

Genetic Diversity Testing for Newfoundland

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 to determine genetic heterogeneity and diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions for specified dog populations. This test panel is useful to dog breeders who wish to use DNA-based testing to track and distribute genetic diversity as a supplement to in-depth pedigrees. Information on genetic heterogeneity and diversity, along with DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions in order to improve the overall genetic health of a breed.

Genetic diversity testing in the Newfoundland has been established, and almost all existing alleles at the 33 STR loci and 7 DLA class I and II regions have potentially been identified. As of October of 2022, 102 Newfoundlands from the United States (n = 94), Canada (n = 7), and Mexico (n = 1) were tested at the VGL to assess genetic diversity in the breed. Allele and DLA haplotype frequencies will be updated as more dogs are tested.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from different regions of the genome were used to assess genetic heterogeneity and existing genetic diversity within an individual as well as across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity and genetic diversity in individuals as well as breed-wide.

DLA haplotypes: Seven STR loci linked to DLA class I and II genes were used to assess genetic diversity within a region that regulates immune responses and self/non-self-recognition. Problems with self/non-self-recognition, along with environmental factors, are responsible for autoimmune disease, allergies, and susceptibility to infectious agents.

Internal Relatedness (IR): The IR value is a measure of the genetic relatedness of an individual's parents. The value 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; two individuals from different sources may have identical IR values, but a quite different genetic makeup.

I. Introduction to the Newfoundland

A. Breed History [1-3, 7]

As its name indicates, the Newfoundland breed was developed in Newfoundland and Labrador, the easternmost province of Canada, around the end of the 17th century. Originally bred by European fishermen to aid in water rescue, Newfoundlands were also used for carting and hauling due to their large and strong bodies. The origins of the breed have been subject of debate throughout its history, with the most prevalent theory indicating that Newfoundlands were developed from crossing native Indian dogs with bear dogs brought to North America by Vikings. Genomic analysis shows that the Newfoundland is closely related to retriever breeds such as the Flat-Coated Retriever, Golden Retriever, and Labrador Retriever.

Due to their beauty, strength and abilities, Newfoundlands have been an integral part of history, either as companions in adventures and expeditions, or as inspiration for artists portraying the dog and its relationship with humans. Arguably, the most famous Newfoundland in history was Seaman, who accompanied Lewis and Clark on their 3-year expedition from the Mississippi to the Pacific and back (**Figure 1**).



Figure 1. Statue of Lewis and Clark with Seaman in St. Charles, Missouri.

The Newfoundland was recognized as a breed by the American Kennel Club (AKC) in 1886 and by the United Kennel Club (UKC) in 1919. It currently ranks 45 out of 284 in popularity among the AKC registries.

B. Appearance [1-4]

According to the AKC's official breed standard, the Newfoundland is a large, heavily coated, well-balanced dog that is deep-bodied, heavily boned, muscular, and strong. The average height for adult males is 28 inches, whereas adult bitches have an average height of 26 inches. Male dogs

weigh between 130 and 150 pounds; adult bitches between 100 and 120 pounds. The head is massive, with a broad skull and strongly developed occipital bone. The eyes are relatively small and dark brown. Ears are relatively small and triangular with rounded tips. Their feet are wide with webbing between the toes for swimming. Adult Newfoundlands have a flat and water-resistant double coat; recognized colors are black, brown, gray, and black and white. Solid-colored dogs may have white at any, some, or all, of the following locations: chin, chest, toes, and tip of tail. Any colors or combinations of colors not specifically described in the breed's official standard, albinism, unilateral or bilateral cryptorchidism, or a short and flat coat constitute disqualifications.

C. Temperament [1-4]

The breed has a sweet disposition, being very docile, easy to train, and generally good with kids. The Newfoundland enjoys outdoor activities and requires a good amount of exercise.

D. Health

1. Lifespan

Like many other large dog breeds, the average life expectancy of a Newfoundland is 9-10 years.

2. Diseases

As is the case of most large and giant dog breeds, Newfoundlands can be affected by hip and elbow dysplasia, which result from unstable joints (hip or elbow sockets) and subsequent degenerative arthritic changes that result from this instability. These conditions can affect young puppies, but more frequently lead to a degenerative, sometimes crippling, arthritis in adult dogs. Cystinuria is an autosomal recessive inherited disorder that leads to the accumulation of cystine in the urine, which causes the formation of crystals or stones in the bladder, kidneys or ureters. Both males and females are affected, and reports suggest that Newfoundlands show symptoms earlier than other breeds (as young as 5 months of age in some cases). Newfoundlands can also suffer from subvalvular aortic stenosis, or SAS. This genetic disorder leads to defective heart valves and can cause sudden death at an early age.

The Newfoundland Club of America requires the following tests be performed for a CHIC designation and strongly recommends these tests for all Newfoundlands:

- 1) Hips and elbow – radiographs submitted to OFA;
- 2) Cystinuria – DNA test by either blood or cheek swab;
- 3) Heart – auscultation or echocardiogram by a Board Certified Veterinary Cardiologist;

Additional tests recommended:

- 1) Eyes: examination by a Board Certified Veterinary Ophthalmologist;
- 2) Patella palpation;
- 3) Thyroid – blood test.

