at: <http://cgjournal.biomedcentral.com/articles/10.1186/s40575-015-0026-5>, and http://www.vetmed.ucdavis.edu/ccah/local-assets/pdfs/Miniature_Poodle_genetic_comparison_May-23-2012.pdf. Although breeders are free to cross Miniature and Standard Poodles, there has been reluctance for breeders to do so. However, there are some breeders who believe that genetic diversity, and therefore health problems, could be improved by increased cross-breeding of Standard and Miniature Poodles.

The Standard Poodle sets the size for the three (or four) different varieties of Poodles. Most kennel clubs state that an adult Standard Poodle must be over 15 inches (38 cm) at the shoulder, while the FIC sets the size for standards at 18-24 inches (45-60 cm). The adult Miniature Poodle must be 11-15 inches (28-38 cm) at the shoulder in registries other than the FIC, where the size range is 11-14 inches (28-35 cm). The slightly smaller size range for Miniature Poodles and higher size range for Standard Poodles in the FIC is to allow for the Medium (Moyen) variety at 14-18 inches (35-45 cm).

Miniature Poodles are said to be dainty but athletic, lively and playful, bright and attentive, easily trainable, and good with strangers and other animals. They also have a curly and low-shedding coat for dog allergy sufferers. However, there are lines of Miniature Poodles, as with

other breeds, that are timid and neurotic, more sensitive to stresses, and more vocal. Some of these negative traits can be overcome with proper socialization and training, but most are inherent in the individual or line.

II. Baseline genetic diversity testing and what it tells us about Miniature Poodles

A. Allele frequencies in Miniature Poodles at select autosomal STR loci

Autosomal STR loci are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The VGL has been experimenting with two different STR marker panels. Panel 1 has markers on 25/39 canine chromosomes and panel 2 has markers on 39/39 chromosomes. Twenty five of the 33 STR markers on panel 1 are also found on panel 2. Twenty markers on each panel are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG). The panel of 58 genomic markers covers all 39 canine chromosomes instead of the 25 chromosomes of the 33-marker panel. The test panel used for baseline genetic testing of Miniature Poodles used two different panels, panel 1 (110 dogs) with 33 STR markers and panel 2 with 58 STR markers (99/110 dogs). Testing has shown that the 33-marker panel gives results that are very close to the results from the 58-marker panel in the various genetic assessments (Fig 1). Therefore, most of the genetic assessments will be from the original 33 [genomic STR loci](#) (Table 1).

B. The use of genomic allele frequencies to determine breed for a breed-wide standard genetic assessment of individual heterozygosity

The diversity of alleles at each STR and their frequency in a population can be used to make several genetic assessments such as principal coordinate analysis (PCoA), genetic assessment indices (average alleles/locus, average effective alleles/locus, observed heterozygosity, expected heterozygosity, index of inbreeding [F], internal relatedness (IR), and adjusted IR (IRVD)). The first 33 STR markers listed were used in the original panel. Genomic STR loci and their frequencies for Standard and Miniature Poodles can be found at: [genomic STR loci](#) (Table 1).

Table 1: STR alleles from 33 genomic loci and their frequencies in different populations

[\(link to table 1\)](#)

The standard genetic assessments such as those first proposed by Wright can be determined from alleles and their frequencies at each STR locus in the genome (Table 1). These measurements include average # alleles/locus (N_a), average # effective alleles/locus (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e) and a coefficient of inbreeding (F) (Table 2). The average number of alleles/loci (N_a) for the Miniature Poodles was 7.58, which is higher than other pure breeds, but not as high as for the parent breed (N_a Standard Poodle = 8.91). The average number of alleles/locus (N_e) that contributed most to heterozygosity was 4.06, again higher than many pure breeds that have been studied to this point, and even higher than for Standard Poodle ($N_e = 3.49$). The observed and expected heterozygosity ($H_o = 0.712$ and $H_e = 0.720$) were also higher than most other breeds. The values for H_o and H_e were not

significantly different from each other, creating a breed-wide coefficient of inbreeding (F) close to zero (0.010). Therefore, standard genetic assessment values indicate that Miniature Poodles have a relatively high genetic diversity and that breeders are doing a good job of maintaining Hardy-Weinberg equilibrium (random breeding). ST/Miniature Poodle crosses were, as expected, not as heterogeneous as Miniature Poodles because they are a smaller population and their F value was negative as expected for an outcrossed population.

