Genetic Diversity Testing for Lakeland Terriers

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 Lakeland Terrier 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 2023, 137 dogs from the United States (n=127) and Canada (n=10) were tested 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 Lakeland Terriers

A. Breed Description [1,2]

Terriers are defined by the Oxford Dictionary as "various usually small energetic dogs originally used by hunters to dig for small game and engage the quarry underground or drive it out". The name “terrier” is derived from medieval Latin *terra* (Earth). The Lakeland terrier is the smallest of the long-legged, black and tan terriers at 33-38 cm (13-15 inches) at the withers, and weighing 7–8 kg (15–17 pounds). The Lakeland is similar in appearance to the slightly larger Welsh terrier, and the even larger Airedale terrier. Lakeland terriers have a thick bushy wiry outer coat and a soft undercoat and come in a variety of acceptable colors including black, black and tan, blue, blue and tan, liver, and tan, grizzle and tan, red, red grizzle, and wheaten. They have an upright tail that is variably docked depending on custom and laws and small V-shaped ears. The eyes are oval, small and dark, except in liver-colored dogs where they are lighter. The nose and pads of the feet are black except in liver colored dogs where the nose and pad coloring will be brown. Shedding is minimal if properly groomed.

B. Breed History [3-9]

Throughout the 1800’s, different counties in the Lake District of northern England, including Cumberland, Northumberland and Westmoreland, developed a wide variety of small hardworking, broken-coated terriers, each named for their small community of origin. The common ancestor of these small terriers was a now extinct long-legged terrier, with possible introgressions from co-evolving 'breeds' such as Welsh-, Border-, Fox- and Bedlington-terriers. These small terriers assisted their farmer-owners and accompanied larger hounds in finding and destroying foxes, badgers, otters, mice, and other vermin found raiding livestock or disturbing the farm. Their tasks were to mark, flush and even attack vermin in their lairs. Offspring of the Lakeland terriers were prized and often given as gifts to friends and fellow hunters, with the best often incorporated into the new owner’s breeding program. Due to the mountainous and rocky terrain of the Lake District of England, hunting on horseback is difficult and perilous. Therefore, it has been suggested that the Lakeland terrier’s considerable amount of stamina arose from hunting along the ground for long distances while accompanying hounds and hunters on foot. The Lakeland terrier is among those breeds still eligible for sanctioned Earthdog trials in the USA and Canada.

The early working dog versions of the Lakeland terriers were also known as the Fell terriers or Patterdale terriers. Interest in these small regional terriers for agricultural shows grew in the late 1890’s. The dogs were judged by Masters of Hounds and other experienced sportsmen. By 1910, people were talking about a "new and improved Fell Terriers". A terrier breed club was formed in 1912 at the Keswick dog show, out of an organized effort by fanciers to recognize the Cumberland County Terriers. After the difficulties in dog breeding imposed by World War I, Cumberland terriers resurfaced in 1921 when nine breed devotees met at Whitehaven, Cumberland County, and agreed on the name Lakeland terrier. The resultant (and now defunct) Lakeland Terriers Association had initial oversight over the breed. However, members were split on whether Kennel Club recognition was desirable, and it was not until 1928 that a majority favored Kennel Club recognition. The Lakeland terrier first appeared in the show ring in 1928, at Crufts, England. Kennel Club recognition led to the appearance of Lakeland terriers in Kennel Club sanctioned shows and championship status was achieved in 1931. The present Lakeland Terrier Club was founded in 1932 and promoted the breed nationally. One of the breed devotees, Thomas Hosking, later migrated to the United States and the breed was accepted for registration into the American Kennel Club's Stud Book in 1934.

The Lakeland terrier has never been a hugely popular breed in the USA; it currently ranks 143 of 200 in AKC's Breed Popularity Ranking.

C. Temperament [1,2]

Like other terriers, Lakelands are active and bold, sometimes stubborn, vocal, but interact well with owners and all family members. They are not typically shy or aggressive. They are quick to learn and easy to train, although they can get easily distracted when excited. They have a strong prey drive, making them not always ideal around other small animals. Daily exercise and playtimes are strongly recommended as outlets for their energy, as well as to prevent misbehavior and straying. They are said to be mostly hypoallergenic, i.e., non-shedding.

D. Health [1,10,11]

The Lakeland terrier is a healthy breed, with a lifespan of 12 to 16 years. Terrier breeds are prone to chronic allergies, which cause itchy skin and scratching that can lead to bacterial infections (hot spots). Hypothyroidism, heart defects (ventricular septal defects), cryptorchidism, and blood-clotting disease (von Willebrand's) can also occur in Lakeland Terriers, although rare. Several eye disorders occur in the breed including distichiasis, cataracts in older dogs, glaucoma, primary lens-luxation, and persistent pupillary membrane. Legg-Calve-Perthe disease occurs in the breed, as it is widespread among small dogs. However, the incidence is low in Lakeland terriers and appears to be decreasing. Like many dogs, routine dental care is often required to minimize tarter accumulation, periodontal disease and tooth loss. Dental problems may be exacerbated by an abnormal bite (underbite/prognathism). The Canine Inherited Disorders Database (CIDD) does not list any serious disease conditions in Lakeland terriers. They list Legg-Calvé-Perthes disease as a disorder of increasing incidence in the breed. Conditions that are possibly heritable and occur sporadically are cataracts, cryptorchidism, and micropthalmia. They list no disorders associated with conformation.

II. Results on Genetic Diversity of Lakeland Terriers

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

STR markers are multiallelic, 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 [12,13]. 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, catastrophes, 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 137 Lakeland terriers used in this study are listed on **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Lakeland terriers (n=137). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	**AHT137**	**AHTH130**	**AHTh171-A**	**AHTh260**	**AHTk211**
94 (0.255)	131 (0.157)	121 (0.226)	219 (0.047)	238 (0.270)	87 (0.274)
96 (0.004)	**137 (0.398)**	127 (0.281)	225 (0.128)	240 (0.131)	89 (0.051)
100 (0.624)	141 (0.047)	**133 (0.485)**	231 (0.084)	244 (0.150)	91 (0.332)
104 (0.117)	149 (0.102)	137 (0.007)	**235 (0.741)**	246 (0.029)	**95 (0.343)**
	151 (0.296)			248 (0.015)	
				250 (0.405)	