II. Results on Genetic Diversity of Newfoundland

A. Population genetics based on 33 STR loci on 25 chromosomes

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes [5,6]. The average number of alleles identified per locus across the dog breeds tested at the VGL to date is 15.4 alleles/locus. Dog breeds, having evolved from a small number of founders and having been exposed to artificial population bottlenecks, will end up with only a portion of the total available genetic diversity found in canids. Artificial genetic bottlenecks can include phenomena such as sire effects, geographic isolation, outbreaks of disease, and variation in popularity, which can lead to a decrease in population size. The alleles identified at each of the 33 STR loci and their relative frequencies for the 102 Newfoundlands are listed on **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Newfoundland (n = 102). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	AHT137	AHTH130	AHTH171-A	AHTH260	AHTk211
96 (0.814)	131 (0.005)	117 (0.005)	219 (0.098)	234 (0.319)	87 (0.819)
100 (0.020)	133 (0.069)	119 (0.074)	221 (0.015)	238 (0.069)	89 (0.123)
102 (0.025)	137 (0.181)	121 (0.848)	225 (0.059)	242 (0.020)	91 (0.025)
104 (0.005)	141 (0.098)	125 (0.059)	227 (0.583)	246 (0.397)	93 (0.029)
108 (0.113)	147 (0.554)	129 (0.015)	229 (0.010)	248 (0.010)	95 (0.005)
110 (0.020)	151 (0.093)		231 (0.196)	252 (0.186)	
112 (0.005)			233 (0.034)		
			241 (0.005)		
AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
286 (0.176)	116 (0.083)	132 (0.574)	148 (0.010)	234 (0.034)	95 (0.382)
288 (0.755)	118 (0.123)	136 (0.270)	152 (0.730)	236 (0.005)	97 (0.397)
290 (0.054)	120 (0.093)	140 (0.005)	156 (0.216)	238 (0.917)	101 (0.078)
292 (0.015)	124 (0.333)	144 (0.020)	160 (0.029)	240 (0.039)	105 (0.142)
	126 (0.078)	148 (0.005)	164 (0.010)	242 (0.005)	
	130 (0.289)	152 (0.127)	168 (0.005)		
INU005	INU030	INU055	LEI004	REN105L03	REN162C04
106 (0.040)	144 (0.069)	208 (0.181)	85 (0.039)	229 (0.005)	202 (0.211)
110 (0.797)	146 (0.113)	210 (0.250)	95 (0.387)	231 (0.191)	204 (0.005)
124 (0.074)	148 (0.064)	212 (0.319)	97 (0.010)	233 (0.471)	206 (0.299)
126 (0.040)	150 (0.706)	216 (0.250)	107 (0.181)	235 (0.211)	208 (0.485)
128 (0.020)	152 (0.039)		109 (0.382)	241 (0.123)	
130 (0.030)	154 (0.005)				
	156 (0.005)				

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
202 (0.054)	160 (0.074)	268 (0.647)	226 (0.104)	145 (0.662)	13 (0.054)
212 (0.299)	162 (0.618)	272 (0.319)	230 (0.005)	147 (0.078)	19.2 (0.127)
216 (0.475)	164 (0.088)	274 (0.020)	232 (0.525)	149 (0.098)	20.2 (0.029)
218 (0.010)	166 (0.005)	276 (0.005)	234 (0.010)	153 (0.162)	21.2 (0.054)
220 (0.162)	168 (0.078)	278 (0.010)	236 (0.302)		23.2 (0.461)
	170 (0.137)		238 (0.020)		24.2 (0.118)
			244 (0.035)		25.2 (0.132)
					26.2 (0.025)
VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
16.1 (0.005)	13 (0.250)	16 (0.005)	16 (0.181)	9 (0.127)	13 (0.044)
17.1 (0.480)	14 (0.333)	17 (0.005)	17 (0.005)	11 (0.039)	14 (0.029)
18.1 (0.049)	15 (0.265)	18 (0.363)	19 (0.755)	13 (0.745)	15 (0.123)
19.1 (0.147)	16 (0.010)	19 (0.010)	20 (0.039)	14 (0.083)	16 (0.152)
20.1 (0.064)	17 (0.010)	20 (0.010)	21 (0.010)	16 (0.005)	17 (0.015)
21.1 (0.039)	18 (0.098)	21 (0.446)	23 (0.010)		18 (0.338)
22.1 (0.196)	19 (0.029)	22 (0.005)			19 (0.294)
23.1 (0.020)	20 (0.005)	25 (0.015)			20 (0.005)
		26 (0.015)			
		27 (0.020)			
		28 (0.049)			
		29 (0.059)			
VGL2918	VGL3008	VGL3235			
13 (0.025)	14 (0.025)	13 (0.132)			
14 (0.108)	17 (0.064)	14 (0.211)			
15 (0.108)	18 (0.225)	16 (0.020)			
16 (0.132)	19 (0.113)	17 (0.005)			
17 (0.005)	20 (0.451)	18 (0.613)			
17.3 (0.069)	21 (0.118)	19 (0.020)			
18.3 (0.078)	22 (0.005)				
19.3 (0.108)					
20.3 (0.358)					
21.3 (0.010)					

The number of alleles identified for each STR locus in Newfoundlands ranged from 4 (AHTk253, INRA21, INU055, REN162C04, and REN64E19) to 12 (VGL1165) (**Table 1**), with an average across loci of 6.18 alleles (**Table 2**). This means that Newfoundlands possess around 40% of the average number of alleles identified across dog breeds tested at the VGL (15.4 alleles/locus). Loss of alleles is a common feature of pure dog breeds; this phenomenon is a direct effect of a large bottleneck event such as the ones listed above. Another consequence of such bottleneck effects is a disproportionately high frequency estimated for one or two alleles at some STR loci, as seen in Newfoundlands (**Table 1**). These alleles have been inherited from founding dogs whose phenotypes (and consequently genotypes) were highly valued for the breed, and therefore have been positively selected throughout the breed's development. In Newfoundlands, a single allele occurred in 50% or more of the study cohort at a staggering 18 out of the 33 loci (**Table 1**), which suggests that these alleles were present in the foundation stock and are linked to breed-defining

phenotypic traits. Therefore, the goal for Newfoundland breeders should be to re-distribute allele frequencies for the 33 STR markers by conserving and breeding rare lines/families.

B. Assessment of population diversity using standard genetic parameters

Based on the alleles identified for each of the 33 STR loci listed in **Table 1** and their respective frequencies in the study cohort, genetic diversity parameters can be estimated for the population (**Table 2**). These parameters include the average number of alleles found at each locus (**Na**); the average number of effective alleles (**Ne**) (i.e., the number of alleles that contribute most to genetic differences); observed heterozygosity (**Ho**); expected heterozygosity (**He**) if the population was in Hardy-Weinberg equilibrium (i.e., randomly breeding); and the coefficient of inbreeding (**F**) derived from **Ho** and **He** values.

