

Genetic Diversity Testing for Flat-Coated 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 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.

Price: \$80

Price reduced to \$70 when combined with a diagnostic test.

[ORDER TEST KITS](#)

Allow 5-10 business days for results.

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.

Internal Relatedness: The IR value is a measure of genetic diversity within an individual that takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog and cannot be compared between dogs. Two dogs may have identical IR values but with very different genetic makeups.

I. Introduction

A. Breed history

The history of the Flat-Coated Retriever is typical of many pure breeds. It usually starts with a kennel that produced a small number of outstanding dogs and a key person or persons that recognized their exceptional nature and decided to use them as a foundation for a new breed. The prototypic Flat-Coated Retriever was widely used on English estates for hunting game birds. These early dogs were “mongrels” that were purportedly bred from stock imported from North America that included breeds such as the now extinct St. John's water dog and the Newfoundland. Introgressions with native Collie-type dogs may have been used to increase the breed's trainability and Setter blood for enhanced scenting ability. Notable examples of the

prototypic breed were kept by gamekeeper John Hull in 1860 and include a bitch named “Old Bouncy” and her daughter “Young Bouncy.” However, the man most credited with the breed's ultimate development was S.E. Shirley, who standardized the desired type through careful selection. H. R. Cooke, whose Riverside Kennel produced many fine field and show Flat-Coats, also helped bring public attention to the breed.

After its introduction into the U.S., the Flat-Coated Retriever began to quickly gain in popularity as a gun dog from 1873 until 1915, when it was officially recognized as a breed. However, soon after, the breed's popularity began to decrease - eclipsed by the Golden Retriever, which was actually bred in part from the Flat-Coated Retriever and other breeds. Only a small number of Flat-Coated Retrievers remained at the end of WWII, and the breed's survival was uncertain. Fortunately, proponents of the breed were able to bring it back to reasonable numbers by the mid-1960's, and the breed has steadily gained in popularity ever since for sport, conformation showing, and companionship. Although its popularity has never again achieved the level of the Labrador and Golden Retriever, the Flat-Coated Retriever is increasingly recognized for its comparatively good health and success in agility, hunting, retrieving, and tracking. It has also been increasingly used for drug-sniffing and as guide dog for the blind, and similar to the Golden Retriever, it is often crossed with Labrador Retrievers for these roles.

The breed has also become popular in shows in recent decades, which has increased its visibility and popularity around the world. “In 2011, 'Sh Ch. Vbos The Kentuckian' (aka Jet), a 9.5-year-old Flat-Coated Retriever from South Queensferry, near Edinburgh, Scotland, won Best in Show at Crufts. ‘Almanza Far and Flyg’ (a.k.a. Simon), from Oslo, Norway, won the Gundog Group at Crufts in 2007. Before that in 2003, a Swedish dog 'Inkwells Named Shadow' had also won the Gundog Group. The last UK dog to win the Gundog Group at Crufts was ‘Sh Ch Gayplume Dream-maker’ in 2002. The only other Flat-Coated Retriever to win Best in Show at Crufts was 'Ch. Shargleam Blackcap' in 1980.” (https://en.wikipedia.org/wiki/Flat-Coated_Retriever, 7/19/2016)

B. Breed standard and appearance

Flat-Coated Retrievers are extremely uniform in appearance as a result of decades of adherence to the breed standard. Google search finds many fine images of individuals, young and old and at work and play, <https://goo.gl/0Xeuaa>. The Flat-Coated Retriever Society of America, Inc. (FCRSA) is highly active in maintaining the standards and health of the breed (<http://www.fcrsainc.org>). Flat-Coated Retrievers come mainly in two solid colors, black and liver (deep reddish-brown). Yellow is an uncommon color. They were recognized by the AKC in 1909 and are currently the 90th most popular pure breed in the US. The Flat-Coated Retriever is a large and athletic dog, with the familiar retriever shape and retriever abilities. Positive traits are listed as a thick coat with feathering on ears, legs and tail; athleticism, proper behavior even with strangers and other animals, and its rarity and uniqueness. Negative characteristics include the need for vigorous exercise, rowdiness and over-exuberance (especially when young or not routinely exercised), excessive chewing, the need for regular brushing to avoid excessive shedding into the environment, and high incidence of cancer that can decrease their average lifespan.

C. Heritable disorders

Glaucoma and progressive retinal atrophy (PRA) occur in about 1% of Flat-Coated Retrievers and are considered to be heritable conditions. The exact mutations are unknown and no genetic tests are available

(<http://www.fcrsainc.org/resources/articles/glaucoma.html>; <http://fcrsainc.org/health/prrarticle.html>). However, routine testing for both conditions should be conducted, and affected animals not used for breeding. Occasionally, epilepsy is also seen in the breed. Bloat (gastric torsion) is a life-threatening condition that is seen occasionally in all large deep-chested dogs.