AHTk253	**C22.279**	**FH2001**	**FH2054**	**FH2848**	**INRA21**
286 (0.653)	116 (0.109)	124 (0.004)	**156 (0.500)**	230 (0.004)	95 (0.172)
288 (0.325)	**118 (0.474)**	**132 (0.690)**	160 (0.285)	232 (0.066)	**97 (0.566)**
292 (0.022)	120 (0.033)	144 (0.128)	164 (0.047)	234 (0.069)	99 (0.007)
	124 (0.069)	148 (0.172)	176 (0.164)	238 (0.004)	101 (0.255)
	128 (0.314)	152 (0.007)	180 (0.004)	240 (0.128)	
				244 (0.730)	

INU005	**INU030**	**INU055**	**LEI004**	**REN105L03**	**REN162C04**
106 (0.365)	**144 (0.555)**	**208 (0.482)**	85 (0.084)	**229 (0.672)**	202 (0.332)
124 (0.515)	148 (0.234)	210 (0.175)	**95 (0.847)**	231 (0.109)	**206 (0.664)**
126 (0.120)	150 (0.077)	218 (0.343)	97 (0.069)	233 (0.219)	210 (0.004)
	152 (0.113)				
	156 (0.022)				

REN169D01	**REN169O18**	**REN247M23**	**REN54P11**	**REN64E19**	**VGL0760**
202 (0.117)	164 (0.343)	266 (0.058)	222 (0.358)	139 (0.281)	**12 (0.434)**
210 (0.069)	168 (0.204)	**268 (0.923)**	232 (0.015)	**145 (0.453)**	20.2 (0.113)
216 (0.814)	**170 (0.453)**	270 (0.007)	**236 (0.595)**	147 (0.266)	21.2 (0.007)
		272 (0.011)	238 (0.033)		22.2 (0.131)
					23.2 (0.303)
					24.2 (0.011)

VGL0910	**VGL1063**	**VGL1165**	**VGL1828**	**VGL2009**	**VGL2409**
15 (0.150)	12 (0.066)	16 (0.018)	16 (0.201)	**10 (0.409)**	**11 (0.365)**
15.1 (0.004)	13 (0.007)	21 (0.044)	17 (0.040)	11 (0.164)	13 (0.018)
17.1 (0.036)	14 (0.040)	25 (0.077)	19 (0.343)	13 (0.026)	15 (0.004)
18.1 (0.011)	**15 (0.456)**	26 (0.117)	22 (0.004)	14 (0.369)	17 (0.358)
19.1 (0.697)	16 (0.153)	27 (0.095)	**23 (0.412)**	15 (0.033)	18 (0.252)
20.1 (0.102)	17 (0.007)	**28 (0.485)**			19 (0.004)
	20 (0.215)	29 (0.128)			
	21 (0.055)	30 (0.036)			

VGL2918	**VGL3008**	**VGL3235**

12 (0.299)	15 (0.077)	14 (0.343)
13 (0.365)	17 (0.015)	**15 (0.577)**
14 (0.157)	**18 (0.478)**	18 (0.077)
15 (0.058)	19 (0.416)	19 (0.004)
17.3 (0.055)	20 (0.015)	
19.3 (0.062)		
20.3 (0.004)		

Table 1 shows an allele distribution within each of the 33 autosomal STR loci that is typical of most pure dog breeds, with one or two predominant alleles (bolded on **Table 1**). The lowest number of alleles identified for an STR locus was three; surprisingly, this number was found in 27% of all the loci tested (n=9/33). Conversely, the highest number of STR alleles identified for an autosomal locus was 8 (VGL1063 and VGL1165). The average number of alleles across all STR loci was 4.6 (**Table 2**). Therefore, genetic diversity across the markers assessed in this study in Lakeland terriers is relatively low. Loss of alleles is a common feature of pure dog breeds, and reflects the small number of founders existing at the time a breed registry is closed. Additionally, the disproportionately high incidence of one or two alleles at each locus shows that some founders contributed more of their genetics to the breed than others did due to their highly valued phenotypes (and consequently genotypes). A single allele occurred in 50% or more of the dogs tested at 17 of the 33 loci (**Table 1**). Additionally, one allele occurred in 84.7% of the dogs at locus LEI004, and 92.3% of the dogs at REN247M23. The dominance of these alleles can be explained by inheritance from founders (or founding lines) that contributed significantly to the development of the breed.

B. Assessment of population diversity using standard genetic parameters

Alleles for each of the 33 STR loci listed in Table 1 and their respective frequencies are used to determine basic genetic parameters for the population (**Table 2**). These parameters include the number of alleles found at each locus (**Na**); the number of effective alleles (**Ne**) per locus (i.e., the number of alleles that contribute most to genetic differences/heterozygosity); observed heterozygosity (**Ho**); expected heterozygosity (**He**) if the existing 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 137 Lakeland terriers based on 33 autosomal STR loci. SE = standard error of the mean.

	Na	**Ne**	**Ho**	**He**	**F**
Mean	4.606	2.533	0.570	0.569	-0.002
SE	0.246	0.122	0.025	0.024	0.010

The average number alleles (Na) known to exist at the 33 STR loci across breeds, based on all dog breeds tested at the VGL so far, is 15.4 (see section IIA). In Lakeland terriers, this number was estimated at 4.6 (**Table 2**). Therefore, approximately 30% of the known canid diversity at these 33 loci has been retained during development and evolution of the Lakeland terrier breed. This figure is lower than that of more modern terrier breeds tested at the VGL, such as the Rat terrier (49.8%) and American Hairless terrier (49.4%).

However, the average number of effective alleles (Ne) constitutes a more important metric for diversity, since these alleles have the greatest genetic influence on heterozygosity. This number was estimated at 2.53 for this cohort, indicating that genetic diversity for Lakeland terriers is determined by roughly half of the alleles segregating in the breed. The Ne value for Lakeland terriers is amongst the lowest estimated for breeds tested at the VGL.

The mean observed heterozygosity (Ho=0.570) was similar to the estimated expected heterozygosity (He=0.569), thus yielding an inbreeding coefficient (F) of approximately zero (F=-0.002, **Table 2)**. This F value indicates that, despite the low allelic diversity found across autosomal STR markers in Lakeland terriers, the cohort tested in this study is in Hardy-Weinberg equilibrium (HWE); i.e., this group of dogs constitutes a random mating population because breeders are doing a good job in picking unrelated sires and dams from the population.