Table 2. Genetic Assessment of 102 Newfoundlands based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	6.18	2.66	0.55	0.57	0.03
SE	0.31	0.17	0.03	0.03	0.01

The average number of alleles across STR loci in Newfoundlands ($Na = 6.18$) corresponds to approximately 40% of those identified across dog breeds tested at the VGL (15.4 alleles/locus) (**Table 2**), which means that less than half of all the known canid diversity has been retained in Newfoundlands. Compared to closely related breeds, this number is higher than that estimated for Flat-Coated Retrievers (37%) and slightly lower than the popular Labrador Retriever (45%). However, the average number of effective alleles (**Ne**) constitutes a more important metric for diversity, since these represent the alleles with the greatest influence on heterozygosity. In Newfoundlands, this number was estimated at 2.66 (**Table 2**), or only approximately 43% of the total number of alleles segregating in the breed. This is typical for most pure dog breeds, and in Newfoundlands, this can be explained by the presence of a high-frequency allele in the majority of STR loci analyzed (**Table 1**). The values for **Ho** (0.55) and **He** (0.57), albeit relatively low, were not significantly different from each other. Because of that, the breed-wide coefficient of inbreeding (**F**) was estimated at 0.03 (**Table 2**). This value indicates a small deficit in heterozygosity from what is expected for a population in Hardy-Weinberg equilibrium (HWE), i.e., Newfoundlands are approximately 3% more inbred than a random mating population and that the average dog is a product of random selection.

However, the aforementioned values were estimated for the entire cohort and not for individual dogs making up the population. Internal Relatedness (IR) scores provide a better picture of heterozygosity for each dog and should be used by breeders to select the most unrelated mates possible (see **section E** below).

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each STR locus (**Table 3**). This provides an estimate of genetic diversity in the genomic regions associated with each STR marker. **Table 3** lists the average **Na**, **Ne**, **Ho**, **He**, and **F** values for each

STR locus estimated for this cohort of Newfoundlands. Loci with the lowest H_o values contribute the least to heterozygosity levels across the breed; they are most likely associated with traits that are important for the breed's phenotypic standard (and thus tend to vary less). Conversely, high H_o values for a particular locus means that it shows greater genetic diversity across the breed, and that these loci might be associated with traits with greater variation among individuals.

Table 3. Standard Genetic Assessment of individual STR loci for 102 Newfoundlands. Individual STR loci with high inbreeding coefficients ($F > 0.1$) are bolded.

Locus	Na	Ne	Ho	He	F
AHT121	7	1.479	0.294	0.324	0.091
AHT137	6	2.757	0.549	0.637	0.138
AHTH130	5	1.373	0.284	0.272	-0.05
AHTH171-A	8	2.543	0.608	0.607	-0
AHTH260	6	3.344	0.725	0.701	-0.04
AHTk211	5	1.456	0.294	0.313	0.061
AHTk253	4	1.655	0.363	0.396	0.084
C22.279	6	4.319	0.755	0.768	0.018
FH2001	6	2.391	0.578	0.582	0.006
FH2054	6	1.721	0.441	0.419	-0.05
FH2848	5	1.186	0.167	0.157	-0.06
INRA21	4	3.028	0.755	0.67	-0.13
INU005	6	1.55	0.347	0.355	0.023
INU030	7	1.918	0.471	0.479	0.017
INU055	4	3.855	0.784	0.741	-0.06
LEI004	5	3.024	0.667	0.669	0.004
REN105L03	5	3.15	0.588	0.683	0.138
REN162C04	4	2.707	0.647	0.631	-0.03
REN169D01	5	2.901	0.735	0.655	-0.12
REN169O18	6	2.383	0.471	0.58	0.189
REN247M23	5	1.92	0.51	0.479	-0.06
REN54P11	7	2.638	0.554	0.621	0.107
REN64E19	4	2.084	0.461	0.52	0.114
VGL0760	8	3.742	0.716	0.733	0.023
VGL0910	8	3.342	0.627	0.701	0.105
VGL1063	8	3.931	0.716	0.746	0.04
VGL1165	12	2.963	0.618	0.662	0.068
VGL1828	6	1.654	0.333	0.395	0.157
VGL2009	5	1.724	0.343	0.42	0.183
VGL2409	8	4.131	0.755	0.758	0.004
VGL2918	10	5.207	0.833	0.808	-0.03
VGL3008	7	3.503	0.657	0.715	0.081
VGL3235	6	2.282	0.559	0.562	0.005

An inbreeding coefficient (F) value of zero means that a population is randomly breeding (no artificial selection). Positive F values indicate non-random selection (inbreeding), while negative values indicate outbreeding (increased heterozygosity). Eight STR loci had high inbreeding coefficients ($F > 0.1$, bolded on **Table 3**), which suggests that they have been under strong positive selection since breed development and are likely associated with breed-defining phenotypic traits. Taken together, these results suggest that this cohort was as unrelated as possible and that Newfoundland breeders have kept a good balance in inbreeding levels across these genomic regions through careful sire and dam selection.

D. Differences in population structure as determined by Principal Coordinate Analysis (PCoA)

The degree of genetic relatedness between individuals within a population can be measured with a PCoA. The genetic data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (coordinates 1 and 2). The closer two individuals cluster together on the plot, the more closely related they are to each other.

Overall, the 102 Newfoundlands clustered as expected for a pure dog breed on the PCoA plot using allele frequency data obtained from the 33 STR marker panel. Individual dogs were reasonably dispersed across all four quadrants of the graph, with several dogs appearing as outliers (i.e., more genetically distinct), seen on the periphery of the plot (**Figure 2**). A few pairs of individuals (circled in red) were genetically more closely related to each other, as suggested by their tight clustering pattern.