Flat-Coated Retrievers have a higher risk of cancer than most dogs. Hemangiosarcoma, lymphoma, osteosarcoma, and malignant histiocytosis are particularly devastating, and occur at higher rates in Flat-Coats than in many other breeds. The first three cancers are common in dogs and are especially prevalent among Golden Retrievers, while malignant histiocytosis is a peculiar cancer shared almost exclusively with the Bernese Mountain Dog. According to studies sponsored by the Flat-Coated Retriever Society of America (FCRSA), the average lifespan of the Flat-Coated Retriever is only about eight years, with a high percentage of deaths due to cancer.

Flat-Coated Retrievers have a very low rate of hip dysplasia and luxating patellas compared to other medium-sized breeds; the Orthopedic Foundation for Animals statistics consistently show a rate of hip dysplasia in the breed of less than 3%. In the 1997 FCRSA health survey, 4.2% of males and 3.2% of females had been diagnosed with luxating patellae.

II. Baseline genetic diversity testing and what it tells about Flat-Coated Retrievers

A. Standard genetic assessments based on 33 STR loci on 25 chromosomes and allele frequencies

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs, those 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. Each STR locus manifests several different genetic configurations known as alleles. Each dog inherits one of these alleles from the sire and the other from the dam. Table 1 lists the alleles recognized at each STR locus among 190 Flat-Coated Retrievers tested to date, as well as listing the frequency of any given allele in the population.

([link to table 1](#))

1. Standard genetic assessment values for individual STR loci

The allele frequencies (Table 1) can be used to do a standard genetic assessment of heterozygosity at each STR locus (Table 2). The value N_a is the number of alleles that are observed at each locus for a specific breed, while N_e is the number of effective alleles observed at each locus. Effective alleles are those alleles that contribute the bulk of the diversity. The N_a

values for individual STR loci for this population of 296 Flat-Coated Retrievers ranged from a low of 3 to a high of 13, while the Ne ranged from 1.071 to 4.35.

Observed heterozygosity (H_o) is based on the actual allele frequencies at each STR locus and their distribution, while the expected heterozygosity (H_e) of a locus is the value that would be predicted if allele frequencies at a specific locus were in Hardy-Weinberg equilibrium (HWE). HWE is achieved when all alleles at a specific locus are segregating randomly. A H_o value of 1.0 would be observed when alleles at each locus are unique to everyone in the population. A H_o value of 0.00 would occur if there is no heterozygosity, e.g. every individual has the same alleles at a given locus. H_o ranged from 0.147 to 0.754, indicating a large range of genetic diversity from one locus to another. The H_e ranged from 0.063 to 0.763 across the 33 STR loci (Table 2). The H_o and H_e values were used to calculate the F value ($1-H_o/H_e$), a measure of deviation from HWE. F values ranged from -0.075 to +0.177. Twenty-five loci had FIS values of 0 or greater, while 8 loci had FIS scores less than 0, indicating an excess of inbred alleles in the population. One allele was virtually fixed at two loci, LE1004 and VGL1828 (90.6 and 96.6% of dogs, respectively) (Table 1). This near fixation of a single allele indicates that these two loci are associated with breed defining traits that have been strongly conserved throughout breed evolution.

Table 2: Genetic assessments for individual STR loci of Flat-Coated Retrievers. Na= alleles/locus; Ne= effective alleles/locus; Ho=observed heterozygosity; He=expected heterozygosity; F=coefficient of inbreeding (deviation from HWE expectation).

#	Locus	N	Na	Ne	Ho	He	F
1	AHT121	546	8	2.874	0.612	0.652	0.062
2	AHT137	546	6	2.987	0.628	0.665	0.056
3	AHTH130	546	4	2.830	0.623	0.647	0.037
4	AHTh171-A	546	5	1.582	0.368	0.368	-0.000
5	AHTh260	546	4	2.109	0.516	0.526	0.018
6	AHTk211	546	5	2.923	0.615	0.658	0.065
7	AHTk253	546	5	2.256	0.542	0.557	0.026
8	C22.279	546	5	2.810	0.661	0.644	-0.027
9	FH2001	546	8	2.260	0.538	0.557	0.034
10	FH2054	546	7	2.067	0.489	0.516	0.053
11	FH2848	546	6	2.242	0.555	0.554	-0.002
12	INRA21	546	6	3.646	0.707	0.726	0.026
13	INU005	546	3	2.471	0.606	0.595	-0.018
14	INU030	546	3	1.561	0.353	0.359	0.016
15	INU055	546	4	1.540	0.333	0.351	0.050
16	LEI004	546	4	1.212	0.178	0.175	-0.015
17	REN105L03	546	4	2.386	0.603	0.581	-0.037

