Genetic Diversity Testing for Labrador Retrievers

Overview

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

Genetic diversity testing of Labrador Retrievers is now in the data collection phase. During this phase, we will continue to test more registered dogs to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their breed. We are accepting Labrador Retrievers from the USA and Canada, as well as from other regions of the world. At time of writing, we have tested 121 Labrador Retrievers - 113 from the US, 6 from Canada, and 2 from Brazil. Although this number of dogs will probably cover 95% or more of the genetic diversity that exists in dogs from North America, the goal is to keep testing dogs until no new genomic alleles or DLA haplotypes are recognized. We are especially interested in testing more Labrador Retrievers from other regions of the world. We anticipate that they will be genetically related to their North American counterparts, but the degree of that relatedness may vary depending on duration of geographic isolation, field vs. bench, type of performance, and introgressions between these types.

Results reported as:

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

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

Internal Relatedness: The IR value is a measure of genetic diversity within an individual that takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. It is also an estimate of the genetic relatedness of a dog's parents. Unlike standard genetic assessments, IR puts more emphasis on heterozygosity over homozygosity and uncommon over common alleles. IR values are unique to each dog and cannot

be compared between dogs. Two dogs may have identical IR values but with very different genetic makeups.

I. Introduction

A. Breed history

The history of the Labrador Retrievers is strangely parallel to many breeds from the Victorian era, involving dogs of foreign lands that gained notoriety for certain functions, influential members of the Royalty that took note of them, importation of a small number of these dogs and the creation of specific kennels to expand and further mold the proto-breed, public notoriety, and ultimately acceptance as a breed by an official organization and subsequent standardization and refinement. The histories of breeds often differ depending on the chronicler (1-11). Ancestors of contemporary Labrador Retrievers were purportedly indigenous working dogs that evolved on the island of Newfoundland in Canada. The coastal land and waters of Newfoundland were traversed by English, Portuguese, French and Spanish fisherman in the 1500's and their dogs were involved primarily with fishing tasks such as retrieving fish that had fallen off their hooks and hauling in fishing lines through the water. Formal occupation of the region was in 1610 by a small group of settlers, who grew in numbers and came to use these indigenous "St John's water dogs" for other tasks as well. One of these tasks was summarized in 1822 by the explorer W.E. Cormack during his hike across the island of Newfoundland. "The dogs are admirably trained as retrievers in fowling and are otherwise useful....... The smooth or short haired dog is preferred because in frosty weather the long-haired kind become encumbered with ice on coming out of the water."

The first "St. John's dogs" were imported to England and promoted by a few aristocratic British sportsmen. The Earl of Malmesbury at Heron Court had used his St. John's dog for the shooting sports in England as early as 1809 and was a key figure in the breed. He started the first kennel of Labradors and was an avid breeder until his death in 1841. These dogs were initially known as the St. John's water dog, St. John's dog, or Lesser Newfoundland. They were first documented under the name Labrador in 1839 to distinguish them from the "Newfoundland," a breed that evolved separately in the more southern Avalon Peninsula. The 5th Duke of Buccleuch started his kennel in Scotland about 1835 independently from Malmesbury. The Duke's brother, Lord John Scott also started importing the St. John's dogs from Newfoundland. Malmesbury reported that he had kept the blood lines as pure as possible with imported St. John's type dogs from Newfoundland and shared these dogs with other kennels. As the breed developed in the UK, the original St. John's dog slowly became extinct in Newfoundland due to a tax levied on all dogs not used for sheep and a rabies quarantine act in 1895 that prohibited dogs from entering Great Britain from rabies areas without a license and a strict six-month quarantine. These two events slowly eliminated most non-sheep dogs in Newfoundland as well as making it extremely difficult to export remaining St. John's dogs to the UK. Dogs from the UK, being rabies free, were easily exported to Newfoundland, thus providing ready replacements for local St. John's dogs. By the 1930's the St. John's dog was rare in Newfoundland, although the 6th Duke of Buccleuch was finally able to import a few more dogs between 1933-1934 to continue the line. The extinction of the St. John's dog came to pass, even though sheep raising never became a mainstay of

Newfoundland. Sadly, the original St. John's dog slowly disappeared, and the last two aged individuals died in 1981 (4).

Labrador Retrievers survived and became common in England by 1870 and recognized by The Kennel Club in 1903. The first American Kennel Club (AKC) registration was in 1917. Although they had a rocky beginning, the Labrador Retriever has become the most popular breed based on registration numbers in Denmark, Australia, Canada, New Zealand, United Kingdom, and the United States since 1991.

Labrador Retrievers are medium-large sized, with males weighing 65–80 lb. (29–36 kg) and females 55–70 lb (25–32 kg) (5). Labrador hair is usually short and straight, and the tail is quite broad and strong. Their interwoven coat is also relatively waterproof and feet amply webbed, providing more assistance for swimming. Solid black was the accepted coat color for the original Labrador Retriever. Yellow and chocolate pups and white spotting occasionally appeared in litters, but such individuals were usually culled. The first recognized Yellow Labrador was born in 1899 and Chocolate Labradors became more accepted in the 1930s. Contemporary Labrador Retrievers are often categorized as English or American. The two types are result of geographical separation, show vs. performance emphasis (8), and regional differences in interpretation of the original breed standards. American labs tend to be more energetic having been bred originally to compete in field trials. The 'blocky' head for which Labrador Retrievers are known is more prominent in the English dogs, whereas American labs tend to be leaner and longer-legged (7). The American Kennel Club (AKC) and the Labrador's breed club have set the breed standard to accommodate phenotypic differences between field-bred Labrador and conformation-bred Labrador Retrievers, but only to a point. As a result, dual champions that excel in both the field and the show ring are becoming increasingly uncommon.

