# Genetic Diversity Testing Irish Wolfhound

## 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 assess 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.

Genetic diversity testing of Irish Wolfhounds is now in the preliminary results phase. During this phase, we continue to test more registered dogs to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their breed. This report is based on testing of 30 carefully selected Irish Wolfhounds; 15 from the USA, 10 from Great Britain, 3 from Norway, and 2 from Denmark. Although this number of dogs is probably not sufficient to do a final assessment of the breed, this selection of individuals should provide a reasonable picture of genetic diversity in contemporary Irish Wolfhounds. Allele and DLA haplotype frequencies will be updated as more Irish Wolfhounds are tested. It is anticipated that new alleles at the 33 STR loci and additional DLA class I and II haplotypes will be identified in the future, but these will tend to be of much lower incidence than those detected in the present population.

**Price: $50.**

**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. History

The Irish Wolfhound is a sighthound that originated in Ireland and was used to hunt and protect against wolves [1]. The breed became extinct as wolf numbers decreased and was presumably recreated as a show breed by Captain George A. Graham in the late 19th century. [2,3] Therefore, the breed has often been described as it existed in two periods: 1) Pre-19th century when it still existed in its original form, and 2) Post-19th century when it was recreated by dog fanciers.

### 1. Pre-19th century

A reference in the 4th century AD by Quintus Aurelius Symmachus, a Roman Consul, described seven dogs from the Gaels (Irish) that were used for fighting lions and bears, and who wrote "all Rome viewed (them) with wonder"[3]. These dogs are mentioned, in Irish laws and literature dating from the 6th century and in the Irish Sagas from 600-900 AD. However, the Irish archaeologist Finbar McCormick stressed that no bones of dogs of Irish Wolfhound size have ever been found in the period between 1000 BC through to the early Christian period to 1200 AD. He concluded, therefore, that even the original Irish Wolfhounds were a relatively modern development [4].

The Irish Wolfhound, similar to a number of hunting breeds from Europe, were originally bred and coveted by tribal chieftains, royalty, and foreign nobles and used for village and estate hunting, field tests, and often gifted between royalty. Edmund Champion, in his *History of Ireland* from 1571, describes the hounds used by important personages for hunting wolves [1]. "They are not without wolves and greyhounds to hunt them, bigger of bone and limb than a colt". With the advent of modern type firearms, the numbers of wolves were steadily decimated, and they eventually became extinct in many parts of Ireland, the UK and Western Europe. Indeed, references to the Irish Wolfhound in the 18th century tell not only of its great size and strength, but of its scarcity. The last wolf in Ireland was presumably killed in 1786 by a pack of wolfdogs kept by a Mr. Watson of Ballydarton [5, 6, 7]. After the demise of the wolf, the need for wolfhounds for hunting ceased, but fortunately a few dogs remained with families as symbols of status rather than used as hunters [5]. Thomas Pennant (1726-1798) found only three wolfdogs when he visited Ireland. Dr. Scouler mentioned the wolfdog in a report entitled *"Notices of Animals which have disappeared from Ireland"*, at the 1936 meeting of the Geological Society of Dublin [8].

## 2. Post-19th century wolfhound

Captain George Augustus Graham (1833-1909) of Rednock House, Dursley, Gloucestershire has been credited for his efforts in recreating the modern Irish Wolfhound breed [1]. He stated that he could not find the breed "in its original integrity" and "that we are in possession of the breed in its original integrity is not pretended; at the same time it is confidently believed that there are strains now existing that tracing back, more or less clearly, to the original breed; and it appears to be tolerably certain that our Deerhound is descended from that noble animal, and gives us a fair

idea of what he was, though undoubtedly considerably his inferior in size and power." Graham deduced that the Irish Wolfhound could be recreated through using the biggest and best examples of the Scottish Deerhound and the Great Dane, two breeds which he believed had been derived earlier from the wolfhound [9]. Into the mix went other breeds such as the Borzoi, Tibetan Mastiff, and Tibetan Wolfdog or Tibetan Ky Apso [8,10]. Captain Graham, along with other breeders, founded the Irish Wolfhound Club in 1885 and established a standard to which all subsequent breeders should aspire [10, 11].

## DNA analysis

Genomic analysis indicates that although there has been some DNA sharing between the Irish Wolfhound with the Deerhound, Whippet, and Greyhound, there has been significant sharing of DNA between the Irish Wolfhound with the Great Dane [12]. One writer has stated that for the Irish Wolfhound, "the Great Dane appearance is strongly marked too prominently before the 20th Century"[8].

### B. Appearance

The Irish Wolfhound is often described as a rough-coated Greyhound-like breed, strong though gracefully built; movements easy and active; head and neck carried high, the tail carried with an upward sweep with a slight curve towards the extremity [11]. It is the tallest dog breed to be recognized by the American Kennel Club, with a minimum height of 32 inches (81 cm) for mature males, 30 inches (76 cm) for females; a minimum weight of 120 pounds (54 kg) for males and 105 pounds (48 kg) for females [11]. In comparison to other breeds, the average height of an Irish Wolfhound should be taller than that of a Great Dane and with a stature similar to that of a Greyhound with a broad and deep chest. Irish Wolfhounds may be grey, brindle, red, black, white, fawn, and wheaten [11].

### C. Temperament

Irish Wolfhounds exhibit a range of traits that gives each dog its own individual personality [13, 14]. Despite its large size, Irish Wolfhounds are not destructive in the house or overly energetic. In general, the breed is more introverted, intelligent, and reserved with a quieter nature. Wolfhounds often create a strong bond with their family and can become quite destructive or morose if left alone for long periods of time. They are not guard dogs by nature and are more protective of their owner than the home. Most Wolfhounds are very gentle with children. The Irish Wolfhound is relatively easy to train and responds best to firm, but gentle, oversight.

