

# 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 that will measure 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 will be useful to dog breeders who wish to use DNA-based testing as a supplement to in-depth pedigrees.

DNA-based testing of the Lakeland terrier from the US and Canada is now in the preliminary results phase with the objective of building a snap-shot of individual- and breed-wide genetic heterogeneity and diversity. This data base will be progressively expanded as more dogs are added with the goal of identifying all the known alleles for the breed at 33 STR loci as well as all existing DLA class I and II haplotypes. We are accepting Lakeland terriers from all parts of the world with a goal of at least 100-150 individuals.

The 48 Lakeland terriers in this study were exclusively from North America, with eight from Canada and 40 from the US. The dogs were from 11 different owners/breeders. These 48 initial dogs may not identify all the genetic diversity that exists in the breed or accurately portray relatedness between individual or population heterogeneity. However, based on work with other breeds, these 48 dogs will identify all major STR alleles and DLA class I and II haplotypes, and any additional types are likely of low incidence.

**Price for initial phase testing: \$50**

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### **Results reported as:**

Short tandem repeat (STR) loci: A total of 33 STR loci from 25/38 autosomes were used to gauge genetic heterogeneity and diversity within an individual and across the breed. Many of the STRs in this panel have been validated for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and others developed by the VGL for forensic purposes. STR loci are highly polymorphic and have great power to determine genetic differences among individuals and breeds.

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

Internal Relatedness: 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 and two individuals from different sources may have identical IR values but a very different genetic makeup.

## **I. Introduction**

### **A. Breed description**

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 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 7–8 kg (15–17lb) The Lakeland is similar in appearance to the slightly larger Welsh Terrier and even larger Airedale (2). Lakeland terriers have a thick bushy wiry outer coat and a soft undercoat and comes 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**

Throughout the 1800s, 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 (1, 2, 9). The common ancestor of these small terriers was a now extinct long-legged terriers with possible introgressions with co-evolving 'breeds' such as Welsh-, Border-, Fox- and Bedlington-terriers (3). These small terriers assisted their farmer-owners and accompanied larger hounds in finding and destroying foxes, badgers, otters 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. Hunting along the ground (earth) for long distances while accompanying hounds required a considerable amount of stamina, and Lakeland terriers is among those breeds still eligible for sanctioned Earthdog trials in the USA and Canada (6).

The early working dog versions of the Lakeland terriers were also known as the Fell Terriers or Patterdale Terriers (2). 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 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. World War I intervened, and the Cumberland Terriers resurfaced in the 1921, when nine breed devotees met at Whitehaven, Cumberland County, and agreed on the name Lakeland Terriers. 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 popular in the USA and its ranking by the AKC has varied 126th in 2001 to 138th out of 192 recognized dog breeds in 2017 (4,5). Its popularity in the UK has declined and it now ranks 73 among 244 dog breeds (7). The Lakeland terrier breed is listed along with 114 of 173 UK breeds as at "critical risk for extinction" based on the small number of registered breeding dogs ( $n \leq 115$ ) (8).

### C. Breed temperament

Like other terriers, Lakelands are active and bold, sometimes stubborn, vocal, but interact well with owners and all family members (2). Shyness is atypical, as is aggressiveness. They are quick to learn and easy to train, although their responses to command can be easily. 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 and to prevent mis-behavior and straying. They are said to be mostly hypo-allergenic, i.e., non-shedding.

## II. Baseline genetic diversity testing and what it tells us about the Lakeland terriers

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

#### 1. Allele and allele frequencies for each of the 33 STR loci

Alleles and their incidence at each of the 33 STR loci are given in Table 1. From three to eight alleles were detected at each of the loci, which is lower than found in most other breeds. The percentage of known dog/wolf alleles at each of the 33 loci that were identified among these 48 dogs ranged from 13 to 57% (average 23%) (Table 1).

**Table 1.** Allele designation and frequency at 33 STR loci in 48 Lakeland terriers. The number of alleles known to exist in all dogs and wolves that have been tested is listed in parenthesis and the percentage of known alleles present at each locus given after the parenthesis.

