

Genetic Diversity Testing for Black Russian Terriers

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of short tandem repeat (STR) markers that will 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 Black Russian Terrier breeders who wish to track and increase genetic diversity of their breed as a long term goal.

Genetic testing of Black Russian Terriers is now in the development phase -please see [Enrolling a Breed](#). During this phase, we continue to test Black Russian Terriers to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their breed. We encourage breeders to submit samples from active dogs to further build the database. The goal is to test enough dogs so that no new alleles or DLA haplotypes are recognized.

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.

The genetic information used to formulate the enclosed tables and graphs came from DNA samples of up to 109 BRT. We believe that the dogs currently tested represent most of the genetic diversity that exists in the breed. These pages will be updated as more dogs are tested, so allele and DLA haplotype frequencies may change over time.

I. Introduction¹⁻⁵

A. Breed history

The Black Russian Terrier is a relatively new breed that had a novel origin. The breed was created in the USSR by the state-run Red Star (Krasnaya Zvezda) Kennel in Moscow specifically for multi-purpose military duties post-WWII. The goal was to make a better military dog, although the desire to have an USSR creation at the level of German Shepherd Dogs or better must have played into the decision. The breeding stock was supposedly imported from countries occupied by the USSR during and after the war, but especially East Germany. It is stated that 17 breeds were ultimately used to create the Black Russian Terrier, but in particular, Giant Schnauzer, Airedale Terrier, Rottweiler, Newfoundland, and the Caucasian Shepherd Dog. A Giant Schnauzer named Roy is considered by some to be at the foundation of the breed. Black Russian Terriers were bred for working ability, rather than appearance. The dogs were bred solely by Red Star Kennels for the military until 1957 when it was breeding true and some puppies could be sold to civilian breeders. These early dogs were rather mundane in appearance, but private breeders quickly altered them to their modern show appearance, while retaining working ability. This refined Black Russian Terrier spread to the other parts of USSR, [Ukraine](#), then to [Finland](#) and other European countries mainly in the Eastern bloc of the time. The Black Russian Terrier finally reached United States, Canada, Australia and other Western countries.

The first working (original) examples of the breed were put on show at an exhibition in Moscow in 1955 and a breed standard was published in 1958 and adopted by the Fédération Cynologique Internationale in 1984. The Russian Federation Working Dog-breeders & Russian Kynological Federation (RKF) adopted a second Standard for the breed in 1996 to bring the original standard more in line with the modern type. And it was also in 1996 that the breed was first introduced into the UK. In 1998 The Kennel Club added the Russian Black Terrier to the Import Register. The Black Russian Terrier currently ranks 119/194 among AKC registered breeds.

B. Breed description

According to the FCI standard, males stand 72 to 76cm and not more than 78cm at the withers and female's 68–72cm and not more than 74cm. Males weigh between 50 and 60kg (110-132lbs), and females between 45 and 50kg (99-110lbs). Even larger individuals are tolerated in Europe if the dog is well proportioned and retains correct movements. The AKC standard is somewhat more rigid and recommends 27-30inches for males with the desired height between 27 and 29inches and 26 and 29inches for females with the desired height between 26 and 28inches. A mature male less than 27inches or more than 30inches at the withers, or a female less than 26inches or more than 29, is considered a serious fault.

The coat is a double with a course outer guard hair over a softer undercoat, low-shedding, and black or black with some scattered gray hairs. The coat should form a beard and eyebrows on the face, and a slight mane around the withers and neck, especially in males.

Although originally bred for military work, the modern dog is calm, confident, courageous and self-assured. They are easily trained and have performed well in sports, such as agility, and

Schutzhund training. As an ex-military breed, they are expected to remain alert, instinctively protective, deeply loyal to family and somewhat aloof to strangers.

II. Baseline genetic diversity testing of the Black Russian Terrier

A. Population genetics based on allele frequencies at 33 STR loci on 25 chromosomes

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The test panel contains 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.