### II. Genetic diversity studies of contemporary Irish Wolfhound

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

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic

purposes. Each of these STR loci is known to contain from 7 to 27 different alleles (ave. 15.4 alleles/locus) when tested across many breeds of dogs. Each breed, having evolved from a small number of founders and having been exposed to artificial genetic bottlenecks will end up with only a portion of the total available diversity. Artificial genetic bottlenecks include such things as popular sire effects, geographic isolation, catastrophes, outbreaks of disease, and ups and downs in popularity and resulting increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies were determined for 30 Irish Wolfhounds and listed in Table 1.

**Table 1.** Alleles and their frequencies for 33 STR loci in Irish Wolfhound (n=30). Highlighted loci have one allele that is found in more than 80% of Irish Wolfhounds that were tested.

| AHT121 | AHT137 | AHTH130 | AHTh171-A | AHTh260 | AHTk211 |
|---|---|---|---|---|---|
| 94 (0.30) | 131 (0.95) | 119 (0.08) | 219 (0.68) | 244 (0.85) | 87 (0.02) |
| 96 (0.08) | 133 (0.05) | 129 (0.90) | 225 (0.22) | 246 (0.13) | 89 (0.32) |
| 98 (0.20) | | 139 (0.02) | 229 (0.02) | 252 (0.02) | 91 (0.60) |
| 104 (0.35) | | | 233 (0.03) | | 95 (0.07) |
| 106 (0.07) | | | 235 (0.05) | | |

| AHTk253 | C22.279 | FH2001 | FH2054 | FH2848 | INRA21 |
|---|---|---|---|---|---|
| 284 (0.18) | 116 (0.02) | 132 (0.20) | 152 (0.02) | 232 (0.03) | 91 (0.07) |
| 286 (0.03) | 118 (0.27) | 136 (0.37) | 156 (0.73) | 236 (0.02) | 99 (0.18) |
| 288 (0.20) | 122 (0.40) | 144 (0.07) | 160 (0.12) | 238 (0.50) | 101 (0.63) |
| 292 (0.58) | 124 (0.32) | 148 (0.28) | 172 (0.13) | 240 (0.03) | 103 (0.12) |
| | | 152 (0.08) | | 242 (0.42) | |

| INU005 | INU030 | INU055 | LEI004 | REN105L03 | REN162C04 |
|---|---|---|---|---|---|
| 122 (0.13) | 144 (0.83) | 208 (0.13) | 85 (0.28) | 231 (0.22) | 202 (0.73) |
| 124 (0.47) | 152 (0.17) | 214 (0.33) | 95 (0.72) | 233 (0.33) | 204 (0.25) |
| 128 (0.32) | | 218 (0.33) | | 241 (0.37) | 206 (0.02) |
| 132 (0.08) | | 220 (0.20) | | 243 (0.08) | |

| REN169D01 | REN169O18 | REN247M23 | REN54P11 | REN64E19 | VGL0760 |
|---|---|---|---|---|---|
| 212 (0.02) | 162 (0.30) | 268 (0.27) | 228 (0.17) | 145 (0.42) | 20.2 (0.40) |
| 216 (0.98) | 164 (0.40) | 270 (0.27) | 232 (0.05) | 147 (0.30) | 21.2 (0.58) |
| | 168 (0.27) | 278 (0.47) | 236 (0.75) | 149 (0.27) | 23.2 (0.02) |
| | 170 (0.03) | | 240 (0.03) | 153 (0.02) | |

| VGL0910 | VGL1063 | VGL1165 | VGL1828 | VGL2009 | VGL2409 |
|---|---|---|---|---|---|
| 16.1 (0.13) | 8 (0.07) | 16 (0.20) | 20 (0.32) | 9 (0.17) | 16 (0.03) |
| 17.1 (0.82) | 13 (0.15) | 17 (0.02) | 21 (0.13) | 13 (0.18) | 17 (0.03) |
| 18.1 (0.05) | 14 (0.02) | 18 (0.10) | 22 (0.53) | 15 (0.63) | 18 (0.48) |
| | 18 (0.65) | 27 (0.23) | 23 (0.02) | 16 (0.02) | 19 (0.35) |
| | 19 (0.12) | 29 (0.08) | | | 20 (0.10) |
| | | 30 (0.35) | | | |
| | | 31 (0.02) | | | |

| VGL2918 | VGL3008 | VGL3235 |
|---|---|---|
| 20.3 (0.37) | 13 (0.40) | 12 (0.30) |
| 21.3 (0.50) | 17 (0.60) | 13 (0.25) |
| 22.3 (0.08) | | 14 (0.08) |
| 23.3 (0.05) | | 16 (0.28) |
| | | 17 (0.08) |

**B. Assessment of population diversity using standard genetic parameters**

Allele and allele frequencies at each of the 33 STR loci are listed in Table 1 and used to determine basic genetic parameters (Table 2), 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 being randomly bred. 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 of the dogs were completely different at each of the 33 loci. A value of 0.00 would be seen if the selection of sires and dams was balanced in reference to the existing gene pool.

The allele frequency data obtained from the 33 STR panels can be used to assess heterozygosity within a population (Table 2). Using the 33-marker panel, the 30 Irish Wolfhounds had an average of 3.82 alleles/loci (Na). Therefore, these 30 dogs retain 3.82/15.4=24.8% of known canid diversity at these loci. However, the average number of alleles is less important than the number of alleles that have the greatest genetic influence on heterozygosity, a figure known as average effective alleles/loci or Ne. The Ne in this group of dogs averaged 2.39 effective alleles per locus. This means that only 2.39/15.4= 15.5% heterozygosity in Irish Wolfhounds was determined by an even smaller portion of available canid diversity.

The observed (actual) heterozygosity of this group of 30 dogs was 0.52, while the expected heterozygosity (He) for a population in Hardy-Weinberg equilibrium (HWE) was also 0.52, yielding a coefficient of inbreeding (F) of 0.00. This suggests that this population of 30 dogs was in HWE, most likely due to careful selection of these 30 individuals to represent all major genetic lineages.