[Link to Table 1](#)

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AHT121 (24) 15%	AHT137 (17) 29%	AHTH130 (20) 15%	AHTH171-A (14) 28%	AHTH260 (28) 21%	AHTk211 (7) 57%
94 (0.24)	131 (0.15)	121 (0.26)	219 (0.04)	238 (0.35)	87 (0.24)
96 (0.01)	137 (0.34)	127 (0.25)	225 (0.14)	240 (0.11)	89 (0.07)

100 (0.57)	141 (0.06)	133 (0.49)	231 (0.09)	244 (0.21)	91 (0.36)
104 (0.18)	149 (0.04)		235 (0.73)	246 (0.02)	95 (0.32)
	151 (0.41)			248 (0.03)	
				250 (0.27)	

AHTk253 (11) 27%	C22.279 (13) 31%	FH2001 (17) 24%	FH2054 (23) 17%	FH2848 (24) 21%	INRA21 (15) 27%
286 (0.66)	116 (0.09)	132 (0.73)	156 (0.57)	230 (0.01)	95 (0.06)
288 (0.33)	118 (0.47)	144 (0.07)	160 (0.26)	232 (0.10)	97 (0.68)
292 (0.01)	124 (0.03)	148 (0.19)	164 (0.01)	234 (0.03)	99 (0.02)
	128 (0.41)	152 (0.01)	176 (0.16)	240 (0.17)	101 (0.24)
				244 (0.69)	

INU005 (11) 27%	INU030 (14) 36%	INU055 (11) 27%	LEI004 (15) 20%	REN105L03 (22) 14%	REN162C04 (14) 14%
106 (0.44)	144 (0.60)	208 (0.55)	85 (0.04)	229 (0.63)	202 (0.31)
124 (0.46)	148 (0.21)	210 (0.15)	95 (0.85)	231 (0.14)	206 (0.69)
126 (0.10)	150 (0.08)	218 (0.30)	97 (0.10)	233 (0.24)	
	152 (0.09)				
	156 (0.01)				

REN169D01 (14) 21%	REN169O18 (14) 21%	REN247M23 (11) 27%	REN54P11 (14) 29%	REN64E19 (12) 25%	VGL0760 (26) 19%
202 (0.13)	164 (0.31)	266 (0.06)	222 (0.34)	139 (0.28)	12 (0.45)
210 (0.05)	168 (0.29)	268 (0.93)	232 (0.02)	145 (0.51)	20.2 (0.05)
216 (0.82)	170 (0.40)	270 (0.01)	236 (0.61)	147 (0.21)	22.2 (0.17)
			238 (0.02)		23.2 (0.32)
					24.2 (0.01)

VGL0910 (27) 22%	VGL1063 (17) 35%	VGL1165 (23) 13%	VGL1828 (22) 42%	VGL2009 (12) 25%	VGL2409 (13) 31%
15 (0.13)	12 (0.09)	16 (0.03)	16 (0.23)	10 (0.50)	11 (0.31)

15.1 (0.01)	13 (0.02)	21 (0.06)	17 (0.03)	11 (0.11)	13 (0.01)
17.1 (0.04)	14 (0.06)	25 (0.07)	19 (0.36)	13 (0.03)	17 (0.38)
18.1 (0.02)	15 (0.56)	26 (0.05)	22 (0.01)	14 (0.30)	18 (0.30)
19.1 (0.72)	16 (0.10)	27 (0.14)	23 (0.36)	15 (0.05)	
20.1 (0.08)	20 (0.09)	28 (0.44)			
	21 (0.06)	29 (0.16)			
		30 (0.05)			

VGL2918	VGL3008	VGL3235
(19) 32%	(18) 28%	(13) 32%
12 (0.29)	15 (0.08)	14 (0.32)
13 (0.42)	17 (0.02)	15 (0.57)
14 (0.10)	18 (0.47)	18 (0.09)
15 (0.07)	19 (0.42)	19 (0.01)
17.3 (0.05)		
19.3 (0.06)	20 (0.01)	

## 2. Using allele frequency data to do standard genetic assessments of the entire population.

A standard genetic assessment of heterozygosity was made from allele frequency data for all 33 STR loci for 48 Lakeland terriers that were tested (Table 2). The average number of alleles ( $N_a$ ) per loci was 4.24 and the number of effective alleles ( $N_e$ ) was 2.46.  $N_e$  is the number of alleles that contribute most to heterozygosity. The observed (actual) heterozygosity of alleles across the 33 STR loci was 0.554 and the heterozygosity expected ( $H_e$ ) if the alleles were in Hardy-Weinberg equilibrium (HWE) was 0.558. HWE is achieved when a given population is in a state of random breeding. The fact that  $H_e$  and  $H_o$  were almost identical indicates that this population of 48 dogs is in HWE and that breeders are doing a good job in picking unrelated sires and dams from the population.

An inbreeding coefficient ( $F$ ) was calculated based on the differences in  $H_e$  and  $H_o$  and in this case,  $F$  was 0.003 (Table 2). A value of -1.0 would mean that no dog in the population shared alleles, while a value of +1.0 would mean that all the dogs were genetically the same. This  $F$  value was essentially zero, indicating that the average dogs in this group was a product of unrelated parents. However, standard genetic assessment values are averages for the entire population and do not accurately measure the degree to which an individual dog is inbred or outbred. It is still possible to have a significant number of very inbred dogs in a population that appears to be in HWE if the contribution of these individuals is countered by an equal population

of more outbred dogs. The actual heterozygosity status of individual dogs is better demonstrated by internal relatedness (IR) values (see section III).