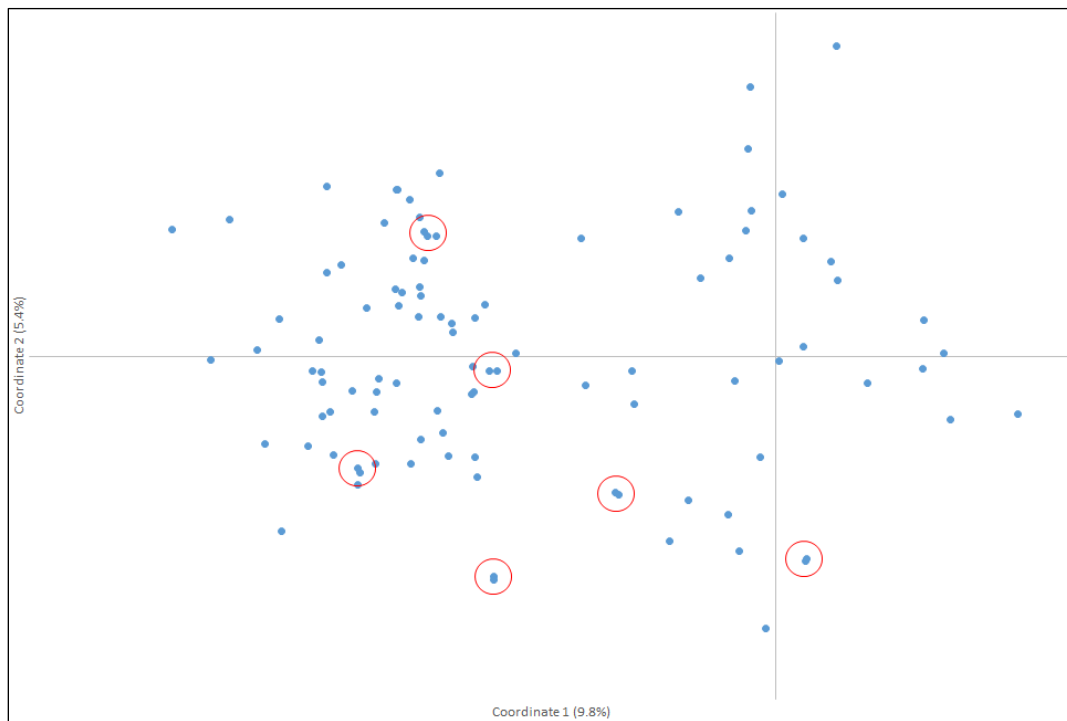


Figure 2. PCoA of Newfoundland ($n = 102$) based on allele frequencies at 33 autosomal STR loci. Closely related pairs of dogs are circled in red.

Principal coordinate analysis can also be used to determine how populations have genetically differentiated from each other over time. **Figure 3** shows a PCoA of the 102 Newfoundlands with a somewhat closely related breed (Labrador Retriever) and a somewhat unrelated breed (Italian Greyhound) [7]. Inter-breed clustering shows separate and well-defined populations, thus indicating that the breeds are genetically distinct as expected. The relationship between Newfoundland (blue dots) and Labrador Retriever (grey dots) can be appreciated from the results: both breeds cluster together mostly below the horizontal or X-axis, and a couple of individuals bridge the two populations, showing a higher degree of genetic relatedness to the other breed than the rest of the cohort.

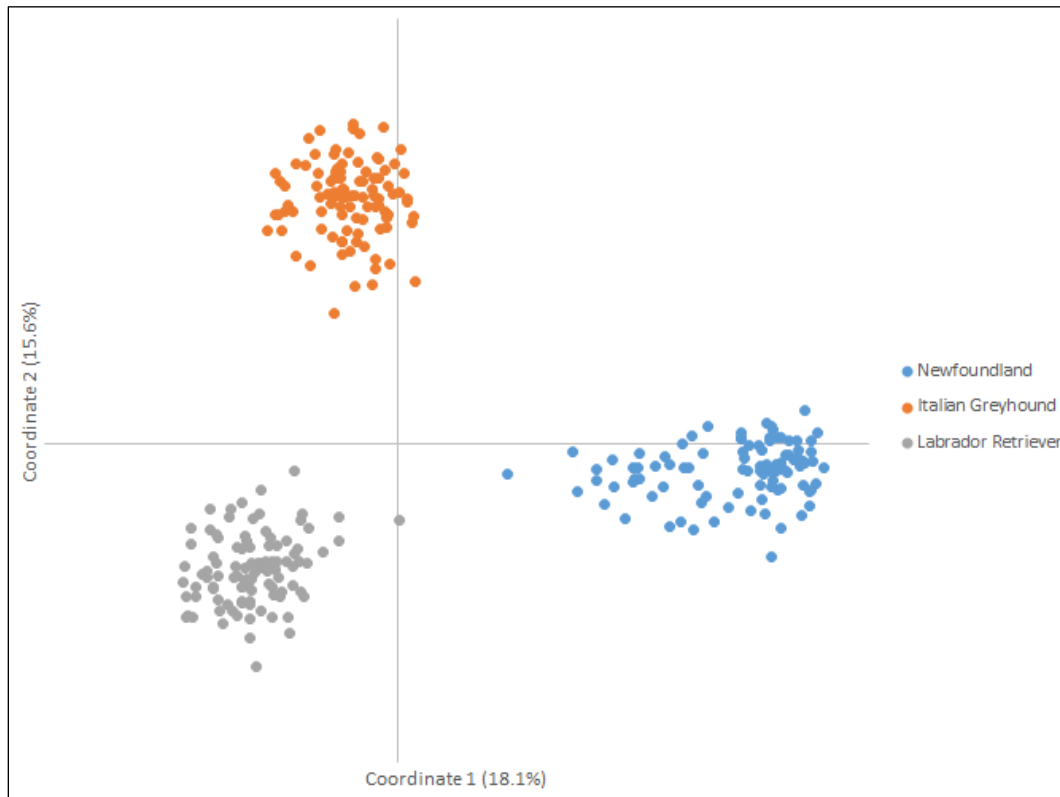


Figure 3. PCoA plot of Newfoundland (blue dots; $n = 102$), Labrador Retriever (grey dots; $n = 100$), and Italian Greyhound (orange dots; $n = 100$).

E. Internal relatedness (IR) scores for Newfoundland

1. IR testing and meaning

Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity inherited by individuals from their parents. Internal Relatedness (IR) is a calculation that is used to determine the degree of relatedness of parents of an individual dog. The IR calculation takes into consideration homozygosity at each of the 33 STR loci in this study and gives more weight to rare and uncommon alleles, which would presumably be identified in less related individuals. 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 an IR value of -1.0 would have

parents that are totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that are genetically identical at all loci. IR values above +0.25 occur when the parents of the full sibling parents are themselves highly inbred. *The higher the IR value is above 0.25 for a particular individual, the more closely related are the parents and grandparents of the sibling parents.* **Table 4** summarizes the IR values for the 102 Newfoundlands.