**Table 2**: Genetic assessment of 30 registered Irish Wolfhound based on allele frequencies at 33 genomic STR loci on 25 chromosomes.

| | N | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|
| **Mean** | 30 | 3.82 | 2.39 | 0.52 | 0.52 | 0.00 |
| **SE** | | 0.19 | 0.15 | 0.03 | 0.03 | 0.02 |

**B. Standard genetic assessment values for individual STR loci**

The allele frequencies can be also used to do a standard genetic assessment of heterozygosity at each STR locus (Table 3). This provides an estimate of genetic similarities in the specific regions of the genome that are associated with each STR marker. Phenotypic differences equate to genotypic differences. Therefore, alleles that are widely shared across the population are indicators that positive selection is occurring for certain desired traits. The Na values for an individual STR locus for this population of 30 Irish Wolfhounds ranged from a low of 2 to a high of 7 alleles per locus, while the Ne ranged from 1.11 to 4.05 alleles per locus. It is important to remember that each STR locus can have from 7-27 different alleles when testing across all dogs. The observed heterozygosity (Ho) for an individual STR locus ranged from 0.035 to 0.860, while He ranged from 0.03 to 0.83 (Table 3). Fourteen loci had positive F values and 19 were negative. Therefore, it appeared that breeders were able to maintain important breed-specific traits (loci with positive F values or inbreeding) by increasing genetic diversity (selection for negative F values or outbreeding) in other regions of the genome not as related to breed specific phenotypes.

**Table 3:** Genetic assessments for individual STR loci of N=30 Irish Wolfhounds. Na=alleles/locus; Ne=effective alleles/locus; Ho=observed heterozygosity; He=expected heterozygosity; F=coefficient of inbreeding (deviation from H-WE expectation).

| # | Locus | N | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|---|
| 1 | **AHT121** | 30 | 5 | 3.79 | 0.73 | 0.74 | 0.004 |
| 2 | **AHT137** | 30 | 2 | 1.11 | 0.10 | 0.10 | -0.053 |
| 3 | **AHTH130** | 30 | 3 | 1.22 | 0.20 | 0.18 | -0.094 |
| 4 | **AHTh171-A** | 30 | 5 | 1.93 | 0.47 | 0.48 | 0.032 |
| 5 | **AHTh260** | 30 | 3 | 1.35 | 0.30 | 0.26 | -0.156 |
| 6 | **AHTk211** | 30 | 4 | 2.15 | 0.57 | 0.54 | -0.059 |
| 7 | **AHTk253** | 30 | 4 | 2.41 | 0.60 | 0.59 | -0.026 |
| 8 | **C22.279** | 30 | 4 | 3.02 | 0.57 | 0.67 | 0.152 |
| 9 | **FH2001** | 30 | 5 | 3.76 | 0.67 | 0.73 | 0.092 |
| 10 | **FH2054** | 30 | 4 | 1.76 | 0.50 | 0.43 | -0.161 |
| 11 | **FH2848** | 30 | 5 | 2.35 | 0.60 | 0.57 | -0.045 |
| 12 | **INRA21** | 30 | 4 | 2.21 | 0.50 | 0.55 | 0.086 |
| 13 | **INU005** | 30 | 4 | 2.92 | 0.67 | 0.66 | -0.014 |
| 14 | **INU030** | 30 | 2 | 1.39 | 0.20 | 0.28 | 0.280 |
| 15 | **INU055** | 30 | 4 | 3.57 | 0.63 | 0.72 | 0.120 |
| 16 | **LEI004** | 30 | 2 | 1.68 | 0.43 | 0.41 | -0.067 |
| 17 | **REN105L03** | 30 | 4 | 3.34 | 0.73 | 0.70 | -0.047 |
| 18 | **REN162C04** | 30 | 3 | 1.67 | 0.40 | 0.40 | -0.001 |
| 19 | **REN169D01** | 30 | 2 | 1.03 | 0.03 | 0.03 | -0.017 |
| 20 | **REN169O18** | 30 | 4 | 3.10 | 0.80 | 0.68 | -0.180 |
| 21 | **REN247M23** | 30 | 3 | 2.78 | 0.63 | 0.64 | 0.010 |
| 22 | **REN54P11** | 30 | 4 | 1.68 | 0.27 | 0.41 | 0.343 |
| 23 | **REN64E19** | 30 | 4 | 2.99 | 0.77 | 0.67 | -0.153 |
| 24 | **VGL0760** | 30 | 3 | 2.00 | 0.53 | 0.50 | -0.068 |
| 25 | **VGL0910** | 30 | 3 | 1.46 | 0.37 | 0.31 | -0.172 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **26** | **VGL1063** | 30 | 5 | 2.16 | 0.60 | 0.54 | -0.118 |
| **27** | **VGL1165** | 30 | 7 | 4.27 | 0.73 | 0.77 | 0.042 |
| **28** | **VGL1828** | 30 | 4 | 2.48 | 0.50 | 0.60 | 0.163 |
| **29** | **VGL2009** | 30 | 4 | 2.16 | 0.50 | 0.54 | 0.069 |
| **30** | **VGL2409** | 30 | 5 | 2.72 | 0.50 | 0.63 | 0.208 |
| **31** | **VGL2918** | 30 | 4 | 2.54 | 0.63 | 0.61 | -0.045 |
| **32** | **VGL3008** | 30 | 2 | 1.92 | 0.47 | 0.48 | 0.028 |
| **33** | **VGL3235** | 30 | 5 | 4.05 | 0.83 | 0.75 | -0.106 |

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

PCoA is a two-dimensional graphic portrayal of how closely individual dogs within a breed are related to each other and the more closely individuals cluster together around the XY axis, the closer related they are to each other. The 30 Irish Wolfhounds are widely dispersed across all quadrants of the graph, and only two individuals (at 11 o'clock) appear closely related (Fig. 1). Therefore, it can be concluded that these 30 dogs were related to the level of a single breed and carefully selected to represent breed-wide genetic diversity. However, there does appear to be a degree of genetic drift between dogs from the USA and Europe (Fig. 1). This effect is a common occurrence in breeds that share a common origin/source and subsequently bred in geographically disparate regions. Although genetically distinguishable, these two populations still do not differ to the level of varieties (e.g., American and Japanese Akita or black vs. peppered Giant Schnauzers.