Demography

In the United States, the breed gained wider recognition following a 1928 American Kennel Gazette article, *"Meet the Labrador Retriever."* Before this time, the AKC had only registered 23 Labradors in the country. The breed gained popularity as hunting dogs during the 1920s and especially after World War II based on their abilities as both game finders and shore/water retrievers. The Russian Retriever Club traces the arrival of Labradors to the late 1960s, as household pets of diplomats and others in the foreign ministry (10). The establishment of the breed in the USSR and later Russian Federation was initially hindered by the relatively small numbers of Labradors and great distances involved, leading to difficulty finding acceptable mates. This led to tacit cross-breeding to other types of retrievers. Home-born dogs are now regularly supplemented by further imports from overseas. Similar difficulties were encountered in countries like Australia, where the breed may have been influenced more by Asian than European or North American dogs.

II. Baseline genetic diversity testing and what it tells us about Labrador Retrievers

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 that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) with additional markers developed by the VGL for forensic purposes

Thirty-three STRs and their alleles were studied in over 100 Labrador Retrievers (Table 1). Allele and allele frequencies were used to determine basic genetic parameters such as the number of alleles found at each STR locus (Na), the number of effective alleles (Ne) per locus, i.e., the number of alleles that contribute most to genetic differences, the observed or actual heterozygosity (Ho) that was found, and the heterozygosity that would be expected (He) if the existing population is in Hardy Weinberg equilibrium (HWE). HWE is achieved when the selection of mates is entirely random and subject to no positive or negative human selection pressure. The value F is a coefficient of inbreeding derived from the Ho and He values. A value of +1.0 would occur only if every individual were genetically indistinguishable at each of the 33 STR loci, while a value of -1.0 would be seen when all the dogs were completely different at each of the 33 loci.

The 33 STR loci chosen from 22 different chromosomes were quite polymorphic with 4-11 alleles per locus (Table 1). AHTh171-A, AHTh171-A, AHTk211, AHTk253, INU030, REN169O18, REN247M23, REN54P11 and REN64E19 were the only loci that had inbreeding coefficients (F) that were greater than 0.100 (Table 2). Almost all loci contained at least one allele that occurred in 25-70% of the Labrador Retrievers tested (Table 2). These high frequency alleles were obviously conserved in the breed and likely associated with founders that typified important breed characteristics.

Table 1. Allele frequencies for 33 STR markers in Labrador Retriever

Table 1 Link

Table 2. Standard Genetic Assessment for Labrador Retriever using 33 STR loci

#	Locus	N	Na	Ne	Ho	He	F
1	AHT121	180	8	2.454	0.567	0.592	0.044
2	AHT137	180	10	4.761	0.722	0.790	0.086
3	AHTH130	180	6	4.815	0.767	0.792	0.032
4	AHTh171-A	180	7	4.142	0.700	0.759	0.077
5	AHTh260	180	10	4.067	0.700	0.754	0.072
6	AHTk211	180	6	3.061	0.567	0.673	0.158
7	AHTk253	180	5	2.888	0.594	0.654	0.091
8	C22.279	180	6	3.591	0.689	0.721	0.045
9	FH2001	180	8	2.196	0.517	0.545	0.051
10	FH2054	180	8	4.076	0.683	0.755	0.095
11	FH2848	180	6	3.835	0.717	0.739	0.031

12 INRA21	180	6	2.582	0.672	0.613	-0.097
13 INU005	180	6	2.345	0.522	0.573	0.089
14 INU030	180	5	2.403	0.539	0.584	0.077
15 INU055	180	7	3.853	0.650	0.740	0.122
16 LEI004	180	6	2.913	0.600	0.657	0.086
17 REN105L03	180	7	2.138	0.489	0.532	0.081
18 REN162C04	180	7	3.152	0.572	0.683	0.162
19 REN169D01	180	9	4.336	0.722	0.769	0.061
20 REN169O18	180	6	2.876	0.567	0.652	0.131
21 REN247M23	180	5	1.749	0.394	0.428	0.079
22 REN54P11	180	9	3.309	0.656	0.698	0.061
23 REN64E19	180	6	3.879	0.689	0.742	0.072
24 VGL0760	180	7	3.302	0.717	0.697	-0.028
25 VGL0910	180	8	4.669	0.756	0.786	0.039
26 VGL1063	180	9	5.206	0.761	0.808	0.058
27 VGL1165	180	11	3.966	0.650	0.748	0.131
28 VGL1828	180	8	4.652	0.761	0.785	0.031
29 VGL2009	180	7	3.443	0.628	0.710	0.115
30 VGL2409	180	6	3.132	0.617	0.681	0.094
31 VGL2918	180	11	4.569	0.756	0.781	0.033
32 VGL3008	180	8	5.836	0.806	0.829	0.028
33 VGL3235	180	8	3.356	0.706	0.702	-0.005

Breed heterozygosity was determined by standard genetic assessment of alleles and allele frequency at each STR locus for all Labrador Retrievers tested (Table 3). At time of writing, (N=117), the average number of alleles per locus was 7, and 3.7 of these alleles had the most genetic effect (Ne). The observed homozygosity (Ho) across the 33 loci was 0.664, while the expected homozygosity (He) for a population in Hardy-Weinberg equilibrium (i.e., state of total random breeding) was 0.705. This difference between Ho and He was used to calculate an inbreeding coefficient (F). The calculated F value for the 117 dogs was 0.059, which was close to 0.00 and indicated that the distribution of alleles was a result of near-random selection. Therefore, the data in Tables 1-3 indicate that Labrador Retrievers are more genetically diverse than many other breeds and that the average dog is a product of random selection.