18 REN162C04	546	5	2.725	0.590	0.633	0.068
19 REN169D01	546	7	4.017	0.758	0.751	-0.010
20 REN169O18	546	8	4.408	0.745	0.773	0.036
21 REN247M23	546	3	1.993	0.469	0.498	0.059
22 REN54P11	546	4	2.857	0.670	0.650	-0.031
23 REN64E19	546	5	3.287	0.650	0.696	0.066
24 VGL0760	546	14	3.681	0.723	0.728	0.007
25 VGL0910	546	9	3.872	0.709	0.742	0.044
26 VGL1063	546	9	3.412	0.685	0.707	0.031
27 VGL1165	546	8	3.951	0.740	0.747	0.009
28 VGL1828	546	5	1.071	0.059	0.066	0.112
29 VGL2009	546	6	1.497	0.333	0.332	-0.004
30 VGL2409	546	5	1.309	0.247	0.236	-0.048
31 VGL2918	546	9	3.169	0.687	0.684	-0.003
32 VGL3008	546	6	1.819	0.454	0.450	-0.009
33 VGL3235	546	6	3.214	0.678	0.689	0.016

2. Using allele frequency data to do standard genetic assessments on the population as a whole.

Allele frequencies across all 33 STR loci taken from Table 1 can also be used to calculate a mean observed heterozygosity (H_o) and expected heterozygosity (H_e) for the Flat-Coated Retriever population (Table 3). The population of 394 Flat-Coated Retrievers which were tested had a mean number of alleles (N_a) of 5.68 across all 33 genomic STR loci. The average number of alleles per locus was low compared to other breeds that have been tested, indicating a low level of genome wide genetic diversity. The mean effective alleles (N_e) per locus were 2.61, also one of the lower N_e scores that we have observed. The low N_e indicates that less than one half of available alleles account for most of the diversity, which is a common proportion for many pure breeds. The mean observed heterozygosity (H_o) was 0.548, which was lower than the expected heterozygosity (H_e) of 0.562. This resulted in an F value (0.024) that is slightly higher than zero, indicating that breed wide heterozygosity is nearly in line with HWE, except for a small proportion of dogs that are more inbred than the total population. Heterozygosity values based on allele frequencies across the entire breed and 33 STR loci indicate that most Flat-Coated Retrievers are products of mating between bitches and studs that are as unrelated as possible, given the limited genetic diversity in the breed. However, H_o and H_e are average scores for the population and may not accurately reflect individuals that are more outbred or inbred than the total population. This is better reflected by the IR scores (see below).

Genetic diversity in the Golden Retriever (Table 4), although considered to be relatively low compared to breeds such as the Standard, Miniature and Toy Poodles, was still greater than for Flat-Coated Retrievers (Table 3). The average number of alleles per locus (N_a) and average number of effective alleles per locus (N_e) was also greater for Golden Retrievers than Flat-

coated Retrievers (8.30 vs. 5.68) as were the observed heterozygosity (Ho) (0.651 vs. 0.548). However, the inbreeding coefficient F for Golden Retrievers was higher than for Flat-Coated Retrievers (0.049 vs 0.024) indicating that the proportion of inbred dogs was higher among Golden Retrievers than Flat-coated Retrievers.

Table 3: Genetic assessment of Flat-Coated Retrievers based on allele frequencies at 33 genomic STR loci on 25 chromosomes.

	N	Na	Ne	Ho	He	F
Mean	546	5.939	2.607	0.549	0.561	0.021
SE		0.393	0.153	0.030	0.031	0.006

Table 4: Genetic assessment of Golden retrievers based on allele frequencies at 33 genomic STR loci.

	N	Na	Ne	Ho	He	F
Mean	713	8.394	3.420	0.652	0.687	0.053
SE		0.463	0.177	0.014	0.013	0.006

3. Using allele frequency data from 33 genomic STR to examine the genetic relationship of individuals within a population.

Principal coordinate analysis (PCoA) uses genetic distance based on allele sharing to demonstrate genetic differentiation between individuals in related or unrelated populations. The resulting data is multi-dimensional but can be accurately portrayed in a two-dimensional graph by selecting values from the two coordinates that represent the greatest proportions of individuals (coordinate 1 and 2 in this case). Figure 1 is a PCoA plot of Flat-coated Retrievers that shows them clustering as a single breed, although the plot is somewhat diffuse with several more distant outliers. This pattern suggests that there is still phenotypic, and therefore genotypic, variation in the breed.