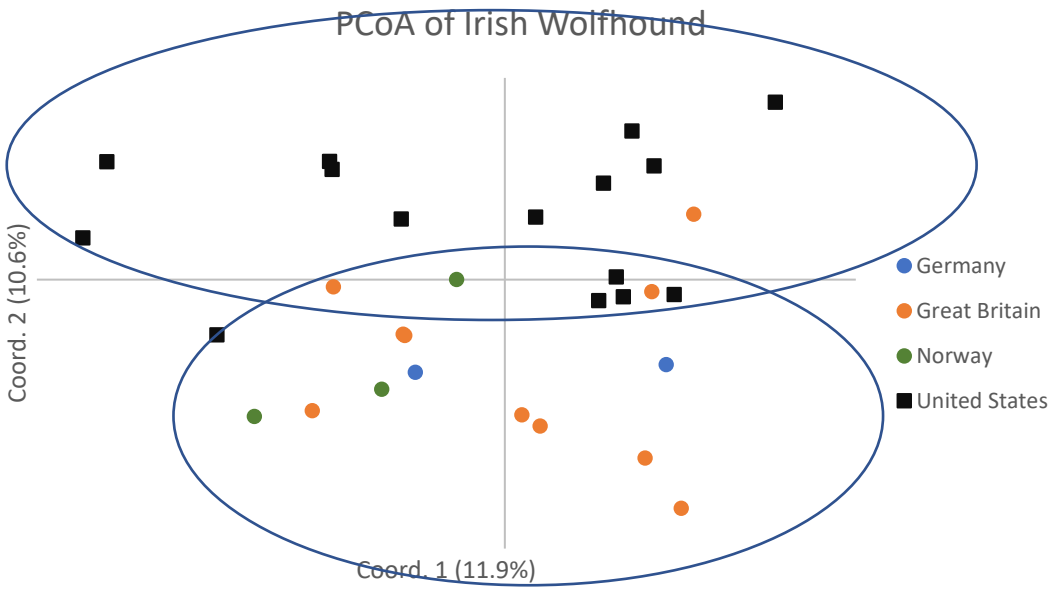



**Figure 1.** PCoA graph portraying the genetic relatedness of 30 Irish Wolfhounds from various parts of the world. The upper circle contains almost all dogs from the USA, while the lower circle contains mostly dogs from European countries.

The degree of relatedness of individuals within a breed can be further emphasized by comparing the 30 Irish Wolfhounds with genetically distinct breeds from Ireland, such as the Irish setter and Irish red and white setter (Fig. 2). These breeds have somewhat similar histories and they are from the same region. This comparison shows the three breeds to be genetically distinct, while tightening the relationship of individuals within each breed. It is noteworthy, however, that one (7 o'clock), and possibly two (2 o'clock), of the Irish Wolfhounds now appear somewhat genetically distant from the bulk of the breed. This suggests the existence of several genotypically (and phenotypically?) distinct bloodlines and will probably be resolved with the testing of more dogs.

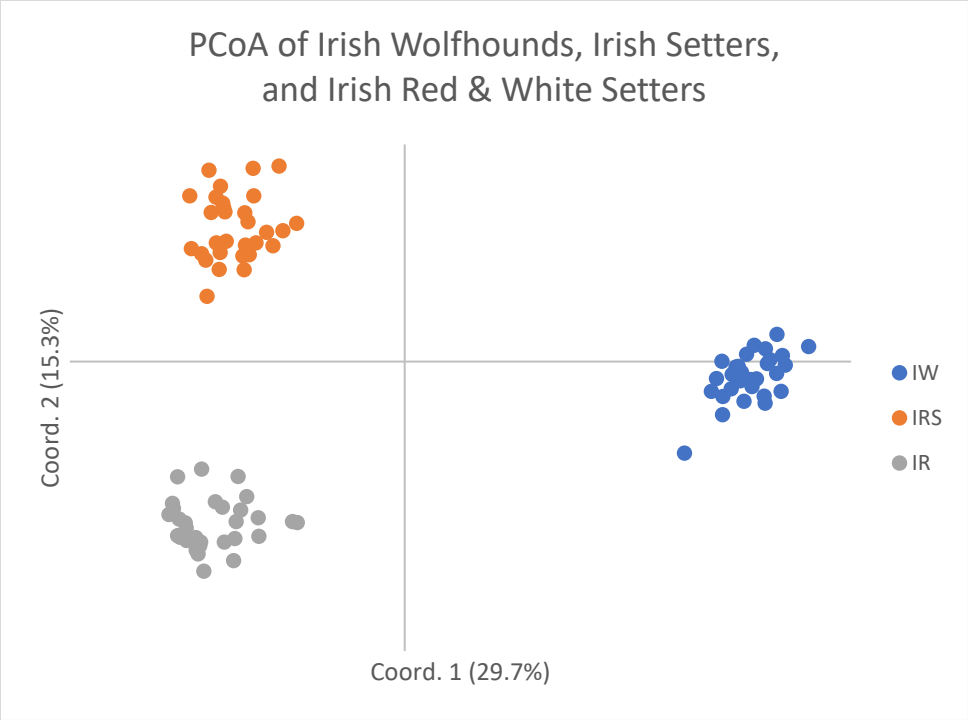



**Figure 2.** A PCoA graph showing the genetic relatedness of three Irish breeds**.**

**D. Internal relatedness (IR) of individuals and the population as a whole**

***1. IR testing***

Genetic assessments such as those presented in Tables 1 to 3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity being provided to individuals by their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree to which the two parents of an individual dog were related. 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 would be found among 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 higher the IR value above 0.25 the more closely related were the parents and grandparents of the siblings.