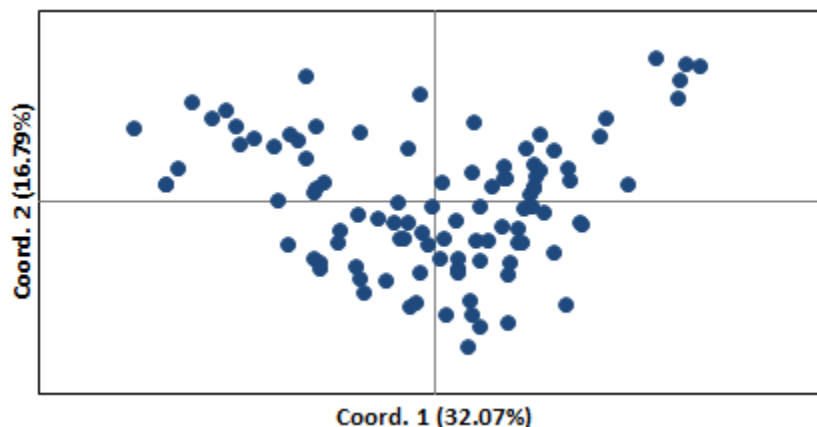
Therefore, Miniature Poodles were genetically more heterogeneous than Standard Poodles. The average effective alleles per locus was higher for the Standard/Miniature Poodles than for Standard Poodles (3.76 vs 3.49) and the F value was -0.058 vs 0.038 demonstrating that the crosses were more outbred than the population as a whole.

Table 2. F-Statistics comparison among Miniature, Standard Poodles, and Miniature-Standard cross based on 33 STR markers

Pop		N	Na	Ne	Ho	He	F
ST	Mean	898	8.909	3.489	0.654	0.680	0.038
	SE		0.473	0.210	0.019	0.019	0.005
ST-Mini	Mean	57	6.879	3.756	0.752	0.709	-0.058
	SE		0.285	0.195	0.021	0.016	0.012
Mini	Mean	110	7.576	4.057	0.712	0.720	0.010
	SE		0.477	0.263	0.018	0.018	0.009

C. Differences in population structure as determined by principal coordinate analysis (PCoA)

Figure 1 shows PCoA results for the same population of Miniature Poodles using allele frequency data obtained from either the 33 or 58 STR marker panels. Both panels provide comparable results.



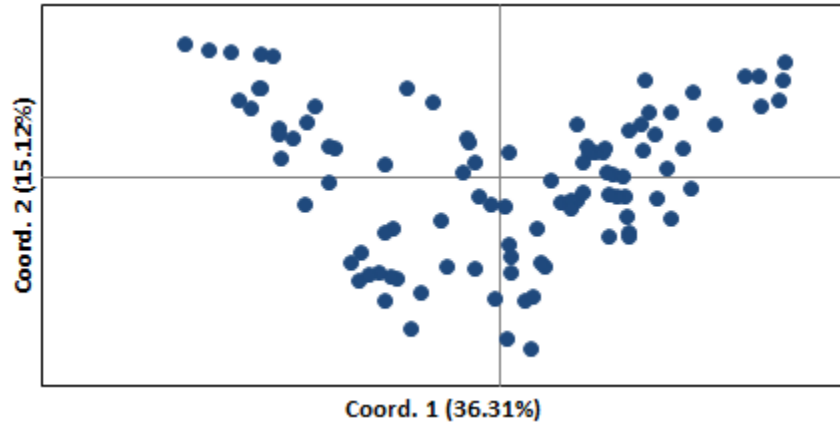


Figure 1. A comparison of PCoA of Miniature Poodles using a 33 vs 58 STR loci panel; the 33 STR loci are found on 25/39 canine chromosomes (top plot), whereas the 58 STR loci panel has markers on 39/39 chromosomes (bottom plot). The results are comparable.