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

The average heterozygosity values for this group of Lakeland terriers indicates that parents are being selected on a random basis from the population. If this were true, one would expect that each region of the genome interrogated by an STR locus would also be in HWE. The data in **Table 3**, which is based on standard genetic assessments of alleles at single STR loci, indicates that this is not the case. In Lakeland terriers, the average number of effective alleles (Ne) ranged from 1.17 (REN247M23) to 3.88 alleles per locus (VGL2918). The lowest average observed heterozygosity (Ho) for an individual STR locus was 0.15 (REN247M23), whereas the highest was estimated at 0.75 (AHTk211). Average expected heterozygosity (He) values ranged from 0.14 (REN247M23) to 0.74 (VGL2918) (**Table 3**).

Loci with the lowest Ho values contribute the least to heterozygosity levels across the breed; they are most likely associated with inherited traits that are important for the breed's phenotypic standard (and thus tend to vary less across individuals). Conversely, high Ho values for a particular locus means that it shows greater genetic diversity across the breed, and that these loci can be associated with phenotypic variation among individuals. The values for Ho and He are used to calculate what is known as inbreeding coefficient (or F), which is a measure of how near that locus is to Hardy-Weinberg equilibrium (HWE). An F value of zero signifies that a population is in HWE, or in other words, is randomly breeding (no artificial selection). Positive F values indicate non-random selection (inbreeding), while negative values indicate outbreeding (increased heterozygosity).

Table 3. Standard Genetic Assessment of individual STR loci for 137 Lakeland terriers. Individual STR loci with high inbreeding coefficients (F > 0.1) are bolded; loci with F values ≤ -0.1 are highlighted in light grey.

Locus	Na	Ne	Ho	He	F
AHT121	4	2.14	0.489	0.532	0.08
AHT137	5	3.53	0.715	0.717	0.002
AHTH130	4	2.73	0.599	0.634	0.056
AHTh171-A	4	1.74	0.438	0.425	-0.03
AHTh260	6	3.6	0.737	0.722	-0.02
AHTk211	4	3.27	0.752	0.694	-0.08
AHTk253	3	1.88	0.438	0.467	0.063
C22.279	5	2.93	0.62	0.659	0.058
FH2001	5	1.92	0.482	0.478	-0.01
FH2054	5	2.78	0.664	0.64	-0.04
FH2848	6	1.79	0.482	0.442	-0.09
INRA21	4	2.41	0.555	0.585	0.052
INU005	3	2.42	0.62	0.587	-0.06
INU030	**5**	**2.62**	**0.56**	**0.62**	**0.1**
INU055	3	2.63	0.679	0.62	-0.1
LEI004	3	1.37	0.285	0.271	-0.05
REN105L03	**3**	**1.96**	**0.44**	**0.49**	**0.11**
REN162C04	3	1.81	0.453	0.448	-0.01
REN169D01	3	1.47	0.314	0.319	0.017
REN169O18	3	2.75	0.628	0.636	0.013
REN247M23	4	1.17	0.153	0.144	-0.07
REN54P11	4	2.07	0.489	0.517	0.054
REN64E19	3	2.82	0.73	0.645	-0.13
VGL0760	6	3.22	0.679	0.689	0.015
VGL0910	6	1.92	0.489	0.48	-0.02
VGL1063	8	3.48	0.737	0.713	-0.03
VGL1165	8	3.52	0.723	0.716	-0.01
VGL1828	5	3.03	0.635	0.67	0.053
VGL2009	5	3.02	0.664	0.668	0.006
VGL2409	6	3.08	0.73	0.675	-0.08
VGL2918	7	3.88	0.715	0.742	0.036
VGL3008	5	2.45	0.591	0.592	0.001
VGL3235	4	2.19	0.518	0.544	0.047

According to **Table 3**, high inbreeding coefficients (F > 0.1) were estimated for two of the 33 STR loci (INU030 and REN105L03). This suggests that these loci have been under strong positive selection since breed development and might be associated with breed-defining phenotypic traits. Conversely, F values around or below zero were estimated for the remaining loci, with two of those loci (INU055 and REN64E19, highlighted in grey) having F values ≤ -0.1. They contain alleles that are likely to be found among the more outbred dogs. The negative and positive effects of these loci balance each other and are why the average inbreeding coefficient (F) reported in **Table 2** is close to zero.

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

PCoA measures the genetic relatedness of individuals within a population. The 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. The 137 Lakeland terriers in this study clustered as expected for a pure dog breed on the PCoA plot, with all individuals reasonably dispersed across all four quadrants (**Figure 1**). A few pairs of individuals (red circles) were found to be more closely related to each other compared to the population-at-large based on 33 autosomal STR markers.