Table 4. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies for 33 STR loci in 102 Newfoundlands.

	IR	IRVD
Minimum	-0.2406	-0.0069
1st Quartile	-0.0665	0.2591
Mean	0.0366	0.3597
Median	0.0185	0.3426
3rd Quartile	0.1330	0.4890
Maximum	0.3457	0.7328

The most outbred dog of the study cohort had an estimated IR score of -0.24, while the most inbred dog had an IR score of +0.35, with a mean IR of +0.04 (**Table 4**). These results show that roughly 25% of the Newfoundland cohort had IR values between 0.13 and 0.35, suggesting that they are products of closely related parents. Therefore, although standard genetic metrics (Tables 2 and 3) indicate that this group of dogs was the product of random mating, IR values for individual dogs suggest that this population is actually composed of inbred and outbred dogs.

The wide range of IR values indicate genetic heterogeneity in the cohort (typical for most pure breeds), and highlights the importance of determining IR values for individual dogs in order to maintain within-breed diversity by selecting the least related individuals possible for mating purposes based on IR.

2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed development

The IR values obtained from known STR alleles and their frequencies can be used to approximate the amount of genetic diversity that has been lost as a breed evolves from its oldest common ancestors to present day. Village dogs that exist throughout the SE Asia, the Middle East and the Island Pacific region are randomly breeding descendants of dogs from which most modern breeds evolved. The known STR alleles and their frequencies of a given breed can be compared with the same alleles and their frequency in modern village dogs to yield an adjusted IR score (IR-village dog or IRVD) (**Table 4** and **Figure 4**, blue line).

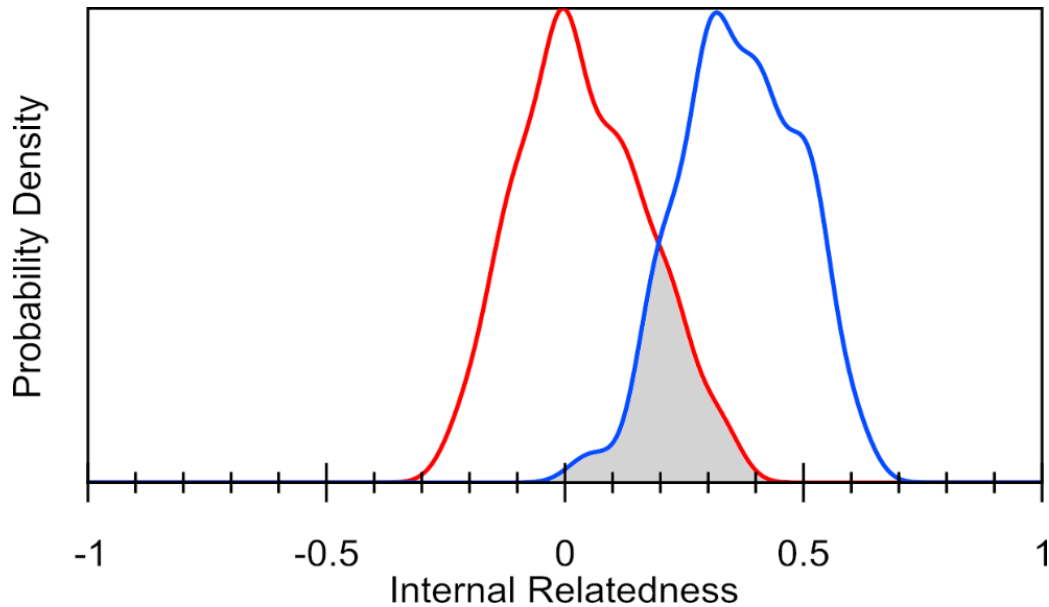


Figure 3. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Newfoundland (n=102). The overlap between the curves (gray area) shows that the breed retains only 22.5% of the genetic diversity existing in randomly breeding village dogs.

The mean IRVD value was calculated at 0.36 for Newfoundlands, ranging from -0.007 for the most outbred dog to +0.73 for the most inbred individual (**Table 4**). The IRVD curve (**Figure 3**, blue line) is shifted to the right when compared to the IR curve (red line), which is typical for all pure breeds of dogs. The overlapping area of the two curves provides an estimate of how much genetic diversity has been lost during breed development. In Newfoundlands, the overlap between IR and IRVD curves was 22.5%, which is lower than the 30% retained genetic diversity calculated for all canids tested at VGL (**section IIB**). This value reflects the relatively low genetic diversity existing in the breed as identified in **sections IIA, IIB, and IIC**.

F. DLA class I and II haplotype frequencies and genetic diversity

The DLA consists of four gene-rich regions that make up a small portion of 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, which can cause autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases. Breeds that lack genetic diversity in the DLA region are often more prone to autoimmune disorders.

The Class I region contains several genes, but only one, *DLA88*, is highly polymorphic (i.e., contains many alleles) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with *DLA88* are linked in various combinations, forming specific haplotypes (**Table 5**).

The class II region also contains several genes, three of which are highly polymorphic: *DLA-DRB1*, *DLA-DQB1* and *DLA-DQA1*. Specific alleles at these three loci associated with the three

class II genes are strongly linked, and often inherited as a single haplotype. An individual inherits one haplotype from each of the parents. It is common for different dog breeds to share common and even rare haplotypes for these loci, depending on common ancestry.

1. DLA class I and II haplotypes existing in the Newfoundland

Thirteen DLA class I and nine DLA class II haplotypes were identified in Newfoundlands (**Table 5**). This number is similar to that found in 394 Flat-Coated Retrievers (11 DLA class I haplotypes and 10 DLA class II haplotypes), but much lower than the number of haplotypes found in Labrador Retrievers (20 DLA class I and 17 DLA class II haplotypes). 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.