Principal coordinate analysis can also be used to determine how two populations have genetically differentiated from each other. Figure 2 shows a PCoA plot of 110 Miniature, 898 Standard and 57 Standard/Miniature Poodle crosses tested using 33 genome-wide STR markers. The two varieties of Poodles are clearly related, given their proximity to each other on the plot, but are genetically distinguishable. Only three dogs registered as a Standard Poodle were found among the Miniature Poodle population. The Standard/Miniature Poodle crosses and several registered Standard Poodles bridged the two populations, as expected. Registered Standard Poodles that segregated with the crosses may well have had both varieties at some point in their pedigrees, but such ancestry was not divulged.

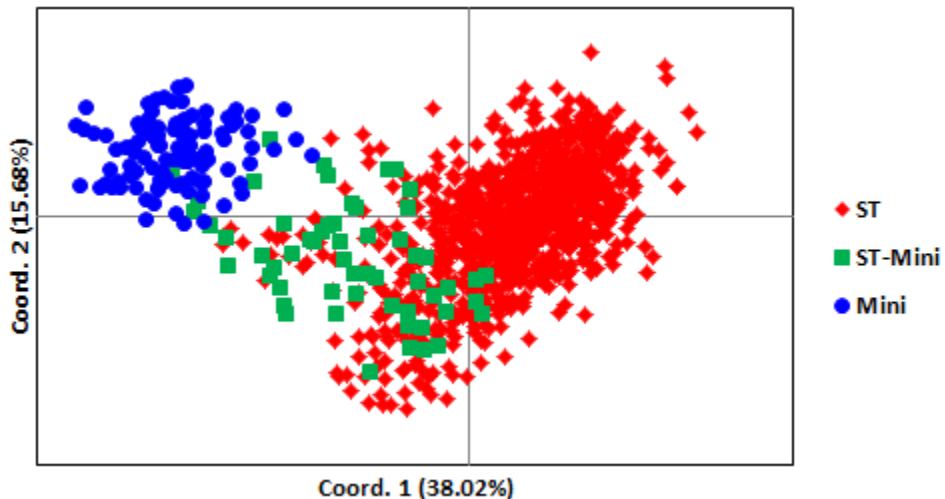


Figure 2. PCoA plot of Miniature Poodles (n=110), Standard Poodles (n=898) and Standard-Miniature cross (n=57) based on 33 STRs.

III. The use of genomic STR loci allele frequencies to determine internal relatedness (IR)

A. Internal relatedness (IR) of individuals and the population as a whole

The genetic assessments given in Table 1 refer to the population, and not to individual dogs. Moreover, assessments based on allele frequency alone do not weight the contributions of common vs rare or uncommon alleles to genetic diversity. Internal Relatedness (IR) is a calculation that gives more weight to rare and uncommon alleles and has been often used as a measure of the genetic differences of an individual's parents. IR values are therefore 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. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with a value of -1.0 would come from parents that were totally unrelated at every locus, while dogs with an IR value of +1.0 have parents that were genetically identical at every locus.

The mean of internal relatedness calculated for 110 Miniature Poodles from North America and other parts of the world was 0.038, with individuals ranging from -.176 to +0.389 (Fig. 3). IR values as high as +0.389 were uncommon and most of the breeds had values below +0.20. A value of +0.25 would apply to offspring of parents that were full siblings, provided that the parents of the full siblings were randomly bred. IR values >0.25 occur when the parents of the full sibling parents were themselves highly inbred.

Although standard genetic assessment values indicate that Miniature Poodles are being randomly bred, the IR values suggest that one-fourth of Miniature Poodles have IR values ranging from 0.10 to 0.389. The effect of these more inbred dogs is counteracted by an equal population of dogs with IR values below -0.10 to -0.176. This leaves one-half of the population with IR values from -0.10 to +0.10, which indicate a reasonable degree of selection for the least related parents.

B. IRVD values as a measure of genetic diversity lost during the entire period of breed evolution from earliest ancestors to present

The IR values can be adjusted in such a way as to provide an estimate of total genetic diversity lost from the earliest ancestors of the breed to present time. This is done by using allele frequencies obtained from DNA of present-day village dogs from the Middle East, SE Asia and Island Pacific nations. These dogs are the most random bred and genetically diverse population that has been studied to date and is the origin of almost all modern breeds. The adjusted IR value is known as IR-village dogs or IRVD.