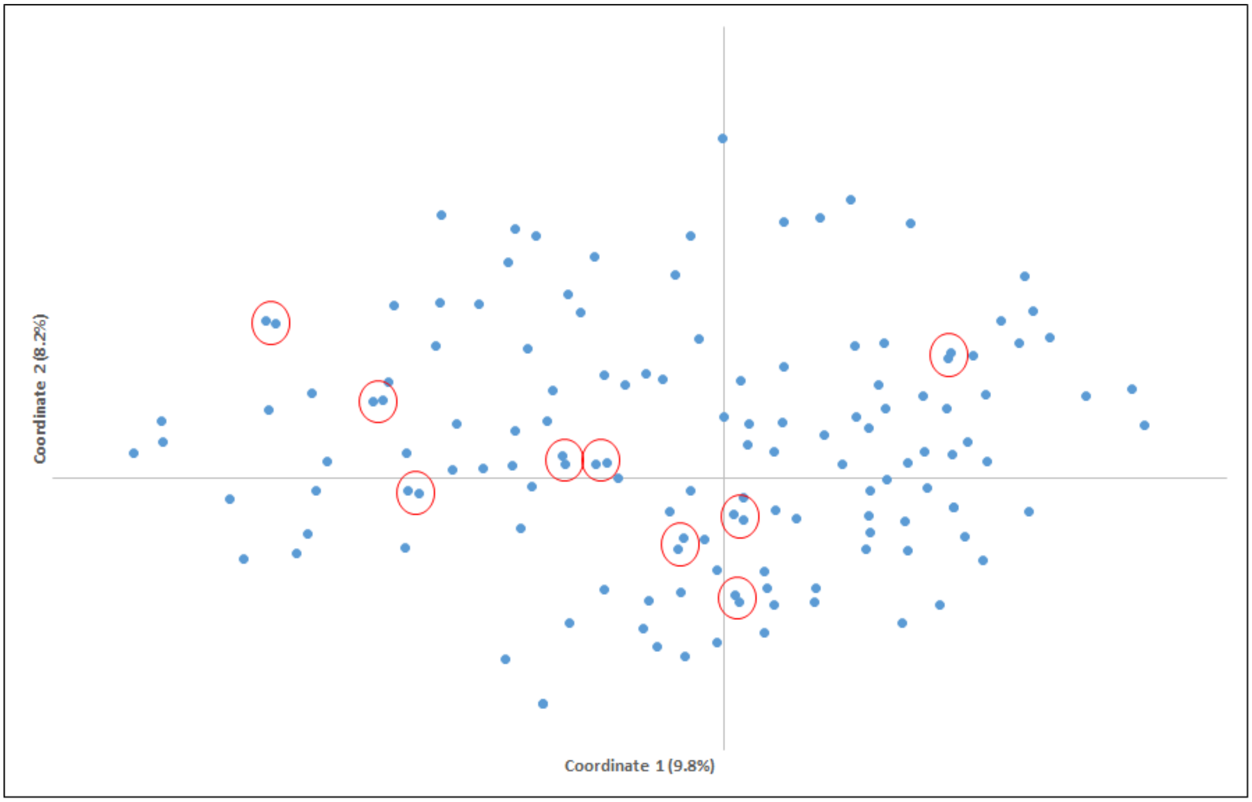


Figure 1. PCoA of Lakeland terriers (n = 137) based on alleles and allele frequencies at 33 autosomal STR loci. Red circles indicate closely related pairs of individuals.

The degree of intra- and inter-breed relatedness can be further assessed by generating a PCoA of the 137 Lakeland terriers with closely related breeds (Black Russian terrier and Giant Schnauzer) [14] (**Figure 2**). Inter-breed clustering shows separate and well-defined populations, indicating that the breeds are genetically distinct as expected. Interestingly, the two terrier breeds cluster together on top of the graph along the Y axis whereas Giant Schnauzers (grey dots) appear in towards the bottom of the graph, with a few individuals clustering between the two terrier breeds. This graph also highlights the relative lack of genetic diversity of the two terrier breeds when compared to Giant Schnauzers due to their tighter clustering in relation to the latter, which disperses further along the Y axis (**Figure 2**).

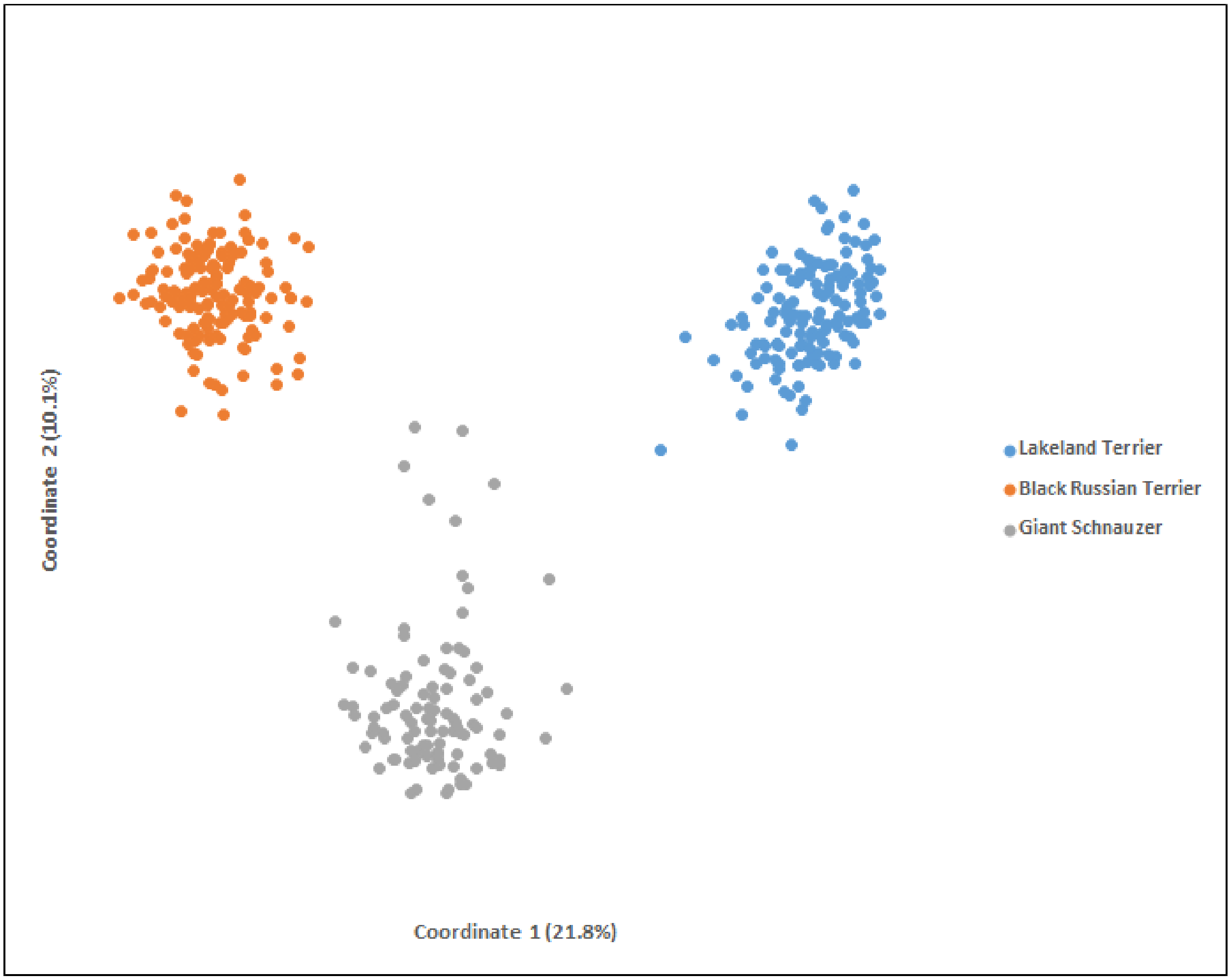


Figure 2. PCoA plot of Lakeland terrier (blue dots), Black Russian terrier (orange dots), and Giant Schnauzer (grey dots).