Table 1. [Table 1 link](#). Allele frequencies for 33 STR markers in Black Russian Terrier

Breed-wide heterozygosity was determined by standard genetic assessment of alleles and allele across all 33 STR loci (Table 2). At time of writing, (N=93), the average number of alleles per locus was 6.3, and 3.5 of these alleles (N_e) had the most effect on heterozygosity. The observed homozygosity (H_o) across the 33 loci was 0.689, while the expected homozygosity (H_e) for a population in Hardy-Weinberg equilibrium (i.e., state of total random breeding) was 0.682. This difference between H_o and H_e was used to calculate an inbreeding coefficient (F). The calculated F value for the 93 dogs was -0.012, which was close to 0.00 and indicated that the distribution of alleles was a result of near-random selection across the study population. However, this assessment is an average (mean) for the population. Based on internal relatedness (IR) calculations, there are individuals in the population that are much more inbred and outbred than the average BRT.

Table 2. Standard Genetic Assessment of Black Russian Terriers based on allele frequencies at each of 33 STR loci.

	N	Na	Ne	Ho	He	F
Mean	133	6.424	3.501	0.691	0.683	-0.013
SE		0.353	0.185	0.019	0.019	0.009

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

1. Genetic relationships of Black Russian Terrier from across the USA

A principal coordinate analysis (PCoA) plot shows the distribution of dogs as they relate to each other (Fig. 1). The actual distribution is three dimensional (i.e., the relationship of individual dogs to each other forms a ball), but for graphic purposes the relationship of individuals is plotted in the two dimensions that most accurately displays their three-dimensional relationships (coordinates 2 on y-axis and coordinate 1 on x-axis). The bulk of the population of BRT is found around the center of the plot with a smaller number of more genetically differentiated dogs forming more distant genetic outliers. The fact that all 93 individuals were found in the same plot

indicates that they are all members of the same breed. The presence of genetic outliers indicates that some individuals in the breed are somewhat less related to the bulk of the population than others, which was suggested by the slightly negative F value.

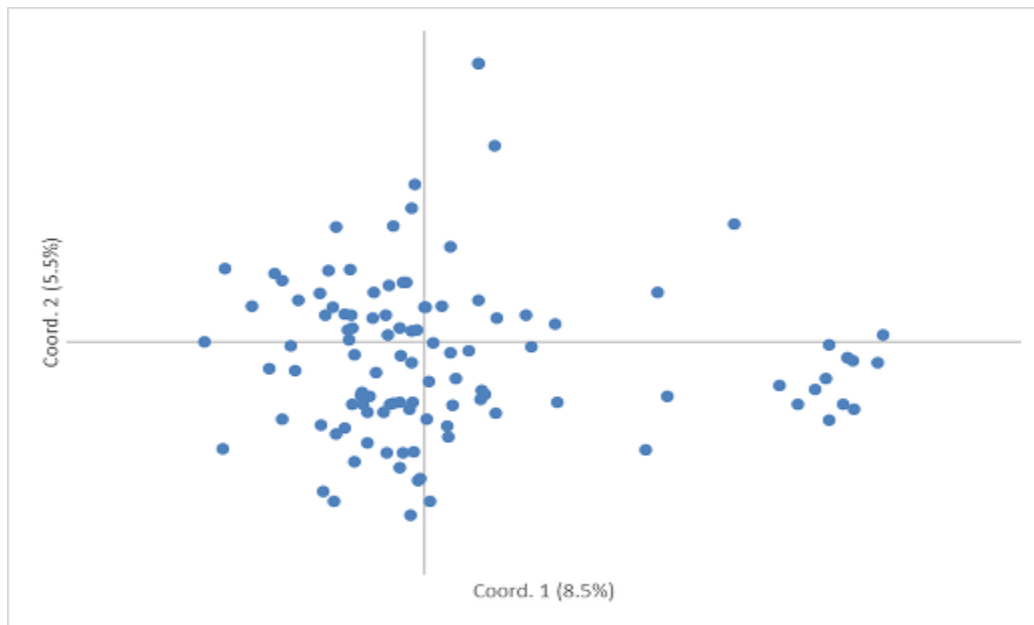


Figure 1. Principal coordinate analysis (PCoA) plot showing how individuals within the population of BRT have genetically differentiated from each other. (n=111)

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 based on information from 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) measures the relatedness (genetic similarity) of an individual dog's parents. IR is also based on allele frequencies at each of the 33 autosomal STR loci, but unlike the standard genetic assessment values, the IR calculation takes into consideration homozygosity at each locus and give more value 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.