The IRVD values for Miniature Poodles are shown in Fig. 3 (blue line). The mean IRVD was 0.149 for the population as a whole, with individuals ranging from -0.085 to 0.525 (Fig. 3). The shift to the right in IRVD values was not nearly as pronounced as it has been for several other breeds that are participating in genetic diversity testing at the VGL and indicates that Miniature Poodles have retained a greater amount of the overall diversity still present in village dogs. An estimate of the amount of diversity in dogdom that still exists in Miniature Poodles can be made

by comparing the blackened area under the two curves with the area of the blue curve. This equals 51%, which is among the highest amount of retained diversity that we have observed.

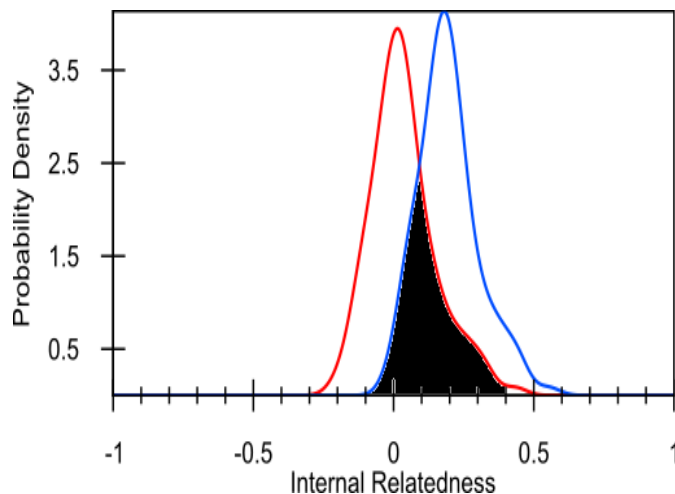


Figure 3. IR and IRVD values for 110 Miniature Poodles. The red curve represents the IR values for all of the Miniature Poodles that have been tested. The blue curve is the IRVD values for these same dogs. The IRVD curve represents how genetic diversity in Miniature Poodles compares to that in a large village dog population, which is the most diverse population we have studied and is from regions containing the direct ancestors of modern breeds. The blackened area represents the amount of genetic diversity in modern village dogs that still exists in contemporary Miniature Poodles (51%).

III. DLA Class I and II Haplotype frequencies

The DLA consists of four gene rich regions (classes I-IV) 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 4). Groups of genes and their alleles 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 inherited as a single block or haplotype (Table 5). One haplotype comes from each of the parents. Specific class I and II haplotypes are often linked to each other and inherited as a genetic block with limited recombination over time. Therefore, DLA class I and II haplotypes can be viewed as reasonable surrogate markers for breed founders.

Determining DLA class I and II haplotypes is often done by sequencing regions containing the desired alleles. This is a cumbersome and expensive procedure. The use of SNPs for measuring diversity in this region is also extremely difficult, given the high level and complexity of genetic polymorphism between various alleles. Fortunately, STRs are strongly associated with specific

genes in the DLA region and can be used as proxy genetically define these regions. Four STR markers are used to identify DLA class I haplotypes and three STRs for DLA class II haplotypes.

The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking with the first haplotypes identified in Standard Poodles being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class II haplotypes. It is common for various dog breeds to share common and even rare haplotypes, depending on common ancestry.

A. DLA class I and II haplotype diversity in Miniature Poodles

We have identified 33 distinct DLA Class I and 23 distinct DLA Class II haplotypes in 242 Miniature Poodles tested to date (Table 3). 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. Given the number of dogs tested, it is unlikely that additional haplotypes will be identified, and if they are, they will be at very low incidence.

The Miniature Poodle has the highest number of DLA class I and II haplotypes of any other pure breed studied to date, confirming that many different founders have been involved in the breed. Many have come from Poodles, but many others have come from other types of dogs. This is supported by the wide range of colors, coats, sizes, leg length, etc., in the breed. Although the 1013 and 1018 DLA class I and 2003 class II haplotypes are higher than expected by chance in Miniature Poodles, the numerous remaining haplotypes are much more random in their incidence. Founders with these haplotypes have obviously played an important role in maintaining predominant phenotypes of the breed and have been highly conserved. A similar situation exists with Standard Poodles, but much more serious. They have a very high incidence of the 1001, 1002, 1003 class I haplotypes and 2001 class II haplotypes. Dogs with these haplotypes have played a very important role in creating the desired phenotype of contemporary Standard Poodles.