E. Internal relatedness (IR) scores for Lakeland terriers

1. IR testing and meaning

Genetic assessments such as those presented in Tables 1-3 are indicators of breed-wide heterozygosity and do not reflect the genetic diversity inherited by individuals from their parents. Internal Relatedness (IR) is a calculation that has been 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 and IRVD (see next section) values for the 137 Lakeland terriers.

Table 4. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele frequencies for 33 STR loci in 137 Lakeland terriers.

	IR	**IRVD**
Minimum	-0.3019	0.0449
1st Quartile	-0.0991	0.2615
Mean	0.0001	0.3492
Median	-0.0085	0.3580
3rd Quartile	0.1012	0.4329
Maximum	0.3566	0.6849

IR scores for the 137 Lakeland terriers ranged from a low of -0.3 (most outbred/parents least related) to a high of 0.36 (most inbred/parents most related), with an average IR value of approximately zero for the cohort. One-fourth of the dogs had IR scores from approximately -0.3 to -0.1; these individuals were more outbred than the average Lakeland terrier. Conversely, one-fourth of the individuals had IR values ranging from 0.1 to 0.35 and were more inbred. Therefore, even though the standard genetic assessment indicated that this group of dogs was from a randomly breeding population, one quarter of the individuals were more outbred than the average and one-fourth more inbred. This wide range of IR values highlights the importance of this genetic diversity metric for mate selection in order to maintain and re-distribute diversity within the breed. This is especially important in a breed with relatively low existing diversity, such as the Lakeland terrier.

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 is lost as a breed evolves from its oldest common ancestors to the 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 3**, blue line).

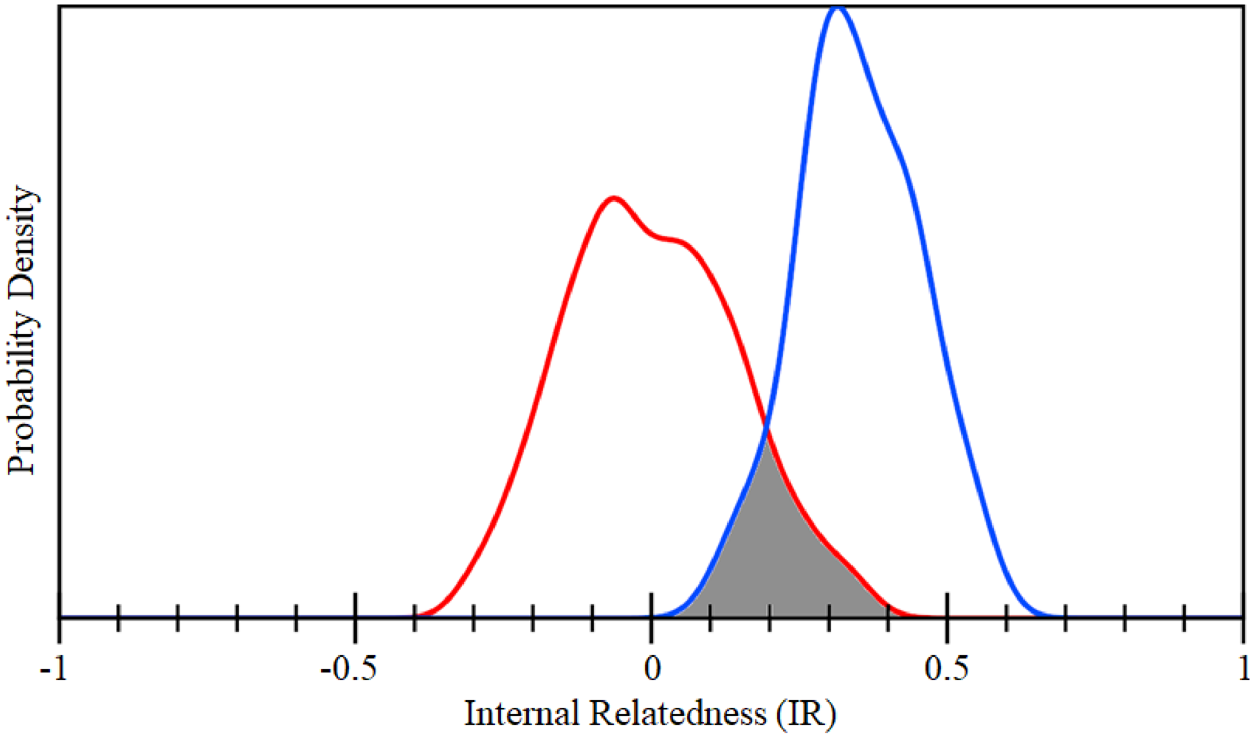


Figure 3. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Lakeland terrier (n=137). The overlap between the curves (gray area) shows that the breed retains 16.7% of the genetic diversity existing in randomly breeding village dogs.

The mean IRVD value was approximately 0.35 for the population, with at least one dog having an IRVD value as low as 0.04 (most outbred) and at least one dog with a value as high as 0.68 (most inbred) (**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 IRVD values are considerably higher than the IR values and reflect the relatedness of these 137 dogs if they were found among a much larger population of village dogs. In essence, this is how breeds are created. A subpopulation of dogs that have the desired traits are removed from the larger population and then further inbred to fix the trait. Based on the IRVD values, three-fourths of the Lakeland terriers appear to be inbred to a much higher degree than indicated by IR values alone.

Finally, the overlapping area of the two curves is an estimate of how much genetic diversity was lost in the creation of a breed. The estimated retention of all available canid genetic diversity in Lakeland terrier was only 16.7%, which is approximately half of the 30% retained genetic diversity calculated for all canids tested at VGL (**section IIB**). More diverse breeds, such as the Labrador Retriever and Toy Poodle, for example, have retained 54% and 60% of the canine genetic diversity, respectively.

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. 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 DLA class I and II genes are strongly linked, and often inherited as a single haplotype (**Table 5**). 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 Lakeland terriers

Seven DLA class I and eight DLA class II haplotypes were identified in Lakeland terriers (**Table 5**); these numbers are amongst the lowest ever identified for a breed at the VGL. Notably, two DLA class I (1012 and 1040) and two DLA class II haplotypes (2003 and 2022) were identified in approximately 95% of the dogs when combined. This means that almost all contemporary North American Lakeland terriers descended from a single founder or founder line. It remains to be determined whether this loss of DLA diversity occurred at the level of the original founders in England, or from artificial genetic bottlenecks that occurred after the registry was closed.