Table 4 lists the IR values for the 30 Irish Wolfhounds that were initially tested. The most outbred dog in the population had an IR score of -0.194, while the most inbred dog in the group had an IR score of 0.321, while the mean (average) IR score for the group was 0.005. The IR curve created from this data was also somewhat triphasic, with one group having IR scores <0.005, a second group with scores 0.005-0.016, and a third group 0.074-0.321 (Fig. 3). This latter group contains some individuals that appear more inbred than offspring of full sibling

parents. Therefore, IR values give a different picture that seen with the average scores determined by the standard genetic assessment (Table 2). While the standard genetic assessments indicated a population in HWE, the IR scores showed a population of individuals that ranged from reasonably outbred to highly inbred. The inbred dogs are balanced by outbred dogs, making it appear that the overall population was in HWE. This is a common feature of all dog breeds.

**Table 4.** Internal relatedness (IR) values calculated using allele numbers and frequencies for 30 Irish Wolfhounds. The IR values can be adjusted to reflect how these same dogs would score if they were to exist in a large population of village dogs.

| | IR | IRVD |
|---|---|---|
| **Min** | -0.194 | 0.145 |
| **1st Qu** | -0.088 | 0.358 |
| **Mean** | 0.005 | 0.393 |
| **Median** | 0.016 | 0.407 |
| **3rd Qu** | 0.074 | 0.459 |
| **Max** | 0.321 | 0.578 |

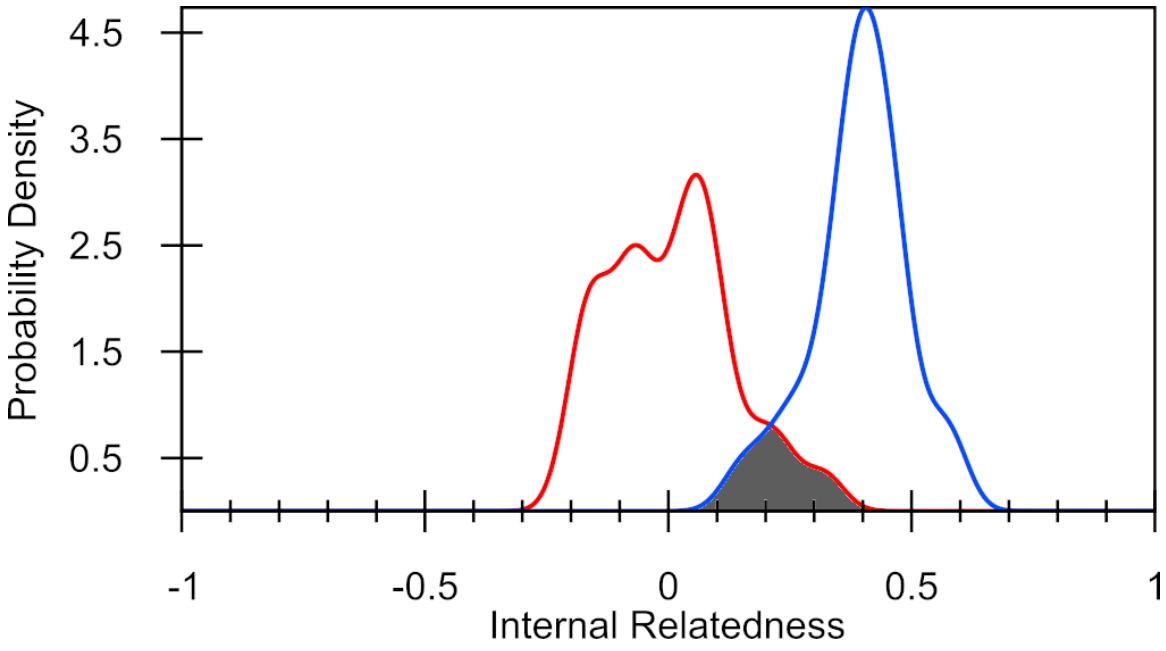



**Figure 3**: Distribution of IR estimates in 30 Irish Wolfhounds based on intra-breed diversity (Red line), compared with IR adjusted for diversity lost during breed development (Blue line). Diversity lost as a result of breed development was determined by comparing allele frequencies at the same loci between Irish Wolfhound and randomly breeding village dogs from the Middle East, SE Asia, and the Pacific Islands. The blackened area shared by both IR and IRVD graphs is an estimate the available genetic diversity present in all dogs that was used to create the breed.

***2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed evolution from time of origin to the present time.***

It is possible to determine the amount of canid genetic diversity a breed has retained as it evolved to present day. This is done by assuming that individual Irish Wolfhound were actually members of the current village dog population found in the Middle East, SE Asia and Island Pacific. The IR values and IR values adjusted to village dogs (IRVD) (Table 4) can then be graphed and the graphs overlaid (Fig. 3).

The IRVD values emphasize the lack of genetic diversity among the 30 dogs compared to randomly breeding village dogs. If these dogs were picked from among from village dogs, all of them would be deemed inbred to the level of offspring of full sibling parents (IRVD ≥0.250). Some would even be equivalent to offspring of village dog parents that were even more closely related than siblings (IRVD=0.250-0.600).

The IRVD curve for the Irish Wolfhounds tested was shifted well to the right of the IR curve, and the area of overlap was only 13.2% (Fig. 3). It can be estimated, therefore, that contemporary Irish Wolfhounds contain around 13.2% of the genetic diversity that exists in village dogs. This estimate is similar to the 15.5% retained canid diversity determined from VGL's entire canid collection and using effective alleles (Ne) of the 33 STR loci (Table 2). This level of retained canid genetic diversity is lower than the 60% or so retained diversity observed in the Toy Poodle or 54% in Labrador Retriever, similar to the 15% found in Doberman Pinchers, and higher than the 7% observed in Swedish Vallhund. All pure breeds of dogs have come from relatively small founder populations and have therefore had limited genetic diversity from the time registries were created and closed - in the case of Irish Wolfhounds, during the 19$^{th}$ century breed recreation. More genetic diversity may have been lost subsequently through artificial genetic bottlenecks such as cataclysmic events (e.g., world wars) or inbreeding for a specific show conformation (e.g., popular sire effects).