As expected, Miniature and Standard Poodles share many class I and II haplotypes, but also possess haplotypes unique to each variety. Miniature Poodles have 10 class I and 9 class II haplotypes that were not found in Standard Poodles. Standard Poodles have even more unique class I and II haplotypes.

Table 3: DLA Class I & II Haplotype Frequencies in Miniature Poodles

DLA Class I Haplotype Frequencies (Updated Oct 9, 2019)				
DLA1 #	STR types			Miniature Poodle (n=287)
1001	380	373	281 182	0.014
1002	380	365	281 181	0.002
1003	387	375	277 186	0.009
1004	393	379	277 183	0.005
1005	389	371	277 181	0.056
1006	387	375	293 180	0.003

1009	382 377 277 184	0.080
1011	376 365 281 180	0.003
1012	388 369 289 188	0.054
1013	392 373 289 186	0.153
1014	375 373 287 178	0.003
1016	382 371 277 178	0.024
1018	375 373 287 186	0.242
1020	388 369 289 184	0.002
1025	380 365 281 186	0.003
1028	376 369 291 186	0.045
1031	382 371 277 186	0.049
1032	382 377 277 178	0.005
1033	382 379 277 181	0.005
1036	389 365 289 180	0.049
1040	380 371 277 186	0.007
1045	376 371 277 186	0.003
1054	382 379 277 184	0.002
1068	380 373 287 181	0.016
1105	382 379 277 178	0.059
1106	395 379 277 178	0.024
1107	376 375 293 183	0.030
1108	382 371 277 180	0.012
1109	381 379 291 186	0.014
1110	382 371 289 184	0.009
1111	387 378 287 182	0.010
1112	393 371 277 181	0.003
1168	382 379 289 186	0.002

DLA Class II Haplotype Frequencies (Updated Oct 9, 2019)

DLA2 #	STR types	Miniature Poodle (n=287)
2001	343 324 284	0.014
2002	343 327 280	0.005
2003	343 324 282	0.514
2004	351 327 268	0.002
2007	351 327 280	0.002
2008	339 327 276	0.080

2009	351 324 280	0.010
2011	345 322 284	0.003
2012	345 322 280	0.063
2014	339 322 284	0.024
2015	339 327 280	0.047
2016	339 323 284	0.030
2021	339 324 268	0.059
2022	339 327 282	0.002
2024	343 323 280	0.010
2025	351 321 280	0.049
2028	345 327 288	0.007
2032	339 323 280	0.012
2037	341 327 280	0.007
2040	345 327 280	0.003
2053	343 324 280	0.016
2066	339 324 280	0.030
2067	343 322 284	0.009
2100	341 324 282	0.002

B. A standard genetic assessment of breed heterozygosity using STRs that define the DLA Class I and II regions

Another way to assess genetic diversity in the DLA class I and II regions is to apply the same types of statistics used to assess diversity across the genome as reported in Table 2. Table 3 shows this same type of genetic diversity assessment using allele frequencies at each of the 7 STR loci that are associated with a much narrower, but very critical, part of the genome, i.e., DLA class I and II regions on chromosome 12. Miniature Poodles possess somewhat fewer average alleles per locus than Standard Poodles (7.00 vs. 7.86), but the average number of effective alleles per loci is higher (3.53 vs. 2.76). Therefore, a greater proportion of alleles are contributing to genetic diversity in the DLA in Miniature Poodles than in Standard Poodles. The observed and expected heterozygosity (H_o and H_e) are higher in Miniature Poodles than in Standard Poodles and are virtually identical in value, giving an inbreeding coefficient (F) in these DLA regions that is slightly less than zero. This indicates that DLA class I and II alleles are in Hardy-Weinberg equilibrium (random breeding), which supports the earlier F values based on genomic markers. In contrast, H_o and H_e values for Standard Poodles are not in balance, giving an inbreeding coefficient (F) of +0.044. Therefore, a subpopulation of Standard Poodles is more inbred in the DLA class I and II regions than the population. This is logical given the marked imbalance in DLA class I and II haplotype frequencies in Standard Poodles. The Standard Poodle/Miniature Poodle crosses are intermediate in most values between the two breeds and the inbreeding coefficient F is negative, mirroring what was seen with the genomic markers; crosses

are more outbred than either parental population, as would be expected for crosses between two genetically disparate breeds.