Table 3. Summary of Standard Genetic Assessment for Labrador Retriever using 33 STR loci

	N	Na	Ne	Ho	He	F
Mean	180	7.333	3.562	0.650	0.696	0.067
SE		0.283	0.167	0.016	0.016	0.009

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

1. Genetic relationships of Labrador Retrievers from across the USA

We tested 117 Labrador Retrievers from the USA. PCoA is a graphic portrayal of how closely individual dogs in a defined population are related to each other. The graph is actually a sphere, but usually graphed in the two-dimensional plane that most accurately depicts relationships between individuals. The Labrador Retrievers that were tested clearly belong to a single breed, but there was evidence of differentiation into two closely related populations (varieties or bloodlines), which are circled. This segregation was not geographical, because almost all dogs were from North America. Rather, it was due to field (performance) rather than bench (show) use.
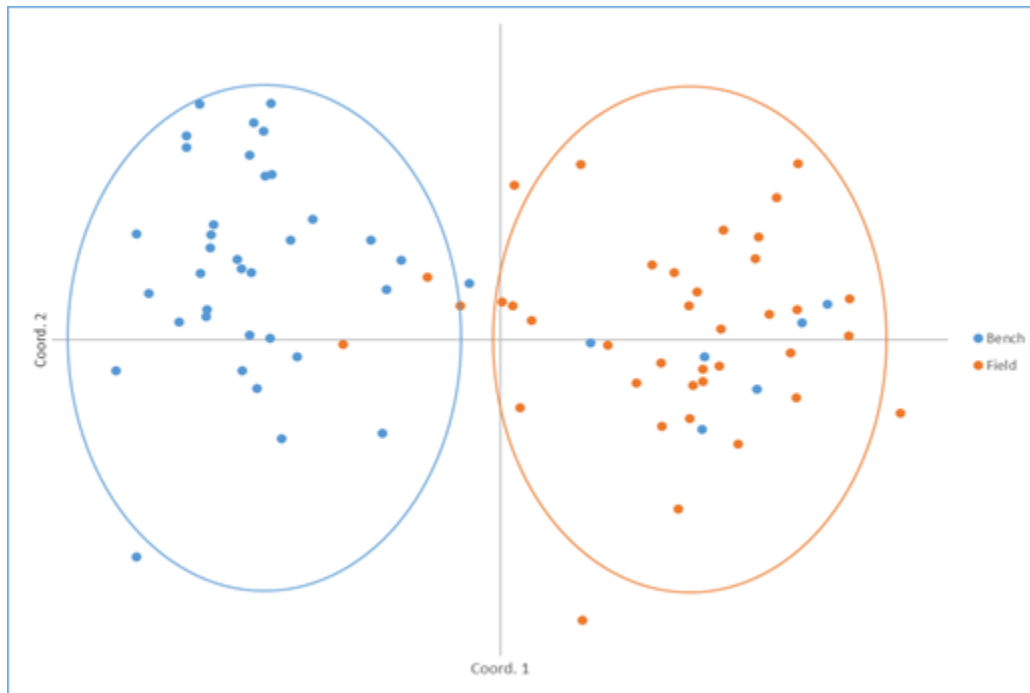

Fig. 1. PCoA of Labrador Retriever (n=117) based on the 33 STRs

2. Genetic relationship between three different breed that may have common ancestors

There is good evidence that many of the contemporary retriever-type breeds share common ancestry (9). We tested this by comparing the relatedness of Labrador Retrievers, Flat Coated Retrievers and Golden Retrievers in PCoA (Fig. 2). It is evident in this plot that the three breeds are genetically distinct when compared against each other.
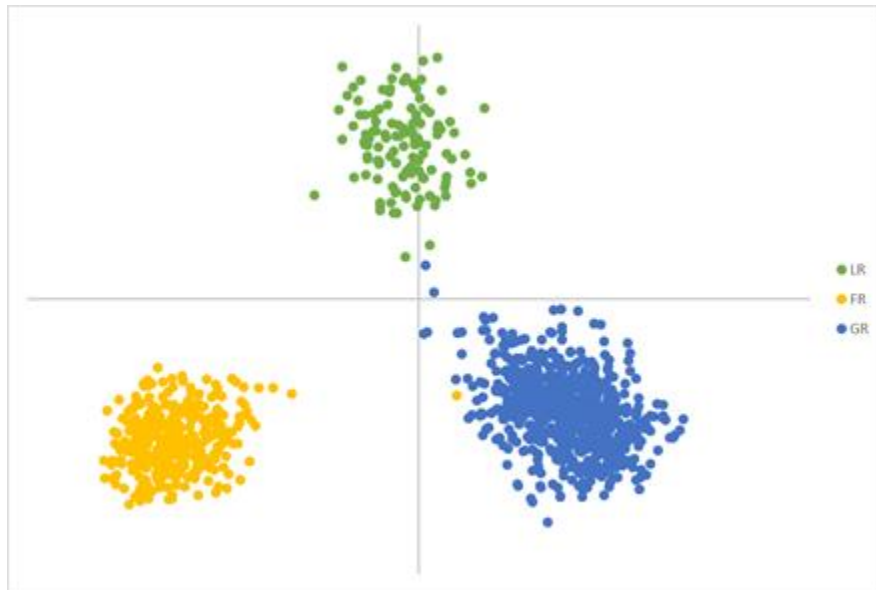

Fig. 2. PCoA plot of genetic distance using 33 genomic STR markers comparing Labrador Retriever (LR), Flatcoat Retriever (FR), and Golden Retriever (GR). The three breeds are genetically distinct, although one Flat Coated Retriever segregated with Golden Retrievers (sampling or breed recognition error?).