**E. 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, allergies, and resistance/susceptibility to infectious 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 the three STR loci associated with the

three Class II genes are strongly linked and also inherited as a single block or haplotype (Table 6). 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. However, there is enough distance between these two regions to allow for a degree of recombination resulting in unusual class I/II combinations. 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 Irish Wolfhound***

The 30 Irish Wolfhounds in this study possessed only five DLA class I and four DLA class II haplotypes (Table 5). Although it is likely that a few additional haplotypes will be identified as more dogs are tested, they will likely occur at a much lower frequency and not appreciably change the relative proportions of the present haplotypes. Only one DLA haplotype, DLA class I -1213, was unique to the breed, while all other class I and all class II haplotypes have been identified in other breeds (Table 6).

The number of DLA class I and II haplotypes found in these 30 Irish Wolfhounds was among the lowest of any breed studied to date. The number of DLA class I (n=5) and II (n=4) haplotypes found in Irish Wolfhounds was comparable to Swedish Vallhund (6/4) and Shiloh Shepherd (7/6); somewhat lower than Giant Schnauzer (14/15), Samoyed (13/12) and Shiba Inu (16/15); and much lower than Golden Retriever (26/23) and Miniature Poodle (33/23). If these 30 dogs are representative of the breed as a whole, it would suggest that four founder lines (breeds?) were involved in the Irish Wolfhound recreation, which is consistent with the breed history.

**Table 5**: DLA class I and II haplotypes identified to date in 30 Irish Wolfhounds. Haplotypes unique to the breed are highlighted.

| DLA class I | Alleles | Incidence |
|---|---|---|
| 1052 | 380 372 289 184 | 0.30 |
| 1068 | 380 373 287 181 | 0.13 |
| 1094 | 395 375 277 176 | 0.23 |
| 1105 | 382 379 277 178 | 0.30 |
| 1213 | 380 372 291 184 | 0.03 |
| **DLA class II** | | |
| 2005 | 339 322 280 | 0.28 |
| 2021 | 339 324 268 | 0.25 |
| 2022 | 339 327 282 | 0.33 |
| 2053 | 343 324 280 | 0.13 |

### *2. DLA haplotype sharing with other dog breeds*

DLA haplotypes are much more conserved than most other regions of the genome and each DLA region inherited as a block of linked genes from each parent and inherited by descent from one generation to the next. Therefore, the number and incidence of DLA haplotypes found in a breed can be used to estimate the founder/founder lines that were used to create a breed and the importance of the various lines in subsequent breed evolution.

The DLA class I and II regions are frequently shared between breeds, reflecting common distant ancestry (Table 6). Only the DLA class I 1213 haplotypes appeared to be unique to Irish Wolfhound, with remaining haplotypes being extensively shared among other breeds (Table 6). Unfortunately, VGL has no results for the breeds that are thought to be most involved in recreation of the Irish Wolfhound, such as the Great Dane and Tibetan Mastiff. However, there is strong sharing of the 1105 haplotype with the Doberman Pinscher, the 1052 haplotype with the Italian Greyhound and Shiloh Shepherd, and the four DLA class II haplotypes with several retriever, setter and shepherd breeds. The class II-2022 haplotype is also commonly found in the Borzoi, but not in the common linkage with class I-1160 (Table 6).

**Table 6**. The occurrence and incidence of specific DLA class I and II haplotypes in various breeds.

| DLA1 # | STR types | Black Russian Terrier (n=131) | Lakeland Terrier (n=63) | Labrador Retriever (n=178) | Irish Red and White Setter (n=51) | Doberman Pinscher (n=576) | Flat Coated Retriever (n=537) | Havanese (n=417) | Samoyed (n=189) | Giant Schnauzer (n=206) | Borzoi (n=31) | English Bulldog (n=163) | Biewer (n=120) | Biewer Yorshire Terrier (n=53) | Biewer Terrier (n=104) | Biro Biewer (n=3) | Italian Greyhound (n=823) | Alaskan Klee Kai (n=534) | Shiloh Shepherd, ISSA (n=179) | Magyar Agar (n=59) | Irish Setter (n=49) | Llewellin Setter (n=91) | Golden Retriever (n=706) | Irish Wolfhound (n=30) | Miniature Poodle (n=279) | Swedish Vallhund (n=219) | Poodle (n=2742) | Toy Poodle (n=139) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1052 | 380 372 289 184 | -- | 0.008 | -- | -- | 0.0017 | -- | 0.007 | -- | -- | -- | -- | -- | -- | -- | -- | 0.1896 | -- | 0.363 | 0.051 | -- | -- | -- | 0.3 | -- | -- | -- | -- |
| 1068 | 380 373 287 181 | -- | -- | 0.048 | -- | -- | 0.27 | 0.018 | 0.042 | 0.036 | -- | -- | -- | 0.009 | -- | -- | -- | -- | 0.243 | -- | 0.01 | -- | 0.051 | 0.13 | 0.016 | 0.349 | -- | 0.011 |
| 1094 | 395 375 277 176 | 0.015 | -- | -- | -- | 0.7604 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.23 | -- | -- | -- | -- |
| 1105 | 382 379 277 178 | -- | -- | 0.056 | -- | 0.0009 | -- | -- | -- | -- | -- | -- | 0.004 | -- | -- | 0.3 | -- | -- | -- | -- | -- | -- | -- | 0.3 | 0.061 | -- | 0.0018 | 0.061 |
| 1213 | 380 372 291 184 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.03 | -- | -- | -- | -- |
| DLA Class II Haplotype Frequencies (Updated Aug 5, 2019) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DLA2 # | STR types | Black Russian Terrier (n=131) | Lakeland Terrier (n=63) | Labrador Retriever (n=178) | Irish Red and White Setter (n=51) | Doberman Pinscher (n=576) | Flat Coated Retriever (n=537) | Havanese (n=417) | Samoyed (n=189) | Giant Schnauzer (n=206) | Borzoi (n=31) | English Bulldog (n=163) | Biewer (n=120) | Biewer Yorshire Terrier (n=53) | Biewer Terrier (n=104) | Biro Biewer (n=3) | Italian Greyhound (n=823) | Alaskan Klee Kai (n=534) | Shiloh Shepherd, ISSA (n=179) | Magyar Agar (n=59) | Irish Setter (n=49) | Llewellin Setter (n=91) | Golden Retriever (n=706) | Irish Wolfhound (n=30) | Miniature Poodle (n=279) | Swedish Vallhund (n=219) | Poodle (n=2742) | Toy Poodle (n=139) |
| 2005 | 339 322 280 | 0.015 | 0.008 | 0.051 | 0.118 | -- | 0.4181 | 0.002 | -- | 0.01 | -- | 0.015 | 0.046 | 0.009 | 0.048 | -- | -- | -- | -- | -- | 0.04 | 0.808 | 0.0163 | 0.28 | -- | -- | 0.0208 | 0.004 |
| 2021 | 339 324 268 | -- | -- | 0.014 | -- | -- | -- | 0.001 | -- | -- | -- | -- | 0.004 | -- | -- | -- | -- | 0.2116 | -- | -- | -- | -- | 0.0921 | 0.25 | 0.061 | -- | 0.002 | 0.061 |
| 2022 | 339 327 282 | -- | 0.175 | 0.076 | -- | 0.0017 | 0.1276 | 0.12 | 0.108 | 0.005 | 0.61 | 0.015 | -- | -- | -- | -- | -- | -- | 0.064 | -- | 0.18 | 0.088 | 0.0007 | 0.33 | 0.002 | 0.055 | 0.0002 | 0.011 |
| 2053 | 343 324 280 | -- | -- | 0.045 | -- | -- | 0.1331 | 0.04 | 0.558 | 0.044 | -- | -- | -- | 0.009 | -- | -- | -- | -- | 0.304 | -- | 0.01 | -- | 0.0297 | 0.13 | 0.016 | 0.495 | -- | 0.014 |