Table 4. Assessment of genetic diversity within DLA region using the frequencies of alleles for each of the 4 STR loci associated with DLA class I and the 3 STR loci associated with class II.

Population		N	Na	Ne	Ho	He	F
ST	Mean	897	7.857	2.760	0.554	0.579	0.044
	SE	<u>0.202</u>	<u>0.937</u>	<u>0.449</u>	<u>0.060</u>	<u>0.063</u>	<u>0.005</u>
ST/Mini	Mean	57	6.000	3.241	0.679	0.652	-0.036
	SE	<u>0.000</u>	<u>0.926</u>	<u>0.428</u>	<u>0.063</u>	<u>0.053</u>	<u>0.026</u>
Mini	Mean	110	7.000	3.532	0.690	0.691	-0.002
	SE	0.000	0.816	0.460	0.027	0.034	0.020

V. Heritable diseases/problems of the Miniature Poodle

Miniature Poodles, like many small dogs, are long-lived. The breed suffers from several complex genetic traits that are common among miniature breeds such as patellar luxation, aseptic necrosis of the femoral head, collapsing trachea, and periodontal disease. Hip dysplasia occurs but is uncommon. Cushing's syndrome and heart valve degeneration increase in frequency with age. Obesity is a problem in the breed and increases with age.

Autoimmune disorders, such as sebaceous adenitis and Addison's disease and common in breeds such as the Standard Poodle, are uncommon in Miniature Poodles. However, Miniature Poodles can suffer from type 1 diabetes, immune mediated thrombocytopenia or hemolytic anemia, and granulomatous meningoencephalitis. Epilepsy is increasing incidence in many breeds in parallel with increasing incidence of autoimmune disorders. Otitis externa is a problem as it is in any dogs with this type of coat, drooping ears, hair growth down into the ear canal and skin allergies. Cancer such as lymphoma is one of the leading causes of death, but not different in frequency than dogs in general. Skin tumors such as basal cell carcinomas are also a problem in the breed is a problem in Miniature Poodles as it is in many pure and random breeds of dogs.

Progressive retinal atrophy and von Willebrand's disease type 1 are simple recessive genetic disorders in the breed. Cataracts often occur within the first three years of life, also suggesting a heritable origin.

One of the emerging problems in Miniature Poodles is intravertebral disc disease (IVDD), something that is common in shorter and longer backed breeds such as the Dachshund and Corgi. The Miniature Poodle had two different body types: 1) "Correct or square", with legs long enough for their body height equal their length, and 2) short legs and a long back. The latter body type is much more susceptible to IVDD. Shortening of the legs was first thought to involve a single mutation in what is known as the FGF4 gene. The common mutation leads to what is

called chondrodysplasia, a condition that leads to a shortening of the legs and not associated with IVDD. This mutation has been introduced into many breeds of dogs to decrease the length of long bones and torso and cause some bowing of the front legs. Many Miniature Poodles possess this mutation and its associated phenotype. A second mutation in FGF4 has been recently described (see reference below) that causes a further shortening of the legs and lengthening of the back, but also causes weakness of the cartilage of the spinal discs (Brown et al., 2017). Breeds such as the Dachshund and Corgi have both mutations, explaining the extraordinary shortening of the legs and elongation of the back. The FGF4 mutation has been identified in Miniature Poodles that have suffered IVDD (Brown et al., 2017).

Brown EA, Dickinson PJ, Mansour T, Sturges BK, Aguilar M, Young AE, Korff C, Lind J, Ettinger CL, Varon S, Pollard R, Brown CT, Raudsepp T, Bannasch DL., 2017. FGF4 retrogene on CFA12 is responsible for chondrodystrophy and intervertebral disc disease in dogs. *Proc Natl Acad Sci U S A.* 114(3):11476-11481.