**Table 2.** Summary of Standard Genetic Assessment for Lakeland terriers using 33 STR loci (Updated January 04, 2019)

	N	Na	Ne	Ho	He	F
<b>Mean</b>	48	4.242	2.462	0.554	0.558	0.003
<b>SE</b>		0.227	0.12	0.026	0.024	0.019

### 3. Standard genetic assessment based on allele frequencies at each of the 33 autosomal STR loci

The average heterozygosity values for this group of 48 dogs indicates that parents are being selected on a random basis from among the total population. If this were true, one would expect that each region of the genome interrogated by an STR loci would also be in HWE. The data in Table 3, which is based on standard genetic assessments of alleles at a single STR loci, indicates that this is not the case. If an F value of  $-0.10$  or less, or  $+0.10$  or greater, identifies loci that are either overly outbred or inbred in 10% or more of individuals, 6 loci are under strong positive (inbreeding) selection and 6 loci are under strong negative (outbreeding) selection. The negative and positive effects of these loci balance each other and are why the average inbreeding coefficient (F) is close to zero. The dominant alleles in each of these loci would be found among either the more inbred or outbred dogs.

Table 3. Standard Genetic Assessments of individual autosomal STR loci for 48 Lakeland terriers. F values are  $\geq +0.10$  are highlighted in dark grey and values  $\leq -0.01$  are highlighted in light grey.

#	Locus	N	Na	Ne	Ho	He	F
1	AHT121	48	4	2.398	0.521	0.583	0.106
2	AHT137	48	5	3.225	0.625	0.69	0.094
3	AHTH130	48	3	2.703	0.5	0.63	0.206
4	AHTh171-A	48	4	1.784	0.458	0.439	-0.04
5	AHTh260	48	6	3.895	0.833	0.743	-0.12
6	AHTk211	48	4	3.334	0.729	0.7	-0.04
7	AHTk253	48	3	1.845	0.5	0.458	-0.09
8	C22.279	48	4	2.535	0.583	0.605	0.037
9	FH2001	48	4	1.747	0.479	0.428	-0.12
10	FH2054	48	4	2.378	0.563	0.579	0.029

11	FH2848	48	5	1.952	0.604	0.488	-0.24
12	INRA21	48	4	1.922	0.438	0.48	0.088
13	INU005	48	3	2.425	0.604	0.588	-0.03
14	INU030	48	5	2.357	0.521	0.576	0.095
15	INU055	48	3	2.396	0.625	0.583	-0.07
16	LEI004	48	3	1.347	0.271	0.258	-0.05
17	REN105L03	48	3	2.144	0.417	0.534	0.219
18	REN162C04	48	2	1.753	0.5	0.43	-0.16
19	REN169D01	48	3	1.438	0.271	0.304	0.11
20	REN169O18	48	3	2.946	0.688	0.661	-0.04
21	REN247M23	48	3	1.158	0.146	0.137	-0.07
22	REN54P11	48	4	2.013	0.458	0.503	0.089
23	REN64E19	48	3	2.611	0.688	0.617	-0.11
24	VGL0760	48	5	2.981	0.667	0.664	-0
25	VGL0910	48	6	1.847	0.438	0.459	0.046
26	VGL1063	48	7	2.832	0.667	0.647	-0.03
27	VGL1165	48	8	4.003	0.75	0.75	0
28	VGL1828	48	5	3.13	0.729	0.681	-0.07
29	VGL2009	48	5	2.793	0.729	0.642	-0.14
30	VGL2409	48	4	3.034	0.646	0.67	0.037
31	VGL2918	48	6	3.553	0.646	0.719	0.101
32	VGL3008	48	5	2.495	0.563	0.599	0.061
33	VGL3235	48	4	2.265	0.438	0.559	0.217

## **B. Differences in population structure as determined by principal coordinate analysis (PCoA)**

### **1. Genetic relationships of Lakeland terriers to each other**

Principal coordinate analysis (PCoA) uses genetic distance based on allele sharing to graph relatedness between individuals within and between various populations. The resulting graphic is

multi-dimensional (spherical) but is usually portrayed in two dimensions by selecting two coordinates (planes of the sphere) that represent the greatest proportion of individuals. This usually includes coordinates 1 and 2. We tested 48 Lakeland terriers from North America. All the dogs tested belonged to a single cluster (breed) but with a moderate degree of genetic drift between individuals (Fig. 1). This pattern is consistent with a population that has some degree of phenotypic and therefore genotypic diversity. Populations consisting of closely related individuals will form a much tighter cluster.