Although the three breeds were genetically distinguishable when compared each other, many performance breeds share ancestors further back in their ancestry (9). To test this premise, the three breeds were compared in PCoA with two breeds that have less obvious similarities, Standard Poodles and Italian Greyhounds (Fig. 3). This type of comparison allows breeds to be compared using a lesser degree of relatedness. As expected, Italian Greyhounds formed a distinct population at quite some distance from the other four breeds. The Standard Poodle also formed a genetically distinct population, but because of its retriever origins it was more closely aligned to Golden Retrievers, Labrador Retrievers and Flat Coated Retrievers than to Italian Greyhounds. Indeed, some Standard Poodles (performance type?) segregated with Labrador Retrievers, suggesting outcrossing. Labrador Retrievers, although still genetically distinguishable from the other breeds, were closely aligned to Golden Retrievers and Flat Coated Retrievers, with some individual Golden Retrievers and Labrador Retrievers clustering together. The obvious relationship was between Flat Coated Retrievers and Golden Retrievers, which clustered as distinct subpopulations within one larger population. As expected, breeds that have evolved relatively recently from performance type retrievers are more related (9). It is also noteworthy that the POMC mutation for obesity that is carried by one-quarter of Labrador Retrievers has also been recognized in Flat Coated Retrievers- http://www.ahtdnatesting.co.uk/tests/pomc-mutation-appetite-and-obesity/. The Labradoodle has become a popular cross, although the crosses often suffer the same types of disorders as the Standard Poodle. This may also be associated with the shared risk factors for autoimmune diseases.
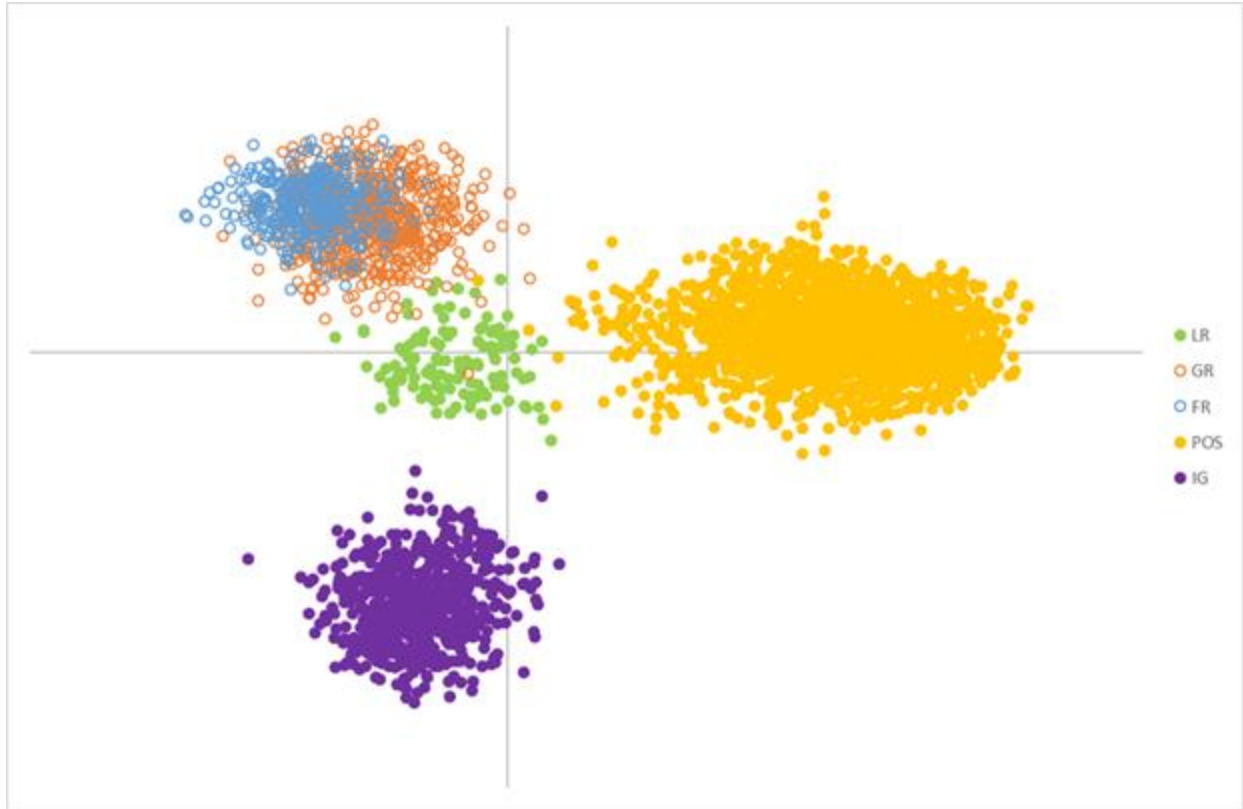
Fig. 3. PCoA plot showing the relatedness of individual Labrador Retrievers, Golden Retrievers, Flat Coated Retrievers, Standard Poodles and Italian Greyhound to each other and to dogs in the other breeds.

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

Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity of individuals within the population. The genetic diversity of an individual dog is largely determined by the diversity inherited from each of its parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree to which the two parents were related (Table 4). 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. IR scores of all individuals in a population can be graphed to form a curve ranging from -1.0 to +1.0. A dog with a value of -1.0 would 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 is expected for offspring of full sibling parents from a random breeding population. IR values >0.25 are expected when the parents were themselves highly inbred.

IR scores ranged from a low of -0.293 (parents least related) to a high of 0.322 (parents most related), with a mean (average) value of 0.060. Therefore, one-half of the population had parents that were related to a greater degree than the average Labrador Retriever tested. One fourth of the population had IR scores from 0.001 to -0.239, and one fourth 0.138 to 0.322. Although the standard genetic assessments made from allele frequencies indicated that the population was randomly breeding, IR values suggest that this assumption is misleading because more outbred dogs are cancelling out more inbred dogs. In truth, one-fourth of Labrador Retrievers tested were products of closely related parents, some being equivalent to half-siblings or closer.

Table 4. IR vs IRVD comparison for Labrador Retriever (n=117)

	IR	IRVD
Min	-0.239	-0.013
1st Qu	0.001	0.017
Mean	0.060	0.250
Median	0.069	0.250
3rd Qu	0.138	0.300
Max	0.322	0.500

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

The IR values can also be used to give an approximation of how much genetic diversity has been lost during breed development and subsequent evolution. This is done by comparing the frequency of a given allele in Labrador Retrievers 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. Contemporary village dogs are largely unchanged from the ancestors of almost all modern dog breeds. The resultant frequencies are then used to calculate the IRVD.