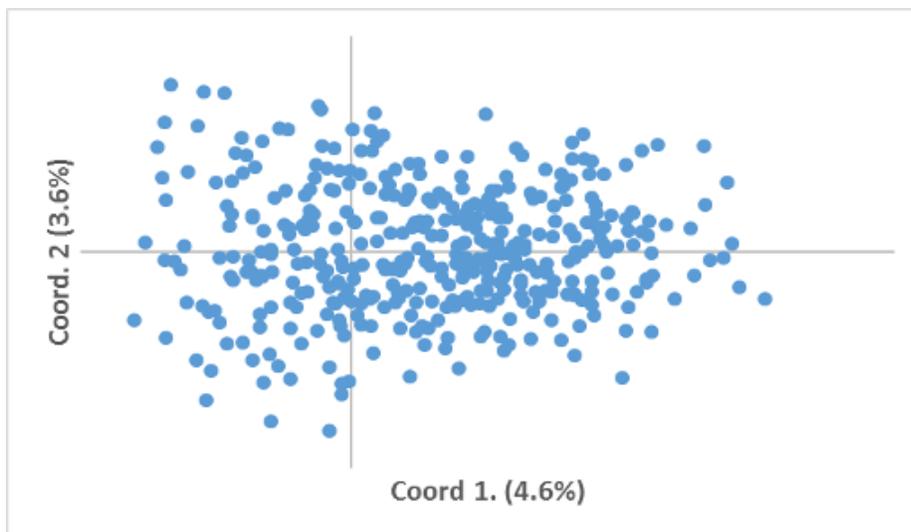


Figure 1: PCoA plot of 402 Flat-coated Retrievers.

It is believed that Flat-coated Retrievers played an important role in the development of the Golden Retriever. Figure 2 is a PCoA plot comparing 686 Golden Retrievers with 296 Flat-coated Retrievers. This PCoA plot shows that the Flat-Coated Retrievers belong to a single breed (population) that is genetically distinct from Golden Retrievers. Although this does not mean that Golden Retrievers are unrelated to Flat-coated Retrievers, it does suggest that the relationship is not as close as assumed. The tight clustering of the Flat-Coated Retrievers compared to Golden Retrievers in this comparative PCoA indicates that Flat-Coats are more related to each (i.e., less genetically diverse) than Golden Retrievers.

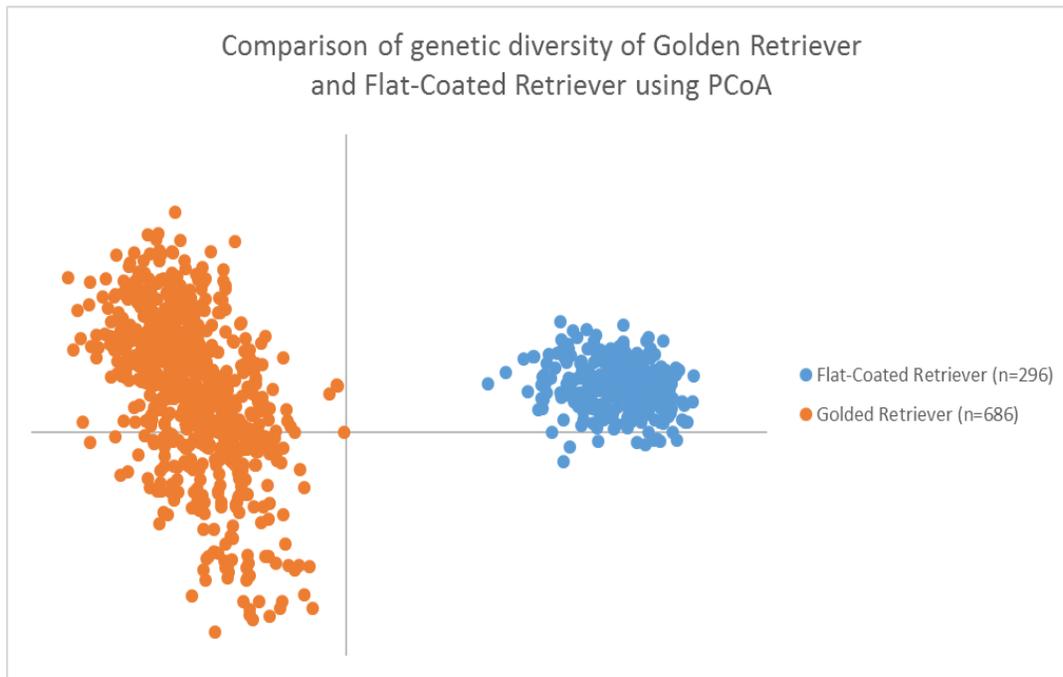


Figure 2: PCoA plot comparing 296 Flat-Coated Retriever (FR) population with 686 Golden retrievers (GR).