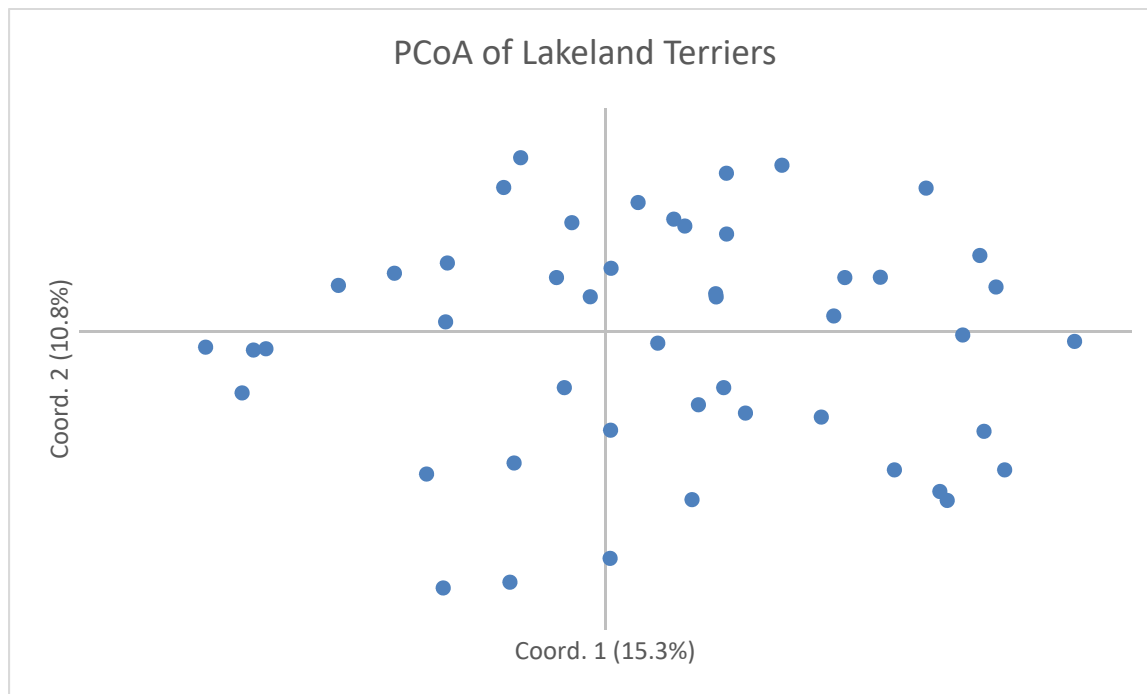


Fig. 1. PCoA of Lakeland Terriers (n=48) based on the 33 STRs

### III. The use of genomic allele frequencies to determine internal relatedness

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

##### 1. IR values

Standard genetic assessments such as presented in Tables 1 and 2 are indicators of population-wide heterozygosity (i.e. a mean or average) and do not show heterozygosity for individual dogs. The heterozygosity of an individual dog is determined by each of its parents, which is usually unknown. Internal Relatedness (IR) is a calculation that has been used to estimate the degree to which the two parents were related by looking only at their offspring. The IR calculation takes into consideration homozygosity at each locus and gives more importance to rare and uncommon alleles. Rare and uncommon alleles would presumably be present in less related individuals. A dog with a value of -1.0 would have parents that were totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were genetically identical at all loci. An IR value of +0.25 would be equivalent to offspring of full sibling parents from a random breeding



population. IR values >0.25 occur only if the parents of the full sibling parents were also highly related.