A comparison of IR values (red curve) and IRVD values (blue curve) can be used as a rough estimate of how much of the genetic diversity available in contemporary village dogs has been maintained in modern Labrador Retrievers. A rough estimate based on areas under the curve (black), indicate that Labrador Retrievers have maintained 54% of the genetic diversity still present among this indigenous dog population.
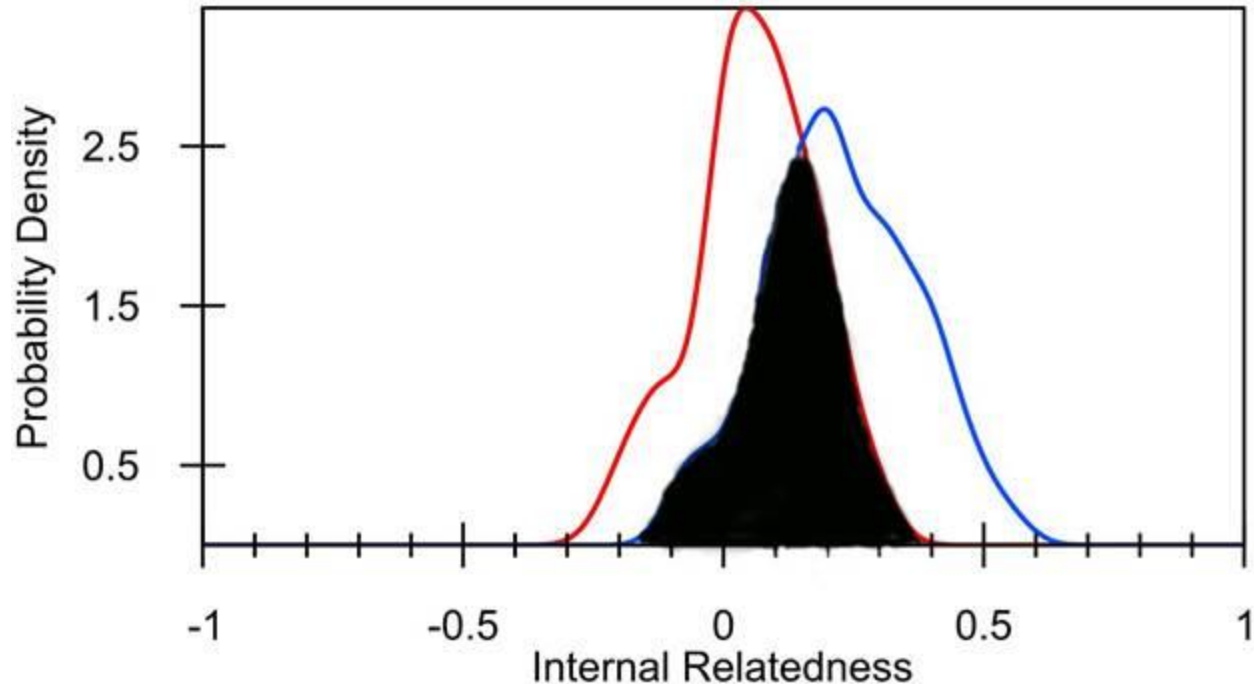
Fig. 4. Distribution of IR estimated in Labrador Retriever (n=117) based on intra-breed diversity (red line). The blue line shows adjusted IR values (IRVD) based on the frequency in village dogs of the same alleles identified in Labrador Retrieves (blue). The black area is a function of alleles shared by Labrador Retrievers and village dogs and their comparative frequency.

IV. DLA Class I and II Haplotype frequencies and genetic diversity

The DLA consists of four gene rich regions (classes I-IV) making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases. The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with the DLA88 are linked together in various combinations, forming specific haplotypes (Table 4). Groups of genes and their alleles inherited as a block, rather than singly, are called haplotypes. The class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at STR loci associated with each of the three Class II genes are strongly linked and inherited as a single block or haplotype (Table 5). One haplotype comes from each of the parents. Specific class I and II haplotypes are often linked to each other and inherited as a genetic block with limited recombination over time. Therefore, DLA class I and II haplotypes can be viewed as reasonable surrogate markers for breed founders.

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

1. DLA class I and II haplotypes existing in Labrador Retrievers

At the time of 123 US Labrador Retrievers in this study, they possessed 20 DLA class I and 17 DLA class II haplotypes (Table 6). DLA class I haplotypes 1175 and 1176 and class II haplotype 2098 have not been seen in other breeds tested to date, but unlike most other breeds, they are relatively uncommon. This suggests that they are either recent introgressions to the breed are have been unintentionally selected against. Two DLA class I haplotypes, 1065 and 1165 occurred an inordinately high proportion of dogs, i.e., 39% and 16% respectively. The 1165 haplotype has been seen before only in Shiloh Shepherds, where it is also a common haplotype. The DLA class II haplotypes 2048 (39%) and 2080 (16%) also have a high incidence in Labrador Retrievers. The 2048 haplotype is also found in Golden Retrievers, where it is a common haplotype, while the 2080 is again common in Shiloh Shepherds. This sharing suggests that these breeds have made heavy use of the same or closely related founders. This was also strongly indicated by PCoA using an entirely different set of genetic loci (Figs.2, 3). A surprisingly high degree of DLA class I and II haplotype sharing also exists between an unlikely breed, the Havanese. This suggests that Havanese are close to these retrieving breeds and their major difference is due to miniaturization.