B. The use of genomic allele frequencies to determine internal relatedness

1. Internal relatedness of individuals and the population as a whole

Genetic assessments such as those presented in Table 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 relative genetic contributions of both parents to an individual. The IR calculation evaluates homozygosity and uses allele frequencies to give more importance to rare and uncommon alleles. 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 would be equivalent to offspring of full sibling parents from a random breeding population. IR values >0.25 occur when the parents of the full sibling parents were themselves highly inbred.

The IR curve calculated for the Flat-Coated Retrievers tested ranged from around -0.278 for the most outbred dog to +0.385 for the most inbred, with a mean value for the population of +0.021 (Table 5, Fig. 3). Therefore, one half of the dogs had IR values over +0.021 and one quarter over +0.096. However, the IR curve was distinctly bimodal, with one quarter of dogs having a peak value nearer to +0.25 and a range from +0.096 to 0.385. A value of +0.25 is comparable to the genetic diversity of offspring of a full-sibling mating. This highly inbred subpopulation is balanced by the one quarter of outbred dogs with IR values of -0.058 to -0.279. This balancing of highly outbred and inbred dogs is why the H_o , H_e and F_{IS} values calculated for the whole population using allele frequencies of the 33 genomic STR loci gave the impression that the dogs tested were all randomly bred.

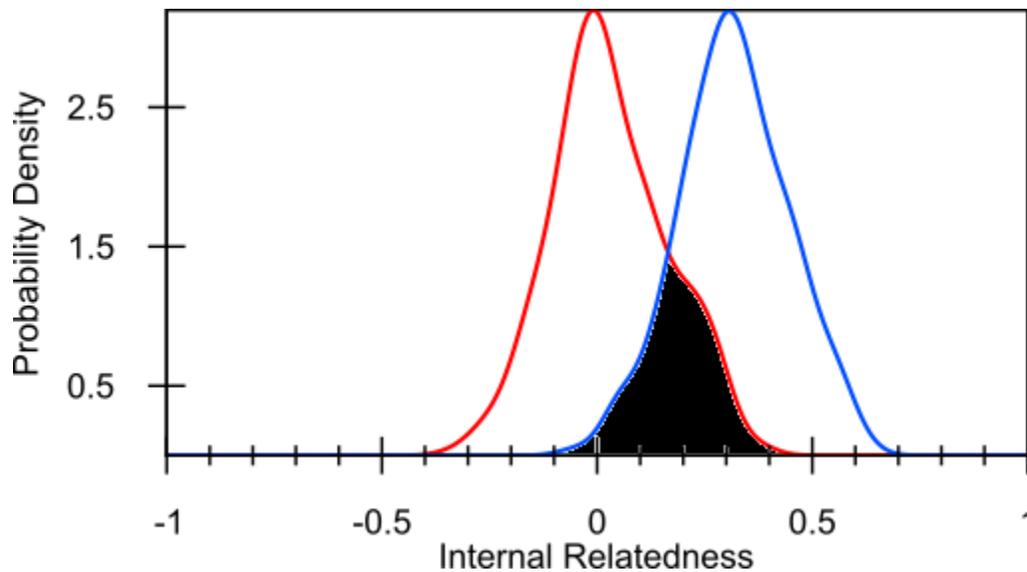


Fig. 3: Distribution of IR estimates in 402 Flat-coated Retrievers based on intra-breed diversity (Red line), compared with IR adjusted for diversity lost during breed development (Blue line). Diversity lost because of breed development was determined by comparing allele frequencies at the same loci between Flat-coated Retriever and randomly breeding village dogs from the Middle East, SE Asia, and the Pacific Islands. The darkened area (35.2%) represents alleles shared between village dogs and Flat-coated Retrievers, while the adjacent white area (64.8%) under the blue curve is an estimate of potential genetic diversity lost in breed evolution.

Table 5: Statistical breakdown of IR and IRVD values used to create population curve shown in Figure 3.

	IR	IRVD
Min.	-0.27884	0.0334
1st Qu	-0.05769	0.2378
Median	-0.0023	0.3038
Mean	0.020774	0.3133

3rd Qu	0.095531	0.3985
Max.	0.385474	0.6327

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

The IR values can be adjusted in such a way as to provide an estimate of the amount of genetic diversity that has been during breed evolution. This is done by using allele frequencies obtained from DNA of present day village dogs from the Middle East, SE Asia and Island Pacific nations, which closely reflect the ancestors of dogs before extensive pure breeding. Village dogs are the most random bred and genetically diverse population that has been studied to date. The adjusted IR value is known as IR-village dogs or IRVD. The IRVD curve was shifted well to the right, reflecting a 70.5% loss of potential genetic diversity during breed development (Fig. 3, Table 5). One half of the dogs, when adjusted for diversity lost since their progeny evolved from village dogs, had IRVD values from +0.304 to +0.633.