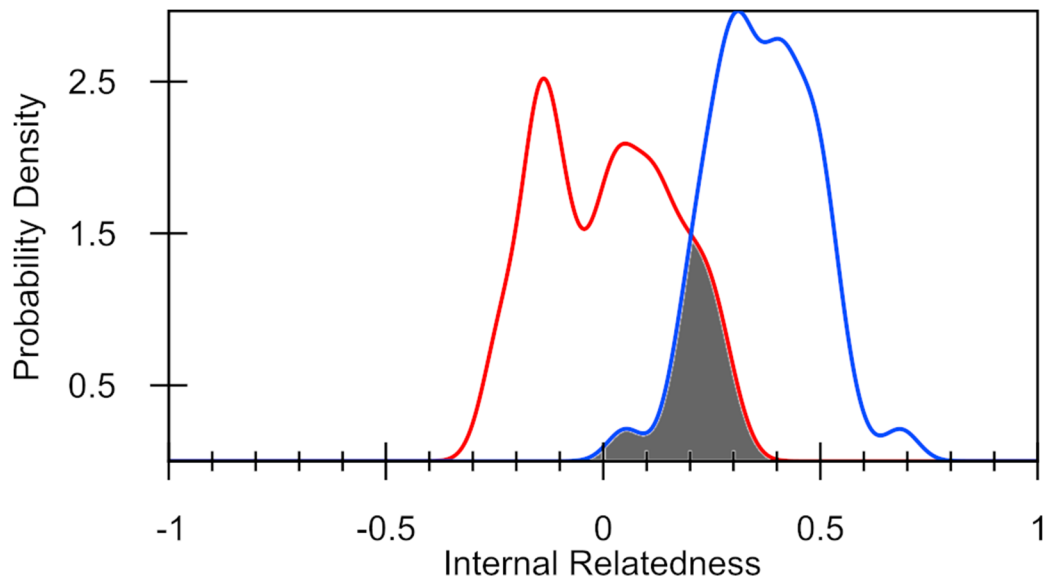
IR scores can be provided for the population (Table 3) or graphed for individual dogs (Fig. 2). Mean IR scores for the 48 Lakeland terriers ranged from a low of -0.259 (most outbred /parents least related) to a high of 0.301 (most inbred/parents most related), with a mean (average) value of -0.007. One-fourth of the dogs had IR scores from -0.133 to -0.224 and were more outbred than the average. Conversely, one-fourth ranged from 0.124 to 0.301 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 indicated and one-fourth more inbred. It is noteworthy the one-fourth inbred dogs had IR scores comparable to offspring of first-cousin (~ 0.125) or full-sibling (0.250) parents from a random breeding population. The few dogs with scores >0.250 were genetically equivalent to offspring of full-sibling parents that were also a product of inbred parents.

The IR curve for these 48 dogs is biphasic (two peaks). This indicates that the population consists of two types of dogs - one group that is on the left of the curve that is more outbred and a second population on the right that is more inbred. This was first observed from an examination of allele frequencies at each of the 33 STR loci (Table 3).

**Table 3.** IR vs IR-Village Dogs (IRVD) comparison for Lakeland terriers (n=48)

	<b>IR</b>	<b>IRVD</b>
<b>Min</b>	-0.2593	0.0504
<b>1st Qu</b>	-0.1330	0.2790
<b>Mean</b>	0.0072	0.3653
<b>Median</b>	0.0068	0.3680
<b>3rd Qu</b>	0.1238	0.4642
<b>Max</b>	0.3011	0.6849

The IRVD values in Table 3 are considerably higher than the IR values and reflect the relatedness of these 48 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.



**Fig. 2.** Distribution of IR estimated in Lakeland terriers (n=48) based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). The darkened overlapping area indicates that 21% of the alleles at the 33 genomic STR loci are shared with village dogs.

## 2. Estimation of genetic diversity lost during breed creation using village dogs as a gold standard

The IR values can also be used to approximate how much genetic diversity was lost during a breed's evolution. This is done by comparing the frequency of a given allele in Lakeland terriers with the frequency of the same alleles in a population of village dogs from the Middle East, SE Asia, Taiwan and other Pacific island nations such as Brunei and the Philippines.

A rough estimate based on areas under the curve (darkened area Fig. 2), indicates that this group of Lakeland terriers possess 21% of the genetic diversity that currently exists in village dogs. If 21% of available genetic diversity has been retained, 79% has been lost in breed creation. The retention of 21% of known genetic diversity is both lower and higher than other breeds. For instance, the Swedish Vallhund has retained only 7% of canine genetic diversity, the Doberman Pinscher 15% and the Shiloh Shepherd 27%, while at the highest level the Miniature Poodle has retained 51%, the Golden Retriever 50.4% in Labrador retriever 54%, and Toy Poodle 60%.

Lost or retained genetic diversity resulting during breed creation can also be estimated from alleles identified in the 33 autosomal STR loci and from existing DLA class I and II haplotypes. Twenty-three percent of the alleles identified at these loci exist in among all the thousands of dogs and wolves tested at the VGL, a figure remarkably close to the 21% identified by the IR/IRVD comparison. This confirms that the village dogs from the Middle East, SE Asia and Island Pacific nations are a reasonable gauge of the total genetic diversity that still exists in canids.

## IV. DLA Class I and II Haplotypes

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 block of genes with limited recombination over time. Therefore, specific DLA class I and II haplotypes are reasonable surrogate markers for a breed founder or closely related founder line.