DLA-I haplotypes 1016, 1040, and 1175 were the most predominant in Newfoundlands, being identified in 32%, 29%, and 15% of the dogs tested, respectively. Similarly, DLA-II haplotypes 2014, 2039, and 2007 were also identified in almost 80% of all dogs analyzed, with frequencies of 34%, 30%, and 15% respectively (**Table 5**). Since the aforementioned DLA-I/DLA-II haplotypes were identified in similar frequencies, it can be inferred that they are in linkage disequilibrium (i.e., inherited together). Therefore, a founder (or founder line) with these combinations of DLA-I/DLA-II haplotypes (1016/2014, 1040/2039, and 1175/2007) has played an important role in establishing important phenotypic trait(s) in Newfoundlands, and thus these DLA haplotypes have been retained in high frequencies in the breed.

Table 5. DLA class I and II haplotypes identified in Newfoundland (n = 102) and their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA I haplotype	STR types	Frequency (%)
1016	382 371 277 178	31.9
1033	382 379 277 181	0.5
1040	380 371 277 186	28.9
1062	382 371 277 183	0.5
1068	380 373 287 181	10.3
1069	380 365 281 184	1.0
1087	380 371 277 178	0.5
1092	376 379 277 181	8.3
1094	395 375 277 176	1.5
1159	395 379 277 181	0.5
1175	380 375 293 180	14.7
1284	388 371 277 181	0.5
1285	380 375 277 186	1.0

DLA II haplotype	STR types	Frequency (%)
2003	343 324 282	0.5
2007	351 327 280	14.7
2014	339 322 284	33.8
2016	339 323 284	0.5
2021	339 324 268	0.5
2039	345 327 276	29.9
2045	339 325 284	1.0
2048	339 331 282	8.8
2053	343 324 280	10.3

Newfoundlands share DLA haplotypes with 47 different breeds/varieties tested at the VGL. Interestingly, two DLA class I haplotypes appear to be unique to Newfoundlands (1284 and 1285), but no DLA class II haplotypes were found exclusively in this breed (**Table 6**). DLA haplotypes tend remain mostly unchanged over the generations, and can be used to assess the shared influence of founder lines in different breeds. As expected, Newfoundlands share a high number of DLA haplotypes (especially class II) with their closely related retriever breeds: Flat-Coated Retriever, Golden Retriever, and Labrador Retriever [7] (**Table 6**).

Table 6. Sharing of DLA class I haplotypes between Newfoundland (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=47).