Table 5. DLA class I and II haplotypes identified in Lakeland terriers (n = 137) with their respective frequencies. Haplotypes with the highest frequency are bolded.

DLA1 Haplotype	STR types	Frequency (%)
1012	**388 369 289 188**	**76.3**
1016	382 371 277 178	0.7
1040	**380 371 277 186**	**18.2**
1052	380 372 289 184	1.1
1087	380 371 277 178	0.4
1104	386 373 289 186	2.9
1159	395 379 277 181	0.4
DLA2 Haplotype	**STR types**	**Frequency (%)**
2003	**343 324 282**	**76.3**
2005	339 322 280	0.4
2014	339 322 284	0.7
2015	339 327 280	0.7
2017	343 322 280	2.9
2022	**339 327 282**	**17.5**
2031	339 322 282	0.4
2067	343 322 284	1.1

DLA class I and II regions are in linkage (i.e., are inherited together), forming distinct extended haplotypes. Such examples can be clearly seen in Lakeland terriers based on allele frequencies estimated for DLA class I and II haplotypes. The extended haplotype 1012/2003 was found in 76.3% of the individuals tested, whereas the 1040/2022 combination was identified in approximately 18% of the dogs.

Haplotype sharing between Lakeland terriers and other dog breeds was also assessed in this study (**Table 6**). The combined 1012/2003 haplotype was strongly shared with the Greater Swiss Mountain Dog (92.4%), and to a lesser extent with the English Bulldog and Biewer/Biewer terrier. It is not surprising to see the relationships between Lakeland terriers and many other dog breeds of European origin. The relationship with the Biewer/Biewer terrier was expected; however, the English bulldog relationship is less obvious, and may be explained by early descriptions of the Bulldog as a small aggressive bull-baiting type dog. This activity is similar to the tenacious English-bred terriers called upon to attack vermin in their lairs. The reason for the substantial haplotype sharing between Lakeland terrier and Greater Swiss Mountain Dog remains elusive, and might be attributed to a remote common ancestor of European origin.

Table 6. Sharing of DLA class I and II haplotypes between Lakeland terriers (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=51).

DLA1 #	STR types	Lakeland Terrier (n=137)	American Eskimo, Standard (n=65)	American Hairless Terrier (n=186)	American Akita (n=160)	Japanese Akita (n=582)	Alaskan Klee Kai (n=657)	Barbet (n=68)	Border Collie (n=65)	Berger Picard (n=148)	Bernese Mountain Dog (n=151)	Black Russian Terrier (n=150)	Biewer (n=137)	Biewer Yorshire Terrier (n=54)	Biewer Terrier (n=219)	Borzoi (n=150)	Chinook (n=35)	Collie (n=49)	Doberman Pinscher (n=1241)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=869)	Great Dane (n=74)	Golden Retriever (n=880)	German Shepherd (n=38)	Giant Schnauzer (n=331)	Havana Silk (n=46)
1012	388 369 289 188	0.763	0.023	0.005	--	--	--	--	0.054	--	--	--	0.226	0.157	0.228	--	0.14	--	0.0008	0.414	--	--	--	--	0.0011	--	--	--
1016	382 371 277 178	0.007	0.008	0.005	--	--	--	--	--	--	0.043	0.01	0.022	0.019	0.011	--	--	0.04	0.0205	0.095	0.15	0.14	--	0.209	0.0045	--	0.045	0.1
1040	380 371 277 186	0.182	--	0.043	0.006	--	0.2123	--	--	--	--	--	0.095	0.204	0.078	--	--	--	0.0089	0.04	--	--	--	--	0.0006	--	--	0.01
1052	380 372 289 184	0.011	--	0.022	--	--	--	--	0.015	0.105	--	--	--	--	--	--	0.01	--	0.0008	--	0.02	--	--	--	--	0.47	--	--
1087	380 371 277 178	0.004	0.008	--	0.013	--	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1104	386 373 289 186	0.029	--	--	--	--	--	--	0.131	--	--	--	0.011	0.019	0.014	--	--	--	--	0.003	--	--	--	--	--	--	--	--
1159	395 379 277 181	0.004	--	--	--	--	--	0.103	--	--	--	--	--	--	--	0.047	--	--	0.004	--	--	--	--	--	--	--	0.165	--