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. Table 4 lists the DLA class I and II haplotypes that were identified among the 48 Lakeland terriers. Six class I and 7 class II haplotypes were found among the 48 dogs tested, which is the second lowest number observed for any breed studied to date. The Swedish Vallhund also has six DLA class one haplotypes, but only four DLA class II haplotypes.

**Table 4:** DLA class I and Class II haplotypes and their frequencies in 48 Lakeland terriers (Updated January 04, 2019)

<b>DLA1 #</b>	<b>STR alleles</b>	<b>Incidence</b>
1012	388 369 289 188	0.71
1016	382 371 277 178	0.02
1040	380 371 277 186	0.2
1052	380 372 289 184	0.01
1104	386 373 289 186	0.05
1159	395 379 277 181	0.01

<b>DLA2 #</b>	<b>STR types</b>	<b>Lakeland Terriers (n=48)</b>
2003	343 324 282	0.71
2005	339 322 280	0.01
2014	339 322 284	0.02

2015	339 327 280	0.02
2017	343 322 280	0.05
2022	339 327 282	0.18
2067	343 322 284	0.01

Table 5 lists the DLA class I and II haplotypes inherited by each of the 48 Lakeland terriers that were tested. However, there is one unique feature in the DLA class I and II haplotypes - the same DLA class I (1012) and class II (2003) haplotypes were found in 45/48 of the dogs tested. Twenty-four of these 45 dogs were heterozygous for 1012/2003 and 19/45 were homozygous. If this dominance of the 1012/2003 haplotypes remains after testing more dogs, which is likely, it 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 inherited by each of the 48 Lakeland terriers. Dogs 1-3 (green) are heterozygous for haplotypes other than 1012 and 2003. Dogs 4-28 (yellow) have inherited one copy of the 1012/2003 haplotype (i.e, heterozygous), and dogs 29-48 (blue) have inherited the same 1012/2003 haplotype from their parents (i.e., homozygous).

1	1040	1040	2022	2022	26	1012	1012	2003	2003
2	1040	1040	2022	2022	27	1012	1012	2003	2003
3	1016	1040	2014	2022	28	1012	1012	2003	2003
4	1012	1040	2003	2015	29	1012	1012	2003	2003
5	1012	1040	2003	2022	30	1012	1012	2003	2003
6	1012	1104	2003	2017	31	1012	1012	2003	2003
7	1012	1040	2003	2022	32	1012	1012	2003	2003
8	1012	1040	2003	2022	33	1012	1012	2003	2003
9	1012	1159	2003	2005	34	1012	1012	2003	2003
10	1012	1040	2003	2022	35	1012	1012	2003	2003
11	1012	1104	2003	2017	36	1012	1012	2003	2003
12	1012	1052	2003	2067	37	1012	1012	2003	2003
13	1012	1040	2003	2022	38	1012	1012	2003	2003
14	1012	1040	2003	2022	39	1012	1012	2003	2003
15	1012	1040	2003	2022	40	1012	1012	2003	2003
16	1012	1104	2003	2017	41	1012	1012	2003	2003
17	1012	1104	2003	2017	42	1012	1012	2003	2003
18	1012	1104	2003	2017	43	1012	1012	2003	2003
19	1012	1040	2003	2022	44	1012	1012	2003	2003
20	1012	1040	2003	2022	45	1012	1012	2003	2003
21	1012	1040	2003	2022	46	1012	1012	2003	2003
22	1012	1016	2003	2014	47	1012	1012	2003	2003
23	1012	1040	2003	2022	48	1012	1012	2003	2003
24	1012	1040	2003	2022					
25	1012	1040	2003	2015					

The DLA class I and II haplotypes are subject to limited recombination over time and given the relatively short time that most modern breeds have existed (i.e., 100-200 years), these gene blocks remain much the same and can be used to identify specific founders or founder lines that existed when the registries were created, and breeding closed to all outside dogs. A founder or founder line with this DLA haplotype was either heavily used to produce the first dogs to be registered or strong artificial selection for a trait or traits in this founder line has led to a significant loss of original breed diversity. To date, the VGL has identified 205 unique DLA I and 112 DLA II haplotypes among all dogs. Linkages between known DLA class I and II regions

has led to 355 recognized DLA class I/II haplotypes. Therefore, the 48 Lakeland terriers tested possess only about 3% of known canid DLA class I haplotypes and 6% of class II haplotypes.