The diversity of alleles at each of the [genomic STR loci](#) and their frequency in the population were used to calculate the internal relatedness of each dog and for the BRT population as a whole (Fig. 3). The mean IR value (red line graph) calculated for over 93 BRT was -0.05 and ranged from -0.35 (most outbred) to +3.0 (most inbred). About one-quarter of the population had IR values over +0.1 (most inbred) and one-quarter less than -0.1 (most outbred).

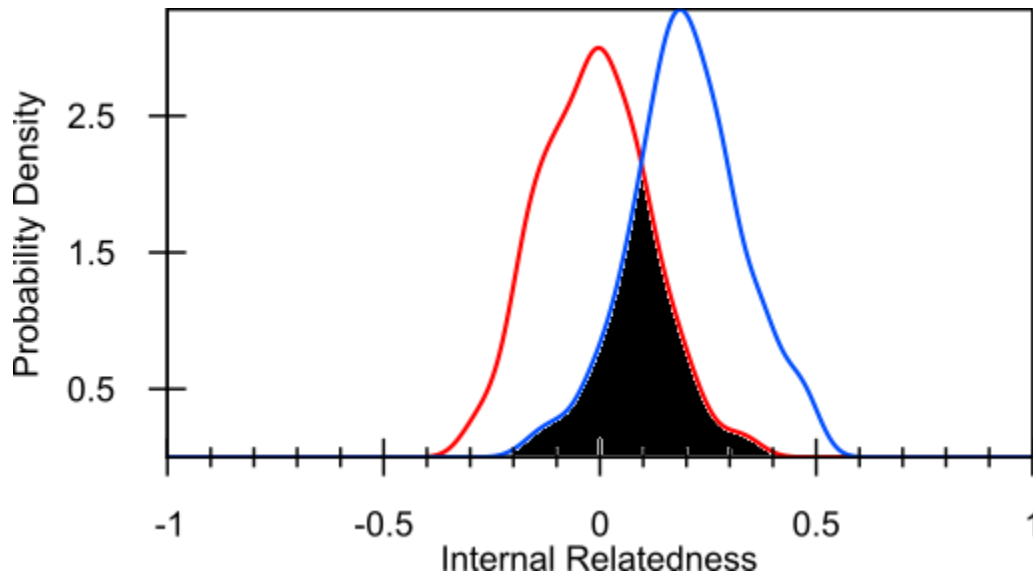


Figure 3. Distribution of IR estimates in Black Russian Terriers (n=111) based on intra-breed diversity (red line). The blue graphs the internal relatedness of individuals in the Black Russian Terrier population if they were compared to village dogs rather than to each other. The blackened area under the area where the two graphs overlap represents the proportion of available genetic diversity that has been retained in the breed (43%).

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 (blue line graph). This is done by comparing the frequency of a given allele in Black Russian Terriers with the frequency of the same alleles in a population of village dogs from the Middle East, SE Asia, Taiwan and other Pacific island nations such as Brunei and the Philippines. 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. The adjusted values reflect how much of dog-wide genetic diversity still exists in a breed and individuals within a breed. Even breeds with a small founder population will be in HWE if average mate selection is random across the population.

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 Black Russian Terriers. A rough estimate based on areas under the curve (black), indicate that Black Russian Terriers have maintained 43% of the genetic diversity that exists in dogdom.

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

In addition to the markers used to estimate relatedness, which reflect genome-wide diversity, a set of STRs associated with specific genes in the dog lymphocyte antigen (DLA) region was identified and used to measure genetic diversity associated with immune function. The DLA is also known as the canine major histocompatibility complex (canine-MHC). 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. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking with the first haplotypes being identified (in Standard Poodles) being named 1001, 1002, ... for class I haplotypes and 2001, 2002, ... for class II haplotypes. It is not unusual for various dog breeds to share common and even rare haplotypes, depending on common ancestry. Therefore, identical haplotypes in other breeds are assigned the same number. The numerical nomenclature used by VGL for DLA class I and II haplotypes does not correlate with numerical rankings used by others and based on direct sequencing.