DLA1 # (cont'd)	STR types (cont'd)	Lakeland Terrier (n=137)	Havanese (n=998)	Italian Greyhound (n=1401)	Irish Setter (n=60)	Irish Red and White Setter (n=107)	Irish Wolfhound (n=78)	Labrador Retriever (n=295)	Llewellin Setter (n=152)	Magyar Agar (n=78)	Newfoundland (n=139)	Polish Lowland Sheepdog (n=83)	Toy Poodle (n=234)	Miniature Poodle (n=419)	Poodle (n=5191)	Rat Terrier (n=44)	Samoyed (n=192)	Saint Bernard (n=90)	Scottish Collie (n=120)	Shiba Inu (n=162)	Shikoku (n=85)	Greater Swiss Mountain Dog (n=59)	Shiloh Shepherd (n=68)	Shiloh Shepherd, ISSA (n=272)	Swedish Vallhund (n=525)	Cardigan Welsh Corgi (n=43)	Whippet (n=110)
1012	388 369 289 188	0.763	0.012	0.0082	--	--	--	--	--	--	--	--	0.021	0.073	0.01493	0.06	0.013	--	0.008	--	--	0.924	--	--	--	0.23	0.036
1016	382 371 277 178	0.007	0.1924	0.0635	--	--	--	0.015	0.03	--	0.327	0.024	0.024	0.027	0.02167	0.05	--	--	0.092	--	--	0.076	--	--	--	--	0.105
1040	380 371 277 186	0.182	0.0155	0.0782	--	--	--	--	--	--	0.277	0.687	0.002	0.005	0.00058	0.17	--	0.011	--	--	--	--	--	--	--	--	0.086
1052	380 372 289 184	0.011	0.0105	0.1867	--	--	0.333	--	--	0.051	--	--	--	--	0.00029	--	--	--	--	--	--	--	0.309	0.382	--	--	--
1087	380 371 277 178	0.004	0.0035	--	--	--	--	0.002	--	--	0.004	--	--	--	--	0.11	--	--	0.017	--	--	--	--	--	--	--	--
1104	386 373 289 186	0.029	--	0.0025	--	--	0.006	--	--	0.128	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.19	0.155
1159	395 379 277 181	0.004	--	--	--	--	--	--	--	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA2 #	STR types	Lakeland Terrier (n=137)	American Eskimo, Standard (n=65)	American Hairless Terrier (n=186)	American Akita (n=160)	Japanese Akita (n=582)	Alaskan Klee Kai (n=657)	Barbet (n=68)	Border Collie (n=65)	Berger Picard (n=148)	Bernese Mountain Dog (n=151)	Black Russian Terrier (n=150)	Biewer (n=137)	Biewer Yorshire Terrier (n=54)	Biewer Terrier (n=219)	Borzoi (n=150)	Chinook (n=35)	Collie (n=49)	Doberman Pinscher (n=1241)	English Bulldog (n=163)	English Mastiff (n=31)	Mastiff (n=21)	Flat Coated Retriever (n=869)	Great Dane (n=74)	Golden Retriever (n=880)	German Shepherd (n=38)	Giant Schnauzer (n=331)	Havana Silk (n=46)
2003	343 324 282	0.763	0.023	0.263	--	--	--	0.132	0.054	--	--	--	0.263	0.213	0.249	--	0.14	--	0.0004	0.598	--	--	0.1461	0.007	0.0193	0.01	0.03	0.25
2005	339 322 280	0.004	--	--	--	--	--	--	0.008	--	0.146	0.013	0.04	0.009	0.023	--	--	--	--	0.015	--	--	0.4177	0.054	0.0136	--	0.008	0.03
2014	339 322 284	0.007	0.008	0.005	--	--	0.067	--	--	--	--	--	--	--	--	--	--	--	--	0.092	0.15	0.14	0.0282	0.034	--	--	0.005	--
2015	339 327 280	0.007	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2017	343 322 280	0.029	--	0.005	0.009	0.0034	--	0.029	0.169	0.105	--	--	--	--	--	0.007	0.01	--	--	0.215	0.21	0.29	0.0006	--	0.0386	0.43	0.002	--
2022	339 327 282	0.175	0.023	0.032	--	--	--	--	0.008	--	--	--	--	--	--	0.587	--	--	0.0008	0.015	--	--	0.1139	0.101	0.0006	0.13	0.003	0.07
2031	339 322 282	0.004	--	--	--	--	--	--	0.008	--	--	0.033	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.041	--
2067	343 322 284	0.011	--	0.022	--	--	--	--	0.069	0.895	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.04	--	--

DLA2 # (cont'd)	STR types (cont'd)	Lakeland Terrier (n=137)	Havanese (n=998)	Italian Greyhound (n=1401)	Irish Setter (n=60)	Irish Red and White Setter (n=107)	Irish Wolfhound (n=78)	Labrador Retriever (n=295)	Llewellin Setter (n=152)	Magyar Agar (n=78)	Newfoundland (n=139)	Polish Lowland Sheepdog (n=83)	Toy Poodle (n=234)	Miniature Poodle (n=419)	Poodle (n=5191)	Rat Terrier (n=44)	Samoyed (n=192)	Saint Bernard (n=90)	Scottish Collie (n=120)	Shiba Inu (n=162)	Shikoku (n=85)	Greater Swiss Mountain Dog (n=59)	Shiloh Shepherd (n=68)	Shiloh Shepherd, ISSA (n=272)	Swedish Vallhund (n=525)	Cardigan Welsh Corgi (n=43)	Whippet (n=110)
2003	343 324 282	0.763	0.2134	0.0075	--	--	--	0.022	--	--	0.004	--	0.425	0.511	0.10441	0.13	0.013	--	0.017	--	--	0.924	--	0.009	--	0.23	0.086
2005	339 322 280	0.004	0.002	--	0.092	0.131	0.199	0.056	0.78	--	--	--	0.002	--	0.01609	0.07	--	0.067	--	--	--	--	--	--	--	--	--
2014	339 322 284	0.007	0.004	0.0014	--	--	--	0.002	--	--	0.349	0.711	0.021	0.026	0.01994	0.02	--	0.117	--	--	--	--	--	--	--	--	--
2015	339 327 280	0.007	--	0.01	0.142	--	--	--	0.003	--	--	--	0.045	0.039	0.00414	0.01	0.016	--	--	0.052	--	--	--	--	--	--	--
2017	343 322 280	0.029	0.011	0.2127	--	--	--	--	--	0.449	--	0.271	0.002	0.001	0.00212	0.02	--	--	--	--	--	--	0.309	0.382	--	0.19	0.241
2022	339 327 282	0.175	0.1137	--	0.15	--	0.353	0.059	0.092	--	--	--	0.006	0.002	0.00048	0.14	0.107	--	--	--	--	--	0.044	0.042	0.0562	--	--
2031	339 322 282	0.004	--	0.0632	--	--	0.013	0.012	--	--	--	--	--	--	--	0.14	--	--	0.017	--	--	--	--	--	--	--	0.105
2067	343 322 284	0.011	--	0.0004	--	--	--	--	--	--	--	0.006	--	0.006	--	--	--	--	--	0.216	0.012	--	--	--	--	--	--

2. Heterozygosity in the DLA region

Due to their physical proximity in canine chromosome 12, the seven loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium (i.e., have a higher probability of being inherited together) when compared to other parts of the genome. 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 as well. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) and averaged across all loci (**Table 8**).

The number of alleles (Na) identified at each DLA locus in Lakeland terriers was low, ranging from 2 (DLA I-4BCT and 5ACA) to 5 (DLA I-3CCA, DLA I-4ACA, and DLA1131). As observed in the 33 STR loci across the genome, the number of effective alleles (Ne) per DLA locus was low, ranging from 1.13 (5BCA) to 1.62 (5ACT). The average observed heterozygosity (Ho) at each locus was similar to the expected heterozygosity (He), yielding F values around zero (**Table 7**).

Table 7. Standard genetic assessment for Lakeland terriers (n=137) using each of the 7 STRs in the DLA class I and II regions.