## 2. DLA class I and II haplotype sharing between Lakeland terriers and other breeds

It is common for various dog breeds to share ancestors and therefore DLA class I and II haplotypes. Table 5 lists several breeds that share the same DLA class I and II haplotypes with Lakeland terriers. The dominant 1012 DLA class I haplotype is found in many breeds, but the highest incidence of sharing is with the English bulldog and the Biewer/Yorkshire terriers. The dominant 2003 DLA class II haplotype is at highest incidence in the Flat coated retrievers, Havanese, Miniature and Toy poodles and Biewer/Yorkshire terriers. The combined 1012/2003 haplotype was strongly shared with the English bulldog and the Biewer/Yorkshire terriers. It is not surprising to see the relationships between Lakeland terriers and many other dog breeds of mainly European origin. The relationship with the Biewer/Yorkshire terriers was expected. However, the English bulldog relationship was less obvious and may be explained by early descriptions of the Bulldog as a small aggressive bull-baiting type dog. This activity is like the tenacious English-bred terriers called upon to attack vermin in their lairs.

**Table 6.** DLA class I and II haplotype sharing between Lakeland terriers and several other pure breeds of dogs

DLA#	STR types	Black Russian Terrier (n=124)	Lakeland Terrier (n=48)	Labrador Retriever (n=151)	High Red and White Setter (n=41)	Doberman Pinscher (n=492)	Flat Coated Retriever (n=42)	Havanese (n=397)	Samoyed (n=187)	Shiba Inu (n=91)	Giant Schnauzer (n=187)	Polish Lowland Sheepdog (n=18)	English Bulldog (n=103)	Biewer Terrier (n=110)	Biewer Yorkshire Terrier (n=3)	Yorkshire Terrier (n=18)	Italian Greyhound (n=768)	Ataskan Klee Kai (n=487)	Shiloh Shepherd USA (n=146)	Magyar Agar (n=44)	English Mastiff (n=18)	American Akita (n=92)	Japanese Akita (n=330)	Hond Akita (n=3)	Golden Retriever (n=69)	Miniature Poodle (n=100)	Sarbel (n=49)	Swedish Vallhund (n=178)	Poodle (n=2391)	Toy Poodle (n=128)		
1012	388 309 389 188	0.71	0.02	0.014	0.014	0.018	0.013	0.217	0.04	0.04	0.04	0.414	0.177	0.151	0.228	0.28	0.051	0.0587	0.223	0.06	0.16	0.014	0.007	0.004	0.023	0.006	0.006	0.006	0.006	0.006	0.006	0.006
1016	382 373 277 178	0.008	0.013	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1040	380 373 277 186	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1051	380 372 289 184	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1054	386 373 289 186	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1159	395 379 277 181	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

DLA#	STR types	Black Russian Terrier (n=124)	Lakeland Terrier (n=48)	Labrador Retriever (n=151)	High Red and White Setter (n=41)	Doberman Pinscher (n=492)	Flat Coated Retriever (n=42)	Havanese (n=397)	Samoyed (n=187)	Shiba Inu (n=91)	Giant Schnauzer (n=187)	Polish Lowland Sheepdog (n=18)	English Bulldog (n=103)	Biewer Terrier (n=110)	Biewer Yorkshire Terrier (n=3)	Yorkshire Terrier (n=18)	Italian Greyhound (n=768)	Ataskan Klee Kai (n=487)	Shiloh Shepherd USA (n=146)	Magyar Agar (n=44)	English Mastiff (n=18)	American Akita (n=92)	Japanese Akita (n=330)	Hond Akita (n=3)	Golden Retriever (n=69)	Miniature Poodle (n=100)	Sarbel (n=49)	Swedish Vallhund (n=178)	Poodle (n=2391)	Toy Poodle (n=128)			
2003	343 324 382	0.71	0.02	0.01	0.01	0.132	0.222	0.013	0.017	0.011	0.017	0.017	0.598	0.223	0.228	0.255	0.38	0.0072	0.31	0.01	0.16	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2005	339 322 280	0.016	0.01	0.01	0.01	0.413	0.003	0.01	0.01	0.01	0.01	0.01	0.015	0.023	0.009	0.054	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
2014	339 322 284	0.02	0.01	0.01	0.01	0.089	0.009	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
2015	339 322 280	0.02	0.01	0.01	0.01	0.089	0.009	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
2017	343 322 280	0.05	0.01	0.01	0.01	0.003	0.009	0.01	0.01	0.01	0.01	0.01	0.38	0.215	0.01	0.01	0.01	0.219	0.39	0.35	0.09	0.016	0.006	0.019	0.017	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
2022	339 322 282	0.18	0.079	0.01	0.01	0.002	0.138	0.151	0.107	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
2067	343 322 284	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	

## V. Health problems of Lakeland terriers

The average lifespan of the Lakeland terriers is said to be 12 to 16 years (9). Terriers 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 (9, 10). 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 and widespread among small dogs. However, the incidence is low and appears to be decreasing. Like many dogs, and smaller breeds, routine dental care is often required to minimize tartar 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 (10). 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 microphthalmia. They list no disorders associated with conformation.