1. DLA class I and II haplotypes existing in Black Russian Terrier

We have identified 10 different STR-associated DLA Class I and 8 DLA Class II haplotypes in BRT (Table 3). Two DLA class I haplotypes (1091 and 1092) occur in 51% and 25% of all the individuals tested, and two class II haplotypes (2033, 2037) at almost identical frequencies. The equal frequencies occur because 1091 is in strong linkage with 2033, forming the 1091/2033 haplotype. Likewise, 1092 is in strong linkage with 2037, forming the extended 1092/2037 haplotype. The high frequency of these two extended haplotypes indicates that two founders or closely related founder lines have been extensively involved in the creation of the breed. It is noteworthy that the 1092 haplotype is also common in Giant Schnauzer and Japanese Akita, while the 2037 haplotype is common in Giant Schnauzer, Alaskan Klee Kai, American Akita, and Japanese Akita (Table 4).

V. What does this assessment of genetic diversity tell us about contemporary Black Russian Terriers

It is apparent from DLA class I and II haplotyping that the Giant Schnauzer has played an important founding role in the Black Russian Terrier. However, the close relationship to the Akita and Alaskan Klee Kai is less obvious. The Akita is a Mastiff-type dog, which makes some sense, because the BRT is not a terrier in the normal sense. The Alaskan Klee Kai is purportedly a miniaturized Husky and the Husky is related to many dogs of the far north, some of which may have been used in the creation of the Black Russian Terrier. The use of indigenous USSR/Russian breeds such as the Caucasian shepherd in development of the breed has been mentioned in passing, but perhaps should be given more weight. The existence of two autosomal recessive disorders in the breed may also give some clue as to breeds involved in the creation of Black Russian Terriers. Juvenile laryngeal paralysis and polyneuropathy (JLPP) may have entered the breed through Rottweilers, where the causative mutation also occurs. Hyperuricosuria may have entered through the Giant Schnauzer, where the mutation also exists.^{9,10}

The genetic diversity of BRT based on autosomal markers is on par with many of the less common pure breeds that we have tested, such as Alaskan Klee Kai, Doberman, and Flat Coat Retriever. These breeds and the BRT lack genetic diversity as evidenced by IRVD comparisons and DLA class I and II haplotype numbers when compared to breeds such as the Golden Retriever, Labrador Retriever, Havanese, Italian Greyhound, Standard Poodle and Miniature Poodle. Nonetheless, the standard genetic assessment of the BRT based on the 33 autosomal STR loci indicates that the breed is randomly breeding and maybe even a little more outbred. Like other pure breeds, this is misleading. Based on IR values, there is a quarter or so of the dogs that are products of closely related parents and they are being balanced in the standard genetic assessment values by a somewhat larger population of outbred dogs. The value of DNA and these type of analyses in identifying dogs that are too inbred should be apparent. It must also be apparent that even highly inbred dogs have value if bred to the appropriate male or female. Many of the puppies from such a mating will much closer to the average internal relatedness for the breed.

VI. Health problems of Black Russian Terrier ⁶⁻¹⁰

The AKC lists the average lifespan as 10-12 years, which is typical for dogs of this size. Black Russian Terriers are known to suffer from several health problems common to larger dogs, including hip dysplasia, elbow dysplasia, and gastric torsion (bloat), which can be considered non-immunologic heritable disorders of ancestral origin and of complex genetic nature. Hypertrophic osteodystrophy, skin and intestinal allergies, and autoimmune diseases such as Addison's disease are immunologic heritable disorders that are also of a complex genetic nature but are fortunately uncommon in the breed. The presence of both ancestral and more recent complex and simple genetic disorders in BRT is not unique and identical, similar or different disorders occur in many other pure breeds. They are indicators of lost genetic diversity and inadvertent positive selection pressures for desirable traits.