## *3. Heterozygosity in the DLA region*

The 7 loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium that many other parts of the genome that are measured by the 33 autosomal STR markers. However, the expectation is that these loci have achieved equilibrium with other loci in the genome over time. This can be tested by doing a standard genetic assessment of each of locus (Table 7), as well as all of the loci taken together (Table 8). Standard genetic assessment of each of the 7 loci demonstrates values for F (inbreeding coefficient) that are moderately positive (0.59-0.244), suggesting that these loci are not in HWE, unlike the rest of the genome (Table 2). This can also be seen in the overall standard genetic assessment of all 7 loci, where F=0.18. It cannot be determined from this limited population of Irish Wolfhounds whether these results are due to the relatively short time period that the breed has existed (i.e., DLA haplotypes have not year reached genome-wide equilibrium), or to non-random selection of the population that was tested for certain haplotypes (e.g., inadvertent selection of individuals from among the most popular bloodlines). The identified haplotypes occur at similar frequencies, which rule out genetic bottlenecks such as popular sire effect.

**Table 7**. Standard Genetic Assessment for Irish Wolfhound using 7 STRs in the DLA region

| # | Locus | N | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|---|
| **1** | **DLA I-3CCA** | 30 | 3 | 2.76 | 0.60 | 0.64 | 0.059 |
| **2** | **DLA I-4ACA** | 30 | 4 | 3.66 | 0.60 | 0.73 | 0.174 |
| **3** | **DLA I-4BCT** | 30 | 4 | 2.54 | 0.47 | 0.61 | 0.231 |
| **4** | **DLA1131** | 30 | 4 | 3.66 | 0.60 | 0.73 | 0.174 |
| **5** | **5ACA** | 30 | 2 | 1.30 | 0.20 | 0.23 | 0.135 |
| **6** | **5ACT** | 30 | 3 | 2.96 | 0.50 | 0.66 | 0.244 |
| **7** | **5BCA** | 30 | 3 | 2.88 | 0.50 | 0.65 | 0.234 |

**Table 8**. Summary of Standard Genetic Assessment for Irish Wolfhound using 7 STRs in the DLA region

| | N | Na | Ne | Ho | He | F |
|---|---|---|---|---|---|---|
| **Mean** | 30 | 3.29 | 2.82 | 0.50 | 0.61 | 0.18 |
| **SE** | | 0.27 | 0.28 | 0.05 | 0.06 | 0.02 |

## III. What does this assessment of genetic diversity tell us about contemporary Irish Wolfhound

This study confirmed that the Irish Wolfhounds that were tested constitute a single breed, albeit with some genetic variation. Although it is not possible at this point to provide a definitive assessment of genetic diversity, the existing findings are sufficient to make several assumptions. The breed originated from a common founder population that appears to be relatively small, perhaps as few as four lineages, each of which share a common DLA type. It will be important to test more dogs to see if the present findings remain unchanged. If new dogs do not significantly change the present results, it means that the Irish Wolfhound is the least genetically diverse breed that VGL has studied to date.

The modern Irish Wolfhound shares genetic diversity with many existing breeds of dogs, which is supported by historical records documenting the breed's recreation. Recent genomic analysis indicates that although there has been some DNA sharing with the Deerhound, Whippet, and Greyhound, the greatest genetic sharing is with the Great Dane [12]. We found strong sharing of the DLA class I-1105 haplotype with the Doberman Pinscher, the 1052 haplotype with the Italian Greyhound and Shiloh Shepherd, and the four DLA class II haplotypes with several retriever, setter and shepherd breeds.

The fact that breeders have this history suggests that they may be more amenable to future introgressions from foundation breeds such as the Scottish Deerhound, Great Dane, Borzoi, Tibetan Mastiff, and Tibetan Wolfdog or Tibetan Ky Apso. Such introgressions should be done with DNA testing rather than pedigrees to assure that new and not old genetic diversity is being introduced. However, great care has to be taken in not introducing heritable traits with such introgressions. The obvious breed for future introgressions would be the Great Dane, a breed known to have several heritable conditions [24]. Not surprisingly, many of these health problems are shared with the Irish Wolfhound.

Although the breed lacks genetic diversity, it is obvious from DNA testing that breeders have been careful in breeding across the full range of genetic diversity that exists and to the full depth of available pedigrees. The fact that the recreation of the breed has been well documented means that these pedigrees are of sufficient depth to include founders. This is an advantage that many dog breeds do not possess.