#### **IV. What does preliminary DNA-based testing tell us about contemporary Lakeland terriers**

This initial study of 48 Lakeland terriers suggests that the breed is extremely 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. Also, this group of 48 dogs is entirely from North America and may not reflect dogs from other parts of the world. These are reasons to test more dogs as well as individuals from other parts of the world, but from historical regions such as the UK or from 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 (8), these characteristics have been maintained. The extreme loss of genetic diversity in the DLA region, if a breed-wide phenomenon, is worrisome but more so if the lost diversity is associated with disorders linked to specific DLA types. Such disorders usually involve autoimmune diseases, autoinflammatory 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 theoretical 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 (11). 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 (11).

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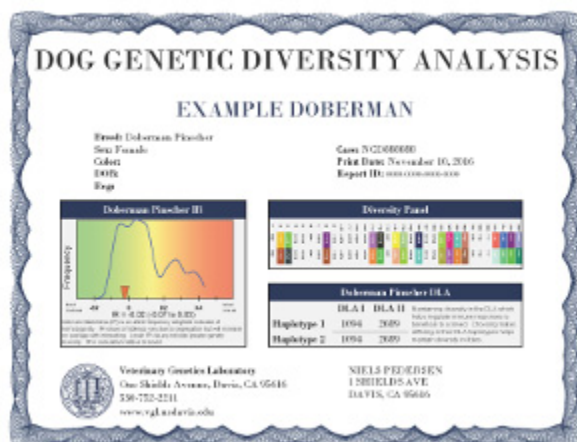
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## VI. Results of Diversity testing

### 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 compared to the results for every dog that has been tested.



### B. What should you do with this information?

The goal for breeders should be to continue to produce puppies with IR scores less than 0, and with time even lower scores. Although most of the individuals tested were randomly bred, there were small subpopulations of dogs that were much more inbred or outbred than the rest of the population. Therefore, there is a possibility to better balance genetic diversity in the breed. Mates should be selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype and encourage the use of dogs with less common genomic alleles or DLA haplotypes.



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, like what is being done by many Standard Poodle breeders. However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

The next step is to compare the DLA class I and II haplotypes. You want to avoid breeding pairs that will produce puppies that will be homozygous for the same haplotypes, and once again, less common haplotypes may offer more diversity than common ones.

Breeders who do not have access to computer programs to predict the outcome of pairings based on IR values of sire and dam can also compare values by manual screening. Potential sires and dams should be first screened for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Some extra weight should be given to rare vs common alleles. This information is included on all certificates and on the breed-wide data on the VGL website.

Puppies, once born, should be tested for their actual IR values, which will reflect the actual genetic impact of each parent on each of their puppies. Considerations of mate choices to maximize heterogeneity across the breed should be balanced with other breeding goals.

## Table

1	1040	1040	2022	2022	26	1012	1012	2003	2003
2	1040	1040	2022	2022	27	1012	1012	2003	2003
3	1016	1040	2014	2022	28	1012	1012	2003	2003
4	1012	1040	2003	2015	29	1012	1012	2003	2003
5	1012	1040	2003	2022	30	1012	1012	2003	2003
6	1012	1104	2003	2017	31	1012	1012	2003	2003
7	1012	1040	2003	2022	32	1012	1012	2003	2003
8	1012	1040	2003	2022	33	1012	1012	2003	2003
9	1012	1159	2003	2005	34	1012	1012	2003	2003
10	1012	1040	2003	2022	35	1012	1012	2003	2003
11	1012	1104	2003	2017	36	1012	1012	2003	2003
12	1012	1052	2003	2067	37	1012	1012	2003	2003
13	1012	1040	2003	2022	38	1012	1012	2003	2003
14	1012	1040	2003	2022	39	1012	1012	2003	2003
15	1012	1040	2003	2022	40	1012	1012	2003	2003
16	1012	1104	2003	2017	41	1012	1012	2003	2003
17	1012	1104	2003	2017	42	1012	1012	2003	2003
18	1012	1104	2003	2017	43	1012	1012	2003	2003
19	1012	1040	2003	2022	44	1012	1012	2003	2003
20	1012	1040	2003	2022	45	1012	1012	2003	2003
21	1012	1040	2003	2022	46	1012	1012	2003	2003
22	1012	1016	2003	2014	47	1012	1012	2003	2003
23	1012	1040	2003	2022	48	1012	1012	2003	2003
24	1012	1040	2003	2022					
25	1012	1040	2003	2015					