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

The DLA consists of four gene rich regions 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.

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. The number of DLA class I and II haplotypes has increased as the number of Flat-coated Retrievers tested has risen from 139 to 394 (Table 6). There are now 11 class I and 10 class II haplotypes, with most of the additions involving dogs with less common haplotypes. DLA class I haplotypes 1017, 1054, 1068, 1142 and DLA class II haplotypes 2003, 2005, 2018, 2022, and 2053 are found in 98% of individuals, while the remaining haplotypes are at low incidence and relatively random in distribution. These findings indicate that four founder or founder-bloodlines were strongly involved in the breed's creation and/or evolution.

The DLA class II 1142, 1143, 1170, and 1171 haplotypes are unique to the breed and most likely go back to the original founders, while the remaining haplotypes are common in other breeds such as the Standard poodle (1017), Italian greyhound (1054) and Golden retriever (1066, 1068). All the DLA class II haplotypes are also found in breeds such as the Standard poodle (2003, 2005, 2014, 2022) and Golden retriever (2046, 2053). The sharing of haplotypes between

Standard poodle, Golden retriever and Flat-Coated Retrievers is understandable given that they were all originally developed as retrievers of fowl in Western Europe. The low number of dominant DLA class I and II haplotypes suggest either a small founder population, or a subsequent loss of genetic diversity due to artificial genetic bottlenecks such as geographic isolation, popular sire effects, popular bloodline effects, catastrophic events such as world wars, etc.

Table 6: DLA class I and class II haplotypes and their frequency in Flat-coated Retriever

DLA Class I Haplotype Frequencies (Updated Oct 9, 2019)		
DLA1 #	STR types	Flat Coated Retriever (n=546)
1017	386 373 289 178	0.4487
1035	386 373 277 184	0.0055
1054	382 379 277 184	0.1190
1065	380 371 277 181	0.0009
1066	376 375 277 178	0.0009
1067	376 373 277 178	0.0009
1068	380 373 287 181	0.2711
1142	376 379 277 180	0.1419
1143	386 373 287 181	0.0055
1170	386 373 277 180	0.0009
1171	380 373 287 184	0.0009
1199	380 373 285 181	0.0037

DLA Class II Haplotype Frequencies (Updated Oct 9, 2019)		
DLA2 #	STR types	Flat Coated Retriever (n=546)
2003	343 324 282	0.1447
2005	339 322 280	0.4194
2014	339 322 284	0.0302
2017	343 322 280	0.0009
2018	339 324 284	0.1401
2022	339 327 282	0.1255
2046	339 329 280	0.0009
2048	339 331 282	0.0009
2053	343 324 280	0.1346
2083	339 324 282	0.0027

F. Using standard genetic assessment parameters and DLA class I and II STR allele frequencies to gauge diversity in the entire DLA region.

It is important to maintain as much genetic diversity in the DLA region as possible and to select mates in a random manner to maintain that diversity. The frequency of haplotypes can be misleading, especially when they are few and certain haplotypes predominate. Genetic diversity in the DLA regions can be more accurately assessed by studying the alleles and allele frequency at the four DLA class I and three DLA class II STR loci. The calculations listed in Table 7 provided one measurement of how the individual alleles that define the DLA class I and II haplotypes are distributed (Table 7).

Genetic diversity in the DLA region can be assessed by studying the frequency of the DLA class I and II alleles of the four DLA class I and three DLA class II STR loci (Table 7), in the same manner as employed with the 33 genomic STR loci. Although these STRs are associated only with the DLA class I and II regions on chromosome 12, the numerous genes and their alleles that make the entire DLA is in strong linkage disequilibrium, meaning that it is inherited as a large block of genes that are less subject to recombination. Therefore, genetic diversity in the DLA region reflects genetic diversity in the genome, but not to the same extent as the 33 genomic STR markers.

Table 7: Standard genetic assessment of the DLA regions using 7 STRs associated with the DLA class I and II regions.

#	Locus	N	Na	Ne	Ho	He	F
1	DLA I-3CCA	546	4	3.091	0.645	0.677	0.047
2	DLA I-4ACA	546	4	1.635	0.381	0.388	0.019
3	DLA I-4BCT	546	4	2.846	0.606	0.649	0.065
4	DLA1131	546	4	3.143	0.641	0.682	0.060
5	5ACA	546	2	1.676	0.377	0.403	0.065
6	5ACT	546	5	2.519	0.544	0.603	0.098
7	5BCA	546	3	2.422	0.570	0.587	0.030

	N	Na	Ne	Ho	He	F
Mean	546	3.714	2.476	0.538	0.570	0.055
SE		0.333	0.217	0.040	0.043	0.009

Flat-Coated Retrievers have a mean of 3.71 DLA class I and II associated alleles at each of the 7 STR loci and 2.48 of these alleles contribute to the bulk of an individual dog's DLA diversity (Table 7). The low number of alleles at each of the DLA loci reflects the low number of DLA class I and II haplotypes in the breed. The Ho for the DLA alleles is like that of the 33 genomic

STRs (0.546 vs. 0.548) as is the H_e (0.571 vs 0.562) (Tables 3, 7). The F value is slightly higher (0.043 vs 0.024) (Tables 3, 7) but both are close to zero. Therefore, both DLA and genomic markers are under random selection breed-wide.