Locus	Na	Ne	Ho	He	F
DLA I-3CCA	5	1.609	0.387	0.378	-0.02
DLA I-4ACA	5	1.612	0.38	0.38	0.001
DLA I-4BCT	2	1.463	0.307	0.316	0.031
DLA1131	5	1.595	0.387	0.373	-0.04
5ACA	2	1.463	0.307	0.316	0.031
5ACT	3	1.618	0.394	0.382	-0.03
5BCA	3	1.125	0.117	0.111	-0.05

The average population-wide inbreeding coefficient based on DLA loci was estimated at -0.01 (**Table 8**), a value that was comparable to that estimated using 33 autosomal STR loci (F=-0.002, **Table 2**). This suggests that the DLA region is not in equilibrium with the genome at large, as expected.

Table 8. Summary of standard genetic assessment for Lakeland terriers (n=137) using seven STRs in the DLA class I and II regions. SE = standard error of the mean.

	Na	Ne	Ho	He	F
Mean	3.57	1.498	0.325	0.323	-0.011
SE	0.49	0.062	0.035	0.034	0.011

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

This study of 137 Lakeland terriers suggests that the breed is lacking in genetic diversity. Although it is possible that additional diversity will be discovered as more dogs from diverse regions are tested, any additional autosomal STR alleles or DLA class I and II haplotypes will be at a comparatively low incidence. In addition, this group of 137 dogs is entirely from North America and may not reflect the genetic diversity existing in dogs from other parts of the world. It would be particularly important to test Lakeland terriers hailing from historical regions, such as the UK or areas where they became established at an earlier time than North American dogs.

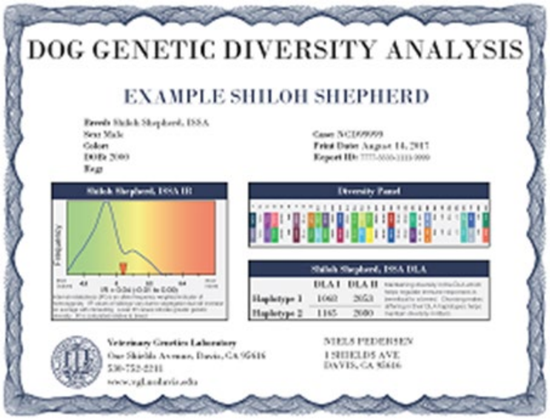
It is important to note that limited genetic diversity is not in itself a bad thing. If the founding population was genetically healthy, the original breed standard strictly adhered to, a reasonable population size sustained, and HWE maintained, the breed should survive in good health. Except for population size, these characteristics have been maintained in the Lakeland terrier. The extreme loss of genetic diversity in the DLA region is worrisome but more so if the lost diversity persists and is associated with disorders linked to specific DLA types. Such disorders usually involve autoimmune diseases, auto inflammatory conditions, increased susceptibility to skin and intestinal allergies, and lack of resistance to certain infections. The breed appears to be historically free, or relatively free, of these types of disorders. Hypothyroidism and skin allergies would be the exceptions. Therefore, DLA homogeneity in Lakeland terriers may be more a long-term theoretical issue than real problem.

Breeds with low genetic diversity are more prone to genetic disorders as well as autosomal recessive mutations, especially when subjected to periods of strong artificial selection for desired conformation traits. Many of these types of disorders involve popular sire effects. A low level of genetic diversity also makes it more difficult, and even impossible, to eliminate genetic mutations from a breed.

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.



DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE SHILOH SHEPHERD

Breed: Shiloh Shepherd, ISSA

Sex: Male

Case: NCD99999

Print Date: August 14, 2017

	DLA I	DLA II
Haplotype 1	1062	2053
Haplotype 2	1165	2080

Veterinary Genetics Laboratory
One Shields Avenue, Davis, CA 95616
530-752-2211
www.vgl.ucdavis.edu

NIELS PEDERSEN
1 SHIELDS AVE
DAVIS, CA 95616

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. <u>However, because IR values reflect the unique genetics of individuals, they cannot be used as the primary criterion for selecting ideal mates.</u> 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

1. Porter R. Profile of the Lakeland Terriers. https://web.archive.org/web/20120312174843/http://lakelandterriersclub.org.uk/profile.htm

2. Wikipedia. Lakeland Terriers. https://en.wikipedia.org/wiki/Lakeland_Terriers

3. The Kennel Club, Breed Information Centre, Lakeland Terriers. https://www.thekennelclub.org.uk/services/public/breed/display.aspx?id=3072.

4. American Kennel Club. AKC Dog Registration Statistics. 2011. https://web.archive.org/web/20120511200854/http://www.akc.org/reg/dogreg_stats.cfm.

5. American Kennel Club. Most popular dog breeds - full ranking list. 2018. https://www.akc.org/expert-advice/news/most-popular-dog-breeds-full-ranking-list/

6. Wikipedia. Earthdog Trial. https://en.wikipedia.org/wiki/Earthdog_trial

7. Pets4Homes. Lakeland Terriers. https://www.pets4homes.co.uk/dog-breeds/lakeland-terriers/.

8. Lewis TW, Abhayaratne BM, Blott SC. 2015. Trends in genetic diversity for all Kennel Club registered pedigree dog breeds. *Canine Genetics and Epidemiology* 2:13. Doi 10.1186/s40575-015-0027-4.

9. Petwave. Lakeland Terriers - History and Health. https://www.petwave.com/Dogs/Breeds/Lakeland-Terriers/Overview.aspx

10. Petwave. Lakeland Terriers - History and Health. https://www.petwave.com/Dogs/Breeds/Lakeland-Terriers/Overview.aspx.

11. Canine Inherited Disorders Database (CIDD), Lakeland terriers. http://cidd.discoveryspace.ca/breed/lakeland-terriers.html

12. Pedersen NC, Pooch AS, Liu H. A genetic assessment of the English bulldog. *Canine Genet Epidemiol.* 2016; 3:6. doi: 10.1186/s40575-016-0036-y.

13. Pedersen NC, Shope B, Liu H. An autosomal recessive mutation in *SCL24A4* causing enamel hypoplasia in Samoyed and its relationship to breed-wide genetic diversity. *Canine Genet Epidemiol.* 2017; 4:11. doi: 10.1186/s40575-017-0049-1.

14. Parker HG, Dreger DL, Rimbault M, Davis BW, Mullen AB, Carpintero-Ramirez G, Ostrander EA. Genomic Analyses Reveal the Influence of Geographic Origin, Migration, and Hybridization on Modern Dog Breed Development. *Cell Rep.* 2017; 19(4):697-708. doi: 10.1016/j.celrep.2017.03.079.

This report was generated by Felipe Avila and Shayne Hughes on 11/03//2023.