DLA1 #	STR types	Newfoundland (n=102)	American Eskimo, Standard (n=63)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Akita (n=158)	Japanese Akita (n=565)	Alaskan Klee Kai (n=634)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=142)	Black Russian Terrier (n=150)	Biewer (n=128)	Biewer Yorkshire Terrier (n=53)	Biewer Terrier (n=201)	Yorkshire Terrier (n=16)	Borzoi (n=147)	Collie (n=49)	Doberman Pinscher (n=1110)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=822)	Great Dane (n=56)	Golden Retriever (n=858)	Giant Schnauzer (n=320)
1016	382 371 277 178	0.319	0.008	0.04	0.11	--	--	--	--	--	0.046	0.01	0.023	0.019	0.012	0.03	--	0.04	0.0212	0.095	0.15	0.14	--	0.188	0.0047	0.047
1033	382 379 277 181	0.005	--	--	--	--	--	--	0.015	--	--	--	--	--	--	--	0.048	--	--	--	0.02	--	--	--	--	--
1040	380 371 277 186	0.289	--	--	--	0.006	--	0.2129	--	--	--	--	0.102	0.208	0.077	--	--	--	0.0095	0.04	--	--	--	--	0.0006	--
1062	382 371 277 183	0.005	--	--	--	--	--	0.2279	--	--	--	--	--	--	--	--	--	--	--	0.187	--	--	--	--	0.0874	--
1068	380 373 287 181	0.103	--	--	--	--	--	--	--	0.221	0.063	--	--	0.009	--	--	--	--	0.0005	--	--	--	0.2835	0.009	0.0472	0.053
1069	380 365 281 184	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0402	--
1087	380 371 277 178	0.005	0.008	--	--	0.013	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--
1092	376 379 277 181	0.083	--	--	--	--	0.3115	--	0.037	0.008	--	0.253	--	0.038	--	0.06	--	--	--	0.006	--	--	0.0012	--	--	0.273
1094	395 375 277 176	0.015	--	--	--	--	--	--	--	--	0.141	0.013	--	--	--	--	0.01	--	0.7432	--	--	--	--	0.027	--	--
1159	395 379 277 181	0.005	--	--	--	--	--	--	0.103	--	--	--	--	--	--	--	0.048	--	0.0041	--	--	--	--	--	--	0.164
1175	380 375 293 180	0.147	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1284	388 371 277 181	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1285	380 375 277 186	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
DLA1 # (cont'd)	STR types	Newfoundland (n=102)	Havana Silk (n=36)	Havanese (n=847)	Italian Greyhound (n=1256)	Irish Setter (n=59)	Irish Red and White Setter (n=74)	Irish Wolfhound (n=78)	Lakeland Terrier (n=130)	Labrador Retriever (n=282)	Llewellyn Setter (n=135)	Magyar Agar (n=78)	Polish Lowland Sheepdog (n=71)	Toy Poodle (n=214)	Miniature Poodle (n=403)	Poodle (n=4818)	Rat Terrier (n=41)	Samoyed (n=191)	Saint Bernard (n=89)	Scottish Collie (n=115)	Greater Swiss Mountain Dog (n=57)	Shiloh Shepherd, ISSA (n=252)	Swedish Vallhund (n=441)	Cardigan Welsh Corgi (n=43)	Whippet (n=107)	
1016	382 371 277 178	0.319	0.08	0.1942	0.0597	--	--	--	0.008	0.016	0.03	--	0.028	0.023	0.029	0.0223	0.05	--	--	0.096	0.07	--	--	--	0.107	
1033	382 379 277 181	0.005	--	0.0018	--	--	--	--	--	0.002	--	0.096	--	--	0.004	0.0032	--	--	--	--	--	--	--	0.08	--	
1040	380 371 277 186	0.289	0.01	0.0183	0.08	--	--	--	0.181	--	--	--	0.683	0.002	0.005	0.0006	0.16	--	0.011	--	--	--	--	--	0.084	
1062	382 371 277 183	0.005	--	0.0006	--	--	--	--	--	0.012	--	--	--	--	--	--	0.06	--	0.062	--	--	--	--	--	--	
1068	380 373 287 181	0.103	0.26	0.0148	--	0.008	--	0.128	--	0.032	--	--	0.007	0.009	0.012	--	--	0.042	0.163	--	--	0.262	0.374	--	--	
1069	380 365 281 184	0.01	--	--	--	0.102	0.027	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
1087	380 371 277 178	0.005	--	0.003	--	--	--	--	--	0.002	--	--	--	--	--	--	--	0.11	--	0.017	--	--	--	--	--	
1092	376 379 277 181	0.083	0.21	0.0945	--	--	--	--	--	--	--	--	--	--	0.0002	0.06	--	--	--	0.004	--	--	--	--	--	
1094	395 375 277 176	0.015	--	--	--	--	--	0.192	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
1159	395 379 277 181	0.005	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
1175	380 375 293 180	0.147	--	--	--	--	0.081	--	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
1284	388 371 277 181	0.005	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
1285	380 375 277 186	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
DLA2 #	STR types	Newfoundland (n=102)	American Eskimo, Standard (n=63)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	American Akita (n=158)	Japanese Akita (n=565)	Alaskan Klee Kai (n=634)	Barbet (n=68)	Border Collie (n=61)	Bernese Mountain Dog (n=142)	Black Russian Terrier (n=150)	Biewer (n=128)	Biewer Yorkshire Terrier (n=53)	Biewer Terrier (n=201)	Yorkshire Terrier (n=16)	Borzoi (n=147)	Collie (n=49)	Doberman Pinscher (n=1110)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=822)	Great Dane (n=56)	Golden Retriever (n=858)	Giant Schnauzer (n=320)
2003	343 324 282	0.005	0.024	0.04	--	--	--	--	0.132	0.057	--	--	0.246	0.208	0.249	0.38	--	--	0.0005	0.598	--	--	0.1436	0.009	0.0198	0.03
2007	351 327 280	0.147	--	--	--	0.082	--	0.0118	--	--	0.081	0.047	--	--	--	--	0.153	--	--	--	--	--	--	0.018	0.0163	0.048
2014	339 322 284	0.338	0.008	0.03	0.07	--	--	0.067	--	--	--	--	--	--	--	--	--	--	--	0.092	0.15	0.14	0.028	0.036	--	0.005
2016	339 323 284	0.005	--	--	--	--	--	--	--	--	--	--	0.078	0.094	0.067	0.09	--	--	--	--	--	--	--	--	--	--
2021	339 324 268	0.005	--	--	--	--	--	0.224	--	--	0.046	--	0.004	--	--	--	--	--	--	--	--	--	--	--	0.0874	--
2039	345 327 276	0.299	--	--	--	0.092	0.1035	--	--	0.156	--	--	--	--	--	--	--	0.95	0.009	--	--	--	--	--	--	--
2045	339 325 284	0.01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0408	--
2048	339 331 282	0.088	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0006	--	0.2663	--
2053	343 324 280	0.103	0.222	0.12	0.04	--	--	--	--	0.074	0.063	--	--	0.009	--	--	--	--	--	0.0014	--	--	0.1472	0.009	0.0286	0.063
DLA2 # (cont'd)	STR types	Newfoundland (n=102)	Havana Silk (n=36)	Havanese (n=847)	Italian Greyhound (n=1256)	Irish Setter (n=59)	Irish Red and White Setter (n=74)	Irish Wolfhound (n=78)	Lakeland Terrier (n=130)	Labrador Retriever (n=282)	Llewellyn Setter (n=135)	Magyar Agar (n=78)	Polish Lowland Sheepdog (n=71)	Toy Poodle (n=214)	Miniature Poodle (n=403)	Poodle (n=4818)	Rat Terrier (n=41)	Samoyed (n=191)	Saint Bernard (n=89)	Scottish Collie (n=115)	Greater Swiss Mountain Dog (n=57)	Shiloh Shepherd, ISSA (n=252)	Swedish Vallhund (n=441)	Cardigan Welsh Corgi (n=43)	Whippet (n=107)	
2003	343 324 282	0.005	0.25	0.2166	0.0072	--	--	--	0.765	0.023	--	--	--	0.421	0.507	0.1035	0.12	0.013	--	0.017	0.93	0.01	--	0.23	0.089	
2007	351 327 280	0.147	0.01	0.0484	--	--	0.155	--	--	0.03	--	--	--	0.002	0.001	0.017	--	0.005	0.006	0.004	--	--	0.246	0.12	--	
2014	339 322 284	0.338	--	0.0047	0.0016	--	--	--	0.008	0.002	--	--	0.711	0.021	0.027	0.0207	0.02	--	0.112	--	--	--	--	--	--	
2016	339 323 284	0.005	--	0.0183	--	--	--	--	--	--	--	0.071	--	0.009	0.03	0.0035	--	--	--	--	--	--	--	--	--	
2021	339 324 268	0.005	--	0.0041	--	--	--	0.295	--	0.012	--	--	--	0.075	0.052	0.0037	0.02	--	0.062	--	--	--	--	--	--	
2039	345 327 276	0.299	--	--	0.0832	--	--	--	--	0.005	--	--	--	--	0.0006	--	--	--	--	0.709	--	0.002	--	0.31	--	
2045	339 325 284	0.01	--	--	--	0.102	0.027	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
2048	339 331 282	0.088	--	--	--	--	--	--	--	0.376	--	--	--	--	--	0.0002	--	--	--	--	--	--	--	--	0.22	
2053	343 324 280	0.103	0.28	0.0319	--	0.008	--	0.128	--	0.03	--	--	0.007	0.012	0.012	--	--	0.56	0.14	--	--	0.329	0.525	--	--	

2. Heterozygosity in the DLA region

Due to their physical proximity on chromosome 12, the seven loci that define the DLA class I and II haplotypes are in strong linkage disequilibrium (i.e., have a higher probability of being inherited together) when compared to the genome-at-large. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome over time, and thus will be inherited randomly. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) as well as averaged across all DLA loci (**Table 8**).