The calculations listed in Table 7 provided one measurement of how the individual alleles that define the DLA class I and II haplotypes are distributed and suggest that diversity in this important region is being maintained in a random fashion. However, the low number of haplotypes and the high frequency of an even smaller number of them increases the chance that even random breeding may lead to a high frequency of homozygosity in DLA haplotypes, something that is generally seen as more likely to be deleterious than beneficial. It is possible to measure the proportion of the population that have inherited the same DLA class I or II haplotype from each parent (i.e., homozygous) (Table 8). About one third of Flat-Coated Retrievers have inherited the same DLA class I haplotype from each parent, one-fourth for the same class II haplotype, and one-fourth for the same linked class I/II haplotypes. This degree of homozygosity is understandable given the low numbers of DLA haplotypes that exist in the breed.

Table 8: Homozygosity vs. heterozygosity for alleles at each of the 4 DLA class I and three DLA class II associated STR loci for 190 Flat-coated Retrievers

	#homozygous	#heterozygous	%homozygous
DLA class I loci			
3CCA	62	128	32.60%
4ACA	108	82	56.80%
4BCT	70	120	36.80%
1131	63	127	33.20%
DLA class II loci			
5ACA	125	65	65.80%
5ACT	80	110	42.10%
5BCA	72	117	38.40%
Class I haplotype	62	128	32.60%
Class II haplotype	51	139	26.80%
Class I/II haplotype	50	140	26.30%

III. Interpretation of genetic diversity test data for Flat-Coated Retrievers

A. Implications of low genetic diversity

Flat-Coated Retrievers have a low level of breed-wide genetic diversity comparable to popular breeds such as the Bulldog and smaller breeds like Black Russian Terrier and Doberman Pinscher. This is understandable given that the breed descended from as few as 4-5 founders or founder-bloodlines that were heavily influenced by a famous mother and daughter. Aspects of these first “flat-coats” were greatly admired by S. E. Shirley, who standardized the desired type and created the ultimate founder population. Such “standardization” usually involves inbreeding

to obtain a uniform physical appearance and behavior, after which the founder population is closed to further genetic introgressions. A lack of genetic diversity is also reflected by the great uniformity between individual Flat-Coated Retrievers. Phenotypic differences, even when small, are associated with genotypic differences, and the more genotypic differences, the greater the genetic diversity.

Although the history of Flat-Coated Retrievers mentions the use of a several breeds in its origin, it must be assumed that either not as many breeds were used as believed or that the diversity provided by many of the initial founders has been subsequently lost. The breed undoubtedly lost some of its diversity during WWII, as did many other European breeds, or from the loss of popularity after the war and until the 1960's when a decision was made to rescue remnants of the breed.

A loss of genetic diversity is often associated with a decline in breed health, but this does not seem to be the case with the Flat-Coated Retriever, as it has been for other breeds. If the foundation stock of a breed possesses good genetic health at the onset, it remains closed to the deliberate or inadvertent introgression of deleterious genes from outside of the breed, good health will usually be maintained over numerous generations, providing that the population remains continuously in what is called Hardy-Weinberg equilibrium. Spontaneous mutations can always occur, and some can be deleterious, but they will remain at low frequency as long as they are not inadvertently amplified by positive selection of a linked beneficial trait. Popular sire or line effects are most likely to cause deleterious mutations to rise to a level of importance. Championships in the show ring over the last decade has increased the popularity of Flat-coated Retrievers, and there is no doubt that the fastest way to attain a certain appearance is to mate dogs of the same desired type. Phenotype is determined by the genotype, and if the phenotypic appearance is similar, the genotypes will be similar. Therefore, even if the pedigrees show two dogs to be unrelated, if they have the same desired traits, they are apt to share the same DNA encoding those traits.

The greatest problem faced by any breed is the loss of health and longevity resulting from the accumulation of numerous complex and simple deleterious genetic traits. Although the Flat-Coated Retriever has a relatively low amount of genetic diversity, founders of the breed and subsequent breeders were wisely chosen to maintain the basic appearance of the normal ancestral dog. The fact that this form was tested in actual performance venues also favored selection for soundness. Form and function are intimately associated, and when form is changed, so is function. An example would be the Bulldog, which is somewhat more genetically diverse than the Flat-Coated Retriever, but nonetheless suffers numerous serious health problems due to an over exuberance towards chondrodysplasia, brachycephaly, and skin wrinkles.