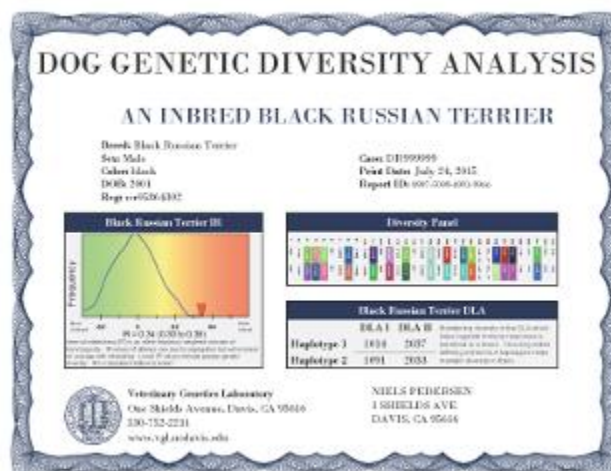
Two deleterious disorders of autosomal recessive inheritance have been documented in the breed. Hyperuricosuria is due to a mutation in the solute carrier gene SLC2A9 and prevents the conversion of uric acid to allantoin.⁸ The mutation was discovered in Dalmatians but has been subsequently detected in several breeds.⁹ Fifty one percent of Black Russian Terriers carry the mutation and 27% excrete high levels of uric acid in their urine.⁸ The mutation causes the excretion of uric acid in the urine and can lead urate stone formation in the bladder. Male dogs may develop life-threatening urinary obstruction when stones lodge in the urethra. A genetic test for the mutation is available and breeding of homozygotes discouraged. Juvenile laryngeal paralysis and polyneuropathy (JLPP) occurs in both Black Russian Terriers and Rottweilers and leads to progressive laryngeal paralysis, swallowing problems, and aspiration pneumonia.¹⁰ Signs of this disorder develop around 3 months of age and most puppies die or are euthanized by 6 months of age. The disorder is caused by a mutation in the Warburg syndrome gene, RAB3GAP1.¹¹ Almost 20% of Black Russian Terriers are heterozygous for the mutation. A DNA test is available for the mutation.

The registry recommends that breeding dogs be radiographed at two years of age for hip and elbow dysplasia's and radiographs sent to the Orthopedic Foundation for Animals (OFA) for evaluation. About 42% of BRT will suffer from hip dysplasia and 28% from elbow dysplasia according to the OFA database. Breeding dogs should also follow OFA guidelines for yearly eye examination by certified veterinary ophthalmologists for congenital eye conditions. Congenital heart disease is reportedly rare in BRT, but if signs of heart disease appear, the cause should be determined by a veterinary cardiologist and abnormal results submitted to the OFA.

VII. How will you be given the results of DNA-based genetic diversity testing on your dog.

A. Certificates with test results for each dog

After testing, 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.



B. What should you do with this information?

The goal for BRT breeders should be to produce a greater and greater proportion of puppies with IR scores less than 0, and with time even lower scores. This will be difficult to achieve given limited genetic diversity genome-wide and in the DLA, small population to choose from and the high incidence of hip and elbow dysplasia and carriers of the hyperuricosuria (*SLC2A9*) and JLPP (*RAB3GAP1*) mutations. Rather than trying to eliminate these simple and complex genetic mutations, it may be preferable to slowly decrease their incidence by selecting against homozygotes (hyperuricosuria and JLPP) and not breeding males and females with highly unfavorable OFA scores for elbow and hip dysplasia. This will make proper mate selection much more difficult. Genetic introgressions may also be helpful in an overall disease control strategy, but such a program is not attractive to pure-breeders and must be managed with great care to assure that new disease is not introduced, and that new diversity is slowly accumulated and uniformly dispersed. This would be best accomplished by combining pedigrees with DNA testing.

Even though genetic diversity is limited, it is nonetheless important to properly manage the diversity that exists in the best possible manner. Maintaining existing diversity will require using different combinations of breeding stock, including even those from inbred lines with high IR values. 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.

Potential sires and dams should be first screened for genetic differences in the genome and in the DLA regions by first comparing allele differences at each STR locus, and then at the DLA class I and II haplotypes. Some thought should be given to rare vs common alleles. This information is included on all certificates and on the website. This preliminary comparison will identify promising pairings and if desired, genetic information on the potential sires and dams can then be used to calculate actual IR expectations for their puppies. 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 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. 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.

VII. References

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