The highest number of alleles (N_a) identified at each DLA locus for Newfoundlands was 7 (DLA1131) whereas the lowest was 4 (DLA I-4BCT and 5ACA). However, as observed in the 33 STR markers across the genome, the number of effective alleles (N_e) per DLA locus was lower, ranging from 1.72 (locus DLA I-4BCT) to 3.9 (DLA1131). Inbreeding coefficients around zero (ranging from 0.03 to 0.08) were estimated for each DLA locus (**Table 7**). Also according to the expectation that the DLA region has achieved an equilibrium with other regions of the genome, we can observe that the average inbreeding coefficient estimated for this region ($F=0.06$, **Table 8**) is similar to that estimated across the 33 STR loci ($F=0.03$, **Table 2**). This suggests that only a small subpopulation (around 6%) of Newfoundlands are more inbred than the population as a whole based on DLA haplotypes. Therefore, the over-representation of three linked DLA-I/DLA-II haplotypes (**Table 5**) probably occurred during the earliest origins of the breed, followed by a longer period of somewhat random breeding.

Table 7. Standard genetic assessment for Newfoundlands ($n=102$) using each of the 7 STRs in the DLA class I and II regions.

Locus	N_a	N_e	H_o	H_e	F
DLA I-3CCA	5	2.31	0.52	0.57	0.08
DLA I-4ACA	5	2.29	0.55	0.56	0.03
DLA I-4BCT	4	1.72	0.40	0.42	0.04
DLA1131	7	3.90	0.69	0.74	0.08
5ACA	4	3.11	0.65	0.68	0.05
5ACT	6	2.99	0.63	0.67	0.06
5BCA	5	3.51	0.68	0.72	0.05

Table 8. Summary of standard genetic assessment for Newfoundlands ($n=102$) using 7 STRs in the DLA class I and II regions. SE = standard error of the mean.

	N_a	N_e	H_o	H_e	F
Mean	5.14	2.83	0.59	0.62	0.06
SE	0.37	0.27	0.04	0.04	0.01

III. What does this assessment of genetic diversity tell us about Newfoundlands

Relatively low levels of genetic diversity were identified in the Newfoundland compared to some other breeds. Additionally, there is evidence that founders or founder lines have had a disproportionately high genetic influence on the breed, and that the genetic imbalance originating

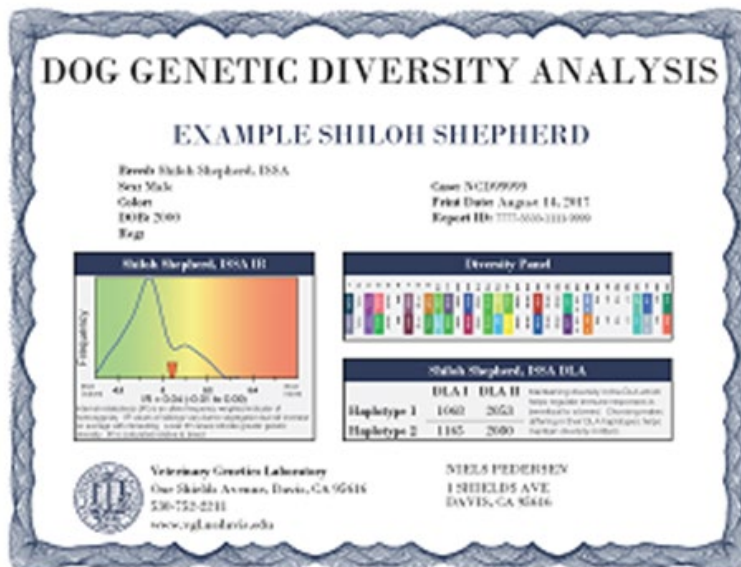
from that phenomenon is being maintained to some degree by artificial selection (breeding practices). These conclusions are supported by results from both the 33 autosomal STR markers and the DLA loci analyzed. However, our results also show that Newfoundland breeders have been doing a good job maintaining the genetic diversity existing in the breed by choosing sires and dams that are as unrelated as possible, as shown by inbreeding coefficients similar to those expected for a random breeding population.

The goal for Newfoundland breeders is to re-distribute the existing genetic diversity by breeding the least related parents possible based on genotypes for the 33 STR markers and the DLA loci. The goal is to produce dogs with IR scores lower than zero.

IV. Results of VGL Canine Diversity Testing

A. How will you be given the results of DNA-based genetic diversity testing on your dog?

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 and 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 reported in relation to others in the population. The alleles at each of the 33 STR loci are presented as numbers that correspond to those found in Table 1. Each locus will have two alleles, which can be different (heterozygous) or the same (homozygous). Each allele is inherited from one of the parents. Dogs from closely related parents will be homozygous for more alleles at each locus, or in regions of the genome that are under strong positive selection for phenotypic trait or traits mostly favored in the breed. Dogs with a predominance of rare (i.e., low frequency) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high frequency) alleles. A sample genetic diversity report is shown below.



B. What should you do with this information?

The goal for breeders should be to continue to produce puppies with IR scores close to zero, and as informed breeding decisions are made, even lower scores. Mates should be preferably selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype; moreover, mating of dogs with less frequent genomic alleles or DLA haplotypes is encouraged. Maintaining existing genomic diversity will require using IR values of potential mates based on the 33 STR loci to assure puppies of equal or greater overall diversity. However, because IR values reflect the unique genetics of individuals, they cannot be used as the primary criterion for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, breeding dogs with high IR values (providing they are genetically different) may produce puppies with much lower IR scores than either parent. A mating between a dog with a high IR value and one with 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 could 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.

The next step is to compare the DLA class I and II haplotypes of the mates. You want to avoid breeding dogs that will produce puppies homozygous for the same haplotypes; once again, less common haplotypes may increase breed diversity in relation to common ones.

Breeders who would like to predict the genetic outcome of puppies of certain sires and dams should screen them for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Rare alleles should be favored over common ones. This information is included on all certificates and on the breed-wide data found on the VGL website.

V. References

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