Although it appears that contemporary Irish Wolfhound have a very narrow genetic base, a lack of genetic diversity is not in itself bad, providing the founder population was relatively free of

deleterious genetic traits and breeders have been judicious in avoiding any artificial genetic bottlenecks that may cause either a loss or imbalance of that original diversity.

The lack of genetic diversity in the DLA class I and II region of these 30 Irish Wolfhounds is troublesome, but it is uncertain what it means. Certain DLA class I and II haplotypes have been associated with specific autoimmune diseases in certain breeds [25], but autoimmune disorders are presumably not a serious problem in the Irish Wolfhound. Nevertheless, it is important that breeders maintain as much diversity and heterozygosity in the DLA region as possible.

Breeds that lack genetic diversity must be managed much more closely to avoid further loss of genetic diversity and have less leeway in dealing with simple recessive or complex polygenic disorders that might arise. Elimination of deleterious traits may result in loss of genetic diversity, especially when diversity is limited.

## IV. Heritable disorders of Irish Wolfhound

### A. Lifespan

Like many large dog breeds, Irish Wolfhounds have a relatively short lifespan. Published lifespan estimations vary between 6 and 10 years with 7 years being the average. In a privately funded study conducted under the auspices of the Irish Wolfhound Club of America and based on an owner survey, Irish Wolfhounds in the United States from 1966 to 1986 lived to a mean age of 6.47, while [15], while a more recent study by the UK Kennel Club puts the average age of death at 7 years [16].

### B. Major disease problems

Irish Wolfhounds have a reported chance of dying of cancer of 89% compared to 31% in the overall dog population, with osteosarcoma occurring at 2.9 times the frequency of other breeds [17]. Irish Wolfhounds may also suffer a higher incidence of lymph node cancer (lymphoma) in early mid-life [18].

Heart disease, along with bone cancer, are leading causes of death in Irish Wolfhounds [18]. Young dogs may develop cardiomyopathy, a weakness of heart muscle that can lead to heart failure. The most common heart irregularity found in older Irish Wolfhounds is an arrhythmia called atrial fibrillation. It may be of no clinical significance or occasionally lead to heart failure.

Orthopedic problems are surprisingly low in Irish Wolfhounds considering their large size. The Orthopedic Foundation of America evaluated the hip X-rays of 2100 Irish Wolfhounds and found 5% to be dysplastic, while the incidence of elbow dysplasia among 900 dogs is higher at 13% [19]. A wobbler's syndrome has also been described uncommonly in Irish Wolfhound, as well as hypertrophic osteodystrophy.

Portosystemic shunts of the liver are a problem seen in some younger Irish Wolfhounds [14, 20]. This is a common problem in small breed dogs but usually uncommon in large breed dogs. It appears to be heritable but of a complex nature. Heritable eye problems such as cataracts, corneal

dystrophy, distichiasis, iris cysts, retinal dysplasia and progressive retinal atrophy have been described in the breed [21]. All of these conditions are found in various dog breeds and the genetic basis for most of them, except for PRA and cataracts, is largely unknown. Cataracts and PRA have been associated with simple autosomal recessive mutations in many breeds, but the genetic basis for them in Irish Wolfhounds has not yet been determined [22]. However, control of eye conditions has been accomplished by routine eye examinations and avoiding breeding of affected dogs.

Irish Wolfhounds, like other sighthounds, are considered to be at higher risk to barbiturate anesthesia and although not often used for induction, the dosage should be reduced to one-half to two-thirds of the normal dog dosage [17].

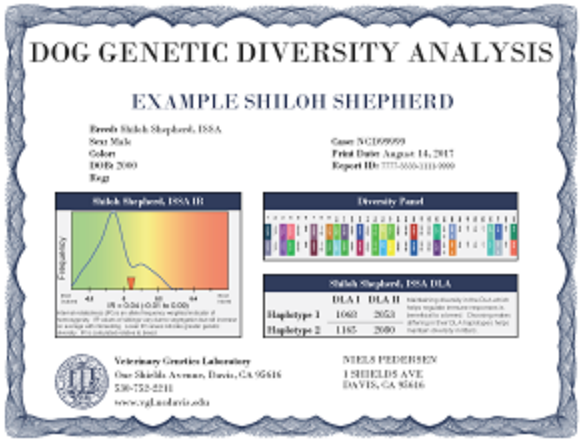
### C. Testing for heritable conditions

The Orthopedic Foundation of America recommends testing for the following heritable disorders: 1) elbow dysplasia (OFA evaluation), 2) eye examination by a boarded veterinary ophthalmologist, 3) hip dysplasia (OFA evaluation -PennHIP), 4) cardiac evaluation (advanced exam including ECG at 24 months of age), 4) serum bile acid test for porto-systemic shunt (optional) [23].

## V. 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 related to the population as a whole. The alleles at each of the 33 STR loci are presented as numbers that correspond to those found in Table 1. Each locus will have two alleles, which are either different (heterozygous) or the same (homozygous). Each allele is inherited from each of the parents. More of the alleles at each locus will be homozygous in dogs from closely related parents or that in regions of the genome that are under strong positive selection for some favored phenotypic trait or traits. Dogs with a predominance of rarer (i.e.,low incidence) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high incidence) alleles.




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

The use of DNA for testing genetic diversity in the Irish Wolfhound has confirmed what has already been anticipated, i.e., that the breed suffers from a lack of genetic diversity due to its origins from a small number of founder individuals/lines. The fact that breeders have maintained accurate and deep pedigrees lessens the need for DNA testing for activities such as ideal mate selection. However, if the breed were to consider increasing genetic diversity by further genetic introgressions, DNA testing of dogs intended for such introgressions would be extremely helpful. DNA testing would also be useful in monitoring the effect of genetic outcrossing on the breed.

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, 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.

Breeders who do not have access to computer programs to predict the outcome of matings 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 v*s* 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.

A more effective use of this study is to contribute the genetic information to a web repository. 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 the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters-https://www.betterbred.com/.

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