Table 5. DLA class I and Class II haplotype and their frequencies

DLA Class I Haplotype Frequencies (Updated Oct 9, 2019)		
DLA1 #	**STR types**	**Labrador Retriever (n=180)**
1003	387 375 277 186	0.003
1006	387 375 293 180	0.039
1008	386 373 289 182	0.064
1016	382 371 277 178	0.011
1017	386 373 289 178	0.033
1030	380 373 293 178	0.031
1033	382 379 277 181	0.003
1034	382 379 277 182	0.006
1045	376 371 277 186	0.008
1054	382 379 277 184	0.075
1062	382 371 277 183	0.014
1063	382 373 289 182	0.003
1065	380 371 277 181	0.372
1066	376 375 277 178	0.003
1068	380 373 287 181	0.047
1070	380 375 291 178	0.006
1105	382 379 277 178	0.056
1134	384 365 291 178	0.008

1142	376 379 277 180	0.003
1165	392 369 281 182	0.203
1175	380 375 293 180	0.003
1176	382 373 289 178	0.003
1180	386 371 277 181	0.003
1189	382 379 289 182	0.003
1226	380 373 277 181	0.003

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

DLA2 #	STR types	Labrador Retriever (n=180)
2001	343 324 284	0.003
2003	343 324 282	0.017
2005	339 322 280	0.050
2007	351 327 280	0.042
2014	339 322 284	0.003
2021	339 324 268	0.014
2022	339 327 282	0.075
2023	341 323 282	0.031
2024	343 323 280	0.053
2031	339 322 282	0.008
2039	345 327 276	0.008
2046	339 329 280	0.003
2048	339 331 282	0.378
2049	339 331 284	0.003
2052	345 321 280	0.056
2053	343 324 280	0.044
2080	339 325 276	0.208
2083	339 324 282	0.003
2098	343 323 282	0.003

Table 6. A comparison of recognized DLA class I and II haplotypes in several different breeds. There is considerable haplotype sharing between breeds, reflecting the common evolution of modern breeds from indigenous dog populations that were greatly expanded during the Neolithic period.

DLA Class I Haplotype Frequencies (Updated Apr 5, 2018)

DLA1 #	STR types	Black Russian Terrier (n=109)	Labrador Retriever (n=121)	Doberman Pinscher (n=403)	Flat Coated Retriever (n=333)	Havanese (n=364)	Samoyed (n=278)	Bulldog (n=1)	English Bulldog (n=163)	Yorkshire Terrier (n=16)	Biewer (n=90)	Biewer Yorshire Terrier (n=50)	Biewer Terrier (n=49)	Biro Biewer (n=3)	Italian Greyhound (n=674)	Alaskan Klee Kai (n=450)	Shiloh Shepherd (n=115)	Magyar Agar (n=27)	Shiloh Shepherd, ISSA (n=127)	American Akita (n=79)	Japanese Akita (n=300)	Blend Akita (n=47)	Golden Retriever (n=688)	Miniature Poodle (n=240)	Poodle (n=2028)
1006	387 375 293 180	0.032	0.029	--	--	0.045	0.004	--	0.003	--	--	--	--	--	--	--	--	--	--	0.089	--	--	0.0145	0.002	0.0483
1008	386 373 289 182	--	0.074	--	--	--	--	--	0.006	0.13	0.011	--	--	--	0.1358	0.061	--	--	--	--	--	--	0.0015	--	0.02
1016	382 371 277 178	0.009	0.012	0.015	--	0.221	--	1	0.095	0.06	0.028	0.04	0.02	--	0.0631	--	--	--	--	--	--	--	0.0015	0.023	0.0145
1017	386 373 289 178	--	0.037	0.088	0.458	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0035
1030	380 373 293 178	--	0.025	0.104	--	0.001	--	--	--	0.63	0.506	0.68	0.98	1	0.0215	--	--	--	--	--	--	--	0.0007	--	0.0032
1033	382 379 277 181	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.15	--	--	--	--	--	0.006	0.0012
1045	376 371 277 186	--	0.012	--	--	--	--	--	--	--	--	--	--	--	--	--	0.004	--	0.004	0.013	--	--	--	0.004	0.0015
1054	382 379 277 184	--	0.091	--	0.119	0.11	--	--	--	--	--	--	--	--	0.0163	--	--	0.11	--	--	--	--	--	0.002	--
1062	382 371 277 183	--	0.021	--	--	--	--	--	0.187	--	--	--	--	--	--	0.214	--	--	--	--	--	--	0.0938	--	--
1065	380 371 277 181	--	0.384	--	0.002	--	--	--	--	--	--	--	--	--	0.0007	--	--	--	--	--	--	--	0.2602	--	--
1066	376 375 277 178	--	0.004	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	0.11	--	--	--	--	0.2805	--	--
1068	380 373 287 181	--	0.041	--	0.266	0.016	0.029	--	--	--	--	--	0.02	--	--	--	0.248	--	0.236	--	--	--	0.0501	0.015	--
1070	380 375 291 178	--	0.008	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0153	--	--
1105	382 379 277 178	--	0.07	0.001	--	--	--	--	--	--	--	--	--	--	0.7	--	--	--	--	--	--	--	--	0.063	0.0002
1134	384 365 291 178	--	0.012	--	--	0.003	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0007	--	--
1142	376 379 277 180	--	0.004	--	0.144	0.001	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1165	392 369 281 182	--	0.161	--	--	--	--	--	--	--	--	--	--	--	--	--	0.235	--	0.232	--	--	--	--	--	--
1175	380 375 293 180	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
1176	382 373 289 178	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

DLA Class II Haplotype Frequencies (Updated Apr 5, 2018)