VI. Interpretation of DNA-based genetic testing

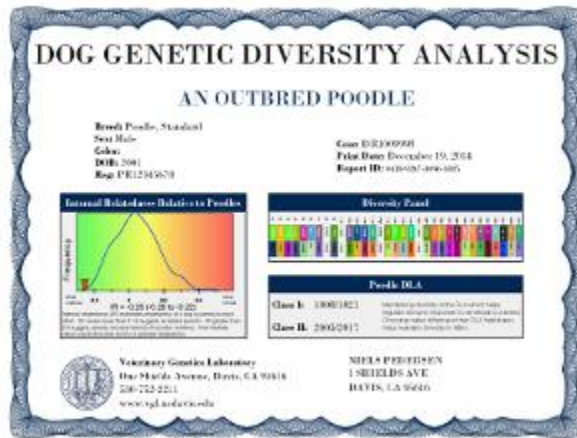
Miniature Poodles have the greatest amount of genetic diversity that has been found in any breed tested to date. This diversity is evident in the genomic autosomes as well as in important regions such as the DLA. This genetic (genotypic) diversity can be attributed to the tremendous phenotypic diversity found between individuals of the “variety.” This can be attributed to a wide genetic base that apparently involved introgressions from several breeds other than Standard or Toy Poodle varieties; their popularity and large population size favoring random mate selection; a relatively loose standard including different coats, coat colors; and a range of sizes and body types. Breeders have been also diligent in selecting the least related parents available to them, as indicated by the small amount of allele and haplotype sharing.

Although breed-wide standard genetic assessments indicate that the breed is very heterogeneous, internal relatedness (IR) scores indicate that there is still a proportion of individual Miniature Poodles that are offspring of parents that are quite related, sometimes to the equivalent level of full siblings. It is important to identify such individuals or bloodlines prior to breeding to find mates that will produce puppies that have much lower IR scores. This should not be a great problem as the breed is has great genetic diversity from which to select the best mates.

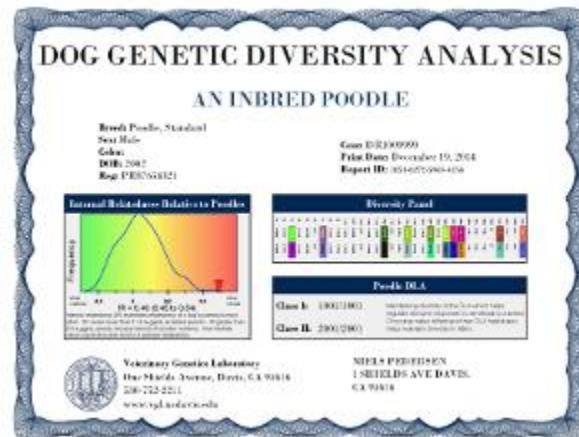
VII. How to use/apply this information

A. Certificates for sharing of genetic information

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 diversity of alleles at each of the genomic STR loci and their frequency in the population were used to determine the genetic relatedness and diversity. The internal relatedness value for the dog being tested is related to the population as a whole.



B. How are DNA based genetic markers best used by breeders?

The goal for Miniature Poodle breeders should be to maintain the large amount of genetic diversity that exists in the breed and to continue to randomly select mating pairs. IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. A breeding pair with identical IR values can have genetically distinct parents and 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 is much different in genomic allele and allele frequencies and DLA haplotypes, 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 because of genetic recombination. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

In brief, 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 individual 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.

An additional goal of this study is to contribute this genetic information to a web repository, hopefully under the control of the registry. This information could be incorporated into a mate selection service that will allow a breeder to identify, among all the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters.

Genetic information on Miniature Poodles and Standard Poodles will be extremely useful for those breeders interested in outcrossing between the two varieties of Poodle. Outcrossing would most benefit Standard Poodles and may be useful in creating desired medium sized dogs. Outcross pairs should be chosen to provide maximum genetic differences in puppies over parents (i.e. low IR scores) and genetic diversity testing used to identify which puppies would be most valuable for further outcrossing or backcrossing. The goal of outcrossing and backcrossing should be to regain the desired phenotype, while maintaining the greatest amount of new diversity.