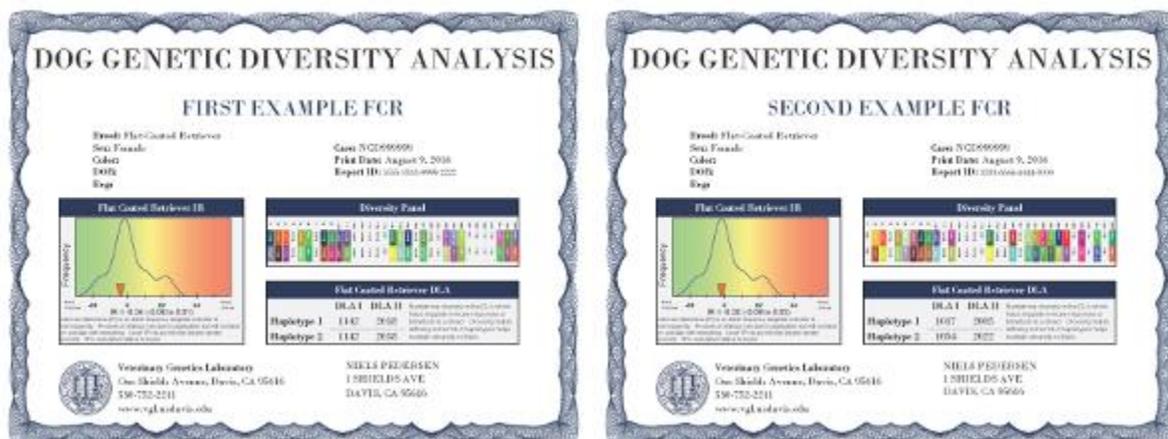
Flat-Coated Retrievers suffer from a low incidence of genetic disorders that are potentially of a simple recessive nature, such as PRA and glaucoma. However, breeders appear to have limited these disorders to a low level by testing their dogs for the earliest signs of these diseases and avoiding using affected dogs for breeding. Orthopedic problems such as hip and elbow dysplasia are also comparatively uncommon, again due to the avoidance of chondrodysplasia and selection for a gait more compatible with performance. The most significant problem in the breed is cancer. Except for malignant histiocytosis, the major cancers of the breed are closely linked to

those of the Golden Retriever, a breed sharing ancestral history. Malignant histiocytosis is also a significant cancer in the Bernese Mountain Dog, which indicates the genetic influence of a common ancestor(s) and inheritance by descent.

Should Flat-Coated Retriever breeders be worried about the small amount of genetic diversity that exists in the breed? If they are pleased with the appearance and health of their breed, they can accept the status quo. However, they must be careful to avoid any further loss of diversity caused by artificial genetic bottlenecks that might involve certain sires, dams, and bloodlines. Inbreeding for a show-winning form may greatly increase the incidence of diseases like PRA and glaucoma and bring other genetic defects to the forefront. Additional genetic diversity can be brought into the breed by outcrossing, but outcrossing is often followed by a period of inbreeding, backcrossing, and an eventual return to random breeding. Therefore, extreme care must be taken in choosing dogs for outcrossing that are free of simple and complex genetic traits, while most closely matching the breed standard. For instance, Flat-Coated Retrievers do not suffer very much from autoimmune diseases, which is a common occurrence with inbreeding into specific sires and blood lines in breeds such as the Standard Poodle and Italian Greyhound. Because the breed lacks diversity, deleterious traits would be manifested much earlier and it would also be more difficult to eliminate them after they are introduced.

B. 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 related to the population as a whole.



C. What should you do with this information?

The goal for Flat-Coated Retriever 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, similar to 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. It is important to note that four class I and five class II haplotypes occur in 98% of the dogs tested, but equally noteworthy that the testing of more dogs has significantly increased the numbers of minor DLA class I and haplotypes that occur in the breed. These minor haplotypes provide great opportunity for breeders to increase DLA diversity in the breed.

Breeders who do not have access to computer programs to predict the outcome of a mating 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 internal diversity. Considerations of mate choices for genetic diversity should be balanced with other breeding goals but maintaining and/or improving genetic diversity in puppies should be paramount.

An additional goal of this study is to contribute this genetic information to a web repository, hopefully under the control of the registry. The best format for such a repository and testing has been provided by Standard Poodle breeders. This information could be incorporated into a mate selection service that will allow a breeder to identify, among all of the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters- <https://www.betterbred.com/>.