DLA2 #	STR types	Black Russian Terrier (n=109)	Labrador Retriever (n=121)	Doberman Pinscher (n=403)	Flat Coated Retriever (n=333)	Havanese (n=364)	Samoyed (n=278)	Bulldog (n=1)	English Bulldog (n=163)	Yorkshire Terrier (n=16)	Biewer (n=90)	Biewer Yorshire Terrier (n=50)	Biewer Terrier (n=49)	Biro Biewer (n=3)	Italian Greyhound (n=674)	Alaskan Klee Kai (n=450)	Shiloh Shepherd (n=115)	Magyar Agar (n=27)	Shiloh Shepherd, ISSA (n=127)	American Akita (n=79)	Japanese Akita (n=300)	Blend Akita (n=47)	Golden Retriever (n=688)	Miniature Poodle (n=240)	Poodle (n=2028)
2003	343 324 282	--	0.021	--	0.129	0.221	0.013	--	0.598	0.75	0.222	0.4	0.43	0.3	0.0067	--	--	--	--	--	--	--	0.0225	0.508	0.092
2005	339 322 280	0.018	0.062	--	0.425	0.003	--	--	0.015	--	0.028	0.02	0.18	--	--	--	--	--	--	--	--	--	0.016	--	0.0234
2007	351 327 280	0.032	0.033	--	--	0.049	0.004	--	--	--	--	--	--	--	--	0.01	--	--	--	0.089	--	--	0.0145	--	0.0148
2021	339 324 268	--	0.021	--	--	0.001	--	--	--	--	--	--	--	--	--	0.217	--	--	--	--	--	--	0.0945	0.063	0.0005
2022	339 327 282	--	0.091	0.002	0.123	0.109	0.092	--	0.015	--	--	--	--	--	--	--	0.057	--	0.051	--	--	--	0.0007	0.002	0.0002
2023	341 323 282	0.005	0.025	0.104	--	0.001	--	--	--	0.63	0.506	0.68	0.98	1	0.0215	--	--	--	--	--	--	--	0.0007	--	0.0032
2024	343 323 280	--	0.066	0.001	--	0.004	0.014	--	0.006	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.008	0.0002
2031	339 322 282	0.023	0.012	--	--	--	--	--	--	--	--	--	--	--	0.0623	--	--	--	--	--	--	--	--	--	--
2039	345 327 276	--	0.012	0.012	--	--	--	--	--	--	--	--	--	--	0.1039	--	0.004	--	0.004	0.165	0.18	0.34	--	--	0.0015
2046	339 329 280	--	0.004	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.2689	--	--
2048	339 331 282	--	0.384	--	0.002	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.258	--	--
2049	339 331 284	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
2052	345 321 280	--	0.058	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0022	--	--
2053	343 324 280	--	0.037	--	0.138	0.038	0.613	--	--	--	--	--	0.02	--	--	--	0.317	--	0.303	--	--	--	0.0291	0.015	--
2080	339 325 276	--	0.161	--	--	--	--	--	--	0.06	0.006	0.02	--	--	--	--	0.235	--	0.232	--	--	--	--	--	--
2083	339 324 282	--	0.004	--	0.003	--	--	--	--	--	--	--	--	0.7	--	--	--	--	--	--	--	--	--	--	--
2098	343 323 282	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Breeds or bloodlines within breeds (e.g., Standard Poodle and Italian Greyhounds) that lack genetic diversity in the DLA region are often more prone to autoimmune disorders that take many clinical forms. Genetic diversity within the DLA class I and II regions of the Labrador Retriever is average for the breeds tested. The number of recognized DLA class I (n=19) and class II (n=19) haplotypes in Labrador Retriever is similar to that of Golden Retrievers (24 and 19, respectively) and Italian Greyhound (21 and 19 respectively); lower than Standard Poodles (48 and 29 respectively); and higher than breeds such as American Akita (11 and 10, respectively), Doberman Pinschers (11 and 10 respectively) and Flat Coated Retriever (11 and 10 respectively).

3. A genetic assessment of allele and allele frequencies of STRs associated with DLA class I and II haplotypes

Although it appears from breed-wide haplotype frequency that there is some positive selection for certain DLA haplotypes (Table 5), this was not confirmed by standard genetic assessment of the individual STR loci that are in linkage (association) with DLA class I and II haplotypes (Table 7). Table 8 provides a genetic assessment of heterozygosity among alleles in the seven DLA-associated STR loci. The average number of alleles for all loci was 6.29, with 3.55 alleles having the most effect on genetic assessment values. The Na and Ne values for the DLA STRs were like those calculated from the 33 genomic STRs. The Ho and He values were almost identical and yielded an F value of -0.03. Therefore, the actual alleles in the seven STR that are associated with the various classes I and II haplotypes were in near Hardy-Weinberg equilibrium across the breed, again corroborating the standard genetic assessment values obtained from the 33 genomic STRs. However, because these STR loci are limited to a region on a single chromosome, they are not good for determining genetic diversity across a much wider region of the genome. Nonetheless, such calculations add power to the assessments made by the 33 STRs used for this purpose.

Table 7. Standard genetic assessment of Labrador Retriever based on 7 STR loci linked to the DLA class I and II regions.

#	Locus	N	Na	Ne	Ho	He	F
1	DLA I-3CCA	180	7	3.385	0.706	0.705	-0.001
2	DLA I-4ACA	180	6	3.771	0.767	0.735	-0.043
3	DLA I-4BCT	180	6	2.691	0.622	0.628	0.010
4	DLA1131	180	7	3.441	0.739	0.709	-0.042
5	5ACA	180	5	1.738	0.417	0.425	0.019
6	5ACT	180	8	4.453	0.783	0.775	-0.010
7	5BCA	180	5	2.685	0.606	0.628	0.035

Table 8. Summary of Standard Genetic Assessment for Labrador Retriever using 7 STRs in the DLA region

	N	Na	Ne	Ho	He	F
Mean	180	6.286	3.166	0.663	0.658	-0.005
SE		0.389	0.308	0.045	0.041	0.010

The average number of alleles (Na) in each of the DLA associated STRs was comparable to that observed in the 33 genomic STRs (Na=6.3) and similarly, about half of the alleles contributed most to the standard genetic assessment values (Ne=3.1). The observed homozygosity breed-wide was almost the same as the expected homozygosity, giving an average inbreeding coefficient close to zero (F=-0.03). However, the same thing applies to these results as those for the 33 genomic STR loci and there are apt to be individuals that are very outbred in the DLA and others that are very inbred.

V. What does this assessment of genetic diversity tell us about contemporary Labrador Retrievers

As is the case with many breeds, analysis of DNA does not always agree with historical facts. The history of the Labrador Retriever suggests that the breed started with a few introductions to the UK of what was known as St. John's retrievers from Newfoundland. However, there is no evidence that one or a small number of related dogs were important in founding the breed. DNA results suggest that the founding population was reasonably large and diverse and that other retrievers may have been involved in the early formation of the breed. The breed has reasonable genetic diversity that is being maintained by current breeding practices. However, individual dogs are products of very inbred as well as outbred parents, masking the problem of excessive inbreeding in a small, but significant, portion of the breed. Identification of such dogs may require DNA testing rather than pedigree examination.

Selection for dogs with desirable bench (show) or field (performance) traits has created phenotypic and genotypically distinguishable bloodlines, explaining the decreasing number of dual show/performance dogs (8). This dichotomy has been well described in several breeds using DNA (9). With time, it is possible that phenotypic and genotypic differences will lead to separate breeds such as the show and performance English Setters.

More information is needed on Labrador Retrievers from outside North America. It is likely, given purported phenotypic differences, that Labrador Retrievers from the UK, Continental Europe, the Russian Federation, Asia and Australasia will constitute separate varieties or bloodlines. Such dogs may be important in increasing and maintaining genetic diversity in the breed, regardless of the country or region of origin.

A standard genetic assessment of the allele frequencies of 33 STRs indicated that the average Labrador Retriever was a product of random selection. However, IR values for individual dogs indicated that there was about one-fourth of dogs that were offspring of more closely related parents and that this population was balanced by an equal number of dogs born to quite unrelated parents. This suggests that there are bloodlines within the breed that may be more inbred than others for a reason. For example, in a study of genetic obesity, the allelic distribution in the affected population (assistance dogs) was markedly out of Hardy-Weinberg equilibrium, indicating positive selection in that population (20). The assistance dogs may also have been under positive selection for a more favorable behavior that also happened to involve obesity.

The high number of deleterious genetic disorders in Labrador Retrievers is troubling given their assumed genetic diversity. Artificial selection for physical or behavioral traits deemed to be favorable in bench or field traits can result in smaller inbred lines within a large breed such as the Labrador Retriever. This is compounded by geographic preferences. Studies of most simple genetic disorders in the breed are frequently concentrated on specific sub-populations of the breed (14-27). Inbreeding to create certain favorable lines often involves popular sires in show or performance trials and leads to the inadvertent selection of undesirable mutations that are often associated with regions of intense selection.

VI. Health problems of Labrador Retriever

A UK breed survey conducted in 2014 reported an average lifespan for the Labrador Retrievers of 12 years and 3 months, with some living up to 19 years of age (13). Therefore, the Labrador Retriever is considered a reasonably healthy breed with few serious problems (11, 12). Most of these occur at a low frequency and frequently occur in other breeds and among random bred dogs. Labrador Retrievers are prone to hip and elbow dysplasia, especially the larger dogs, though not as much as some other breeds. A luxating patella is another orthopedic condition occurring in some individuals. These health problems involve complex genetics, and the predisposing traits may well have been in dogs for centuries or even longer, inherited and concentrated by descent during breed creation. Deafness also occurs in Labradors, either congenitally or later in life. In common with many pure breeds, Labrador Retrievers suffer a low incidence of autoimmune diseases, a problem shared with many breeds. Epilepsy, another complex genetic disorder that is becoming increasingly more common in many dog breeds also occurs in Labrador Retrievers.

Although the health of the breed has been attributed to its genetic diversity, a surprising number of genetic diseases have been identified in the breed over the last decade or so (Table 9). Progressive retinal atrophy, cataracts, corneal dystrophy and retinal dysplasia are specific genetic problems in the breed. Hereditary myopathy, a rare autosomal recessive disorder that causes a deficiency in type II muscle fiber and a "bunny hopping" gait has been identified in the breed. It is caused by mutation in the PTPLA gene. Some Labradors suffer from exercise induced collapse (EIC), a syndrome that causes hyperthermia, weakness, collapse, and disorientation after short bouts of exercise. It is caused by an autosomal recessive mutation. A mutation of the urate transporter gene occurs in Labrador Retrievers, as well as many other breeds, but at very low frequency (<1%). Obesity in Labrador Retrievers has been associated with a mutation in the POMC gene. Twenty-three percent of Labradors in the UK carry at least one copy of the mutation. Each copy of the mutation purportedly increases a dog's weight by 1.9kg. Progressive rod-cone degeneration (prcd) is type of progressive retinal atrophy that occurs in Labrador Retrievers and other several breeds.

Table 9. List of published genetic disorders that have been identified in Labrador Retrievers

Disorder	Gene mutation	Mode of inheritance	Reference	DNA Test
Cornification skin	NSDHL	X-linked	14	?
Myopathy (CNM)	MTML	X-linked	15	Yes
Masking	M264V	AD	16	?
Copper toxicosis	ATP7A	AD	17	YES?
Dwarfism	COL11A2	AR	18	?
Collapse (EIC)	DNM1	AR	19	Yes
Nasal parakeratosis	SUV39H2	AR	20	Yes
Myopathy	PTPLA	AR	21	?
Obesity	POMC	AR?	22	Yes
Myasthenia	COLQ	AR	23	?
Corneal dystrophy	CHST6	AR	24	?
Neurodegeneration	GFAP	AR	25	?
Hip dysplasia	Polymorphic	Complex	26	No
prcd-PRA	PRCD	AR	27	Yes

AR=autosomal recessive; X-linked =found mainly in males; AD=autosomal dominant;= not regularly available

Several examinations and tests have been recommended for Labrador Retrievers to be used for breeding. OFA evaluations are recommended for hip dysplasia and elbow dysplasia, an eye examination by a board certified veterinary ophthalmologist for any ocular abnormality, and DNA-based tests for the causative mutations of exercise induced collapse, the D (dilute) locus, centronuclear myopathy and prcd-progressive retinal atrophy. Results should be recorded in the OFA or CHIC. Cardiac examinations are recommended for congenital or advanced cardiac disease breeding dogs in dogs used for breeding.

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