

Genetic Diversity Testing for Greater Swiss Mountain Dog

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 to determine genetic heterogeneity and diversity across the genome and in the Dog Leukocyte Antigen (DLA) class I and II regions for specified dog populations. This test panel is useful to dog breeders who wish to use DNA-based testing to track and increase genetic diversity as a supplement to in-depth pedigrees. DNA based information on genetic heterogeneity and diversity, along with DNA testing results for desired phenotypes and health traits, can aid in informing breeding decisions.

Genetic diversity testing of the Greater Swiss Mountain Dog is now in the preliminary results phase. During this phase, we will continue to test more registered dogs to build the genetic database necessary to provide an accurate assessment of genetic diversity. This report is based on 34 registered Greater Swiss Mountain Dogs from the USA (n=22), Czech Republic (n=4), Canada (n=2), Russia (n=2), Belgium (n=1), Netherlands (n=1), France (n=1), and Switzerland (n=1). Although results reported herein are preliminary, this selection of individuals should provide a reasonable picture of genetic diversity in the breed. Allele and DLA haplotype frequencies will be updated as more dogs 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 for the Greater Swiss Mountain Dog, but these will tend to be of lower incidence than those detected in this initial population.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from carefully selected regions of the genome were used to assess genetic heterogeneity and existing genetic diversity within an individual as well as across the breed. The alleles inherited from each parent are displayed graphically to highlight heterozygosity and genetic diversity in individuals as well as breed-wide.

DLA haplotypes: Seven STR loci linked to the DLA class I and II genes were used to identify genetic differences in a region that regulates immune responses and self/non-self-recognition. Problems with self/non-self-recognition, along with non-genetic factors in the environment, are responsible for autoimmune disease, allergies, and susceptibility to infectious agents.

Internal Relatedness: The IR value is a measure of the genetic relatedness of an individual's parents. The value takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog; two individuals from different sources may have identical IR values, but a quite different genetic makeup.

I. Introduction to the Greater Swiss Mountain Dog

A. Breed History [1-4]

The Greater Swiss Mountain Dog is the oldest and largest of the four Alpine mountain dog breeds originating from Switzerland, also called *Sennenhund* from the German *Senne* (“alpine pasture”) and *Hund* (dog). The other closely related Sennenhund breeds are the Bernese Mountain Dog, the Appenzeller Mountain Dog, and the Entlebucher Mountain Dog. These breeds have the same coat color and markings but differ in body size and coat length.

Several theories exist regarding the origin of the Greater Swiss Mountain Dog (and Sennenhund breeds alike); the most popular one states that they are descended from Molossian hounds (large, Mastiff-like dogs) brought by Roman legions upon their invasion of the Swiss Alps over 2,000 years ago. Mating of Molossian hounds with native farm dogs likely originated the ancestors of modern Greater Swiss Mountain Dogs.

Early ancestors of the Greater Swiss Mountain Dog were used by alpine farmers, herdsman and merchants in Switzerland and adjacent countries. Due to its size and strength, the breed was commonly used as a draft dog to pull heavy carts; other uses included guarding, herding, cattle droving, and as family companion.

One of the most popular early dog breeds in Switzerland, the Greater Swiss Mountain Dog almost completely disappeared in the late 19th century due to being replaced by machines or other dog breeds in their work functions. However, at the 1908 show of the Swiss Kennel Club (SKG), which commemorated its 25th anniversary, two dogs entered as ‘short-haired Bernese Mountain Dogs’ were shown by Franz Schertenlieb to Dr. Albert Heim, Swiss geologist and Sennenhund expert (**Figure 1**).

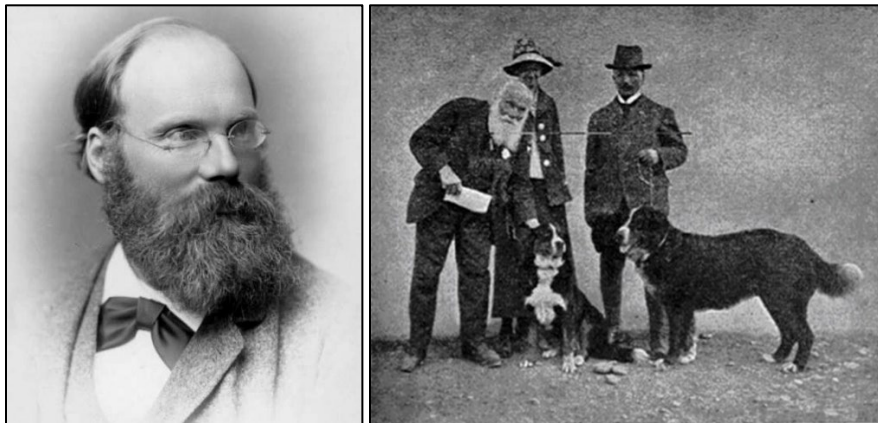


Figure 1. *Left:* Dr. Albert Heim (1849-1937), Swiss geologist and Sennenhund expert.
Right: Dr. Heim (far left) with a pair of Bernese Mountain Dogs in the 1920's.

One of the dogs shown by Franz Schertenlieb to Dr. Heim, Bello vom Schlossgut (**Figure 2**), was strong, tall, and shorthaired. Dr. Heim, having seen dogs with similar characteristics across Switzerland in the 1860s, recognized Bello to be a member of the larger and once hugely popular variety of Swiss Mountain Dogs, and began advocating for their recognition as a separate breed. Heim wrote the first breed standard based on Bello, and Franz Schertenlieb started breeding him to two shorthaired bitches. As a result, the Greater Swiss Mountain Dog was recognized as a breed by the Swiss Kennel Club in 1909 and entered in Volume 12 of the Swiss studbook. Subsequently, the first Greater Swiss Mountain Dog breed club was formed in 1912.



Figure 2. Bello vom Schlossgut, shown in 1908 as a ‘short-haired Bernese Mountain Dog’, was the first member of the Greater Swiss Mountain Dog breed.

It is reported that Greater Swiss Mountain Dogs contributed to the development of many other breeds, including the Saint Bernard and the Rottweiler. The breed grew slowly both in popularity and number of dogs throughout the 20th century in Europe, being used as draft dogs by the Swiss army during World War II. In 1945, there were between 350 and 400 Greater Swiss Mountain Dogs in existence. In 1968, the breed was imported to the United States by Frederick and Patricia Hoffman, and shortly thereafter, the Greater Swiss Mountain Dog Club of America (GSMDCA) was formed. In 1983, the breed registry reported 257 registered dogs in the US. The Greater Swiss Mountain Dog was recognized as a breed by the AKC in 1995, as part of the Working group. Currently, it is ranked 74 of 202 in popularity among the AKC registries.

B. Appearance [2,4]

The Greater Swiss Mountain Dog shares its coat color and markings with the three other Swiss Mountain Dog breeds. The topcoat is dense and black, approximately 1¼ to 2 inches long. The markings are rich rust and white, preferably symmetrical. On the head, rust appears over each eye, each cheek, and the underside of the ears. On the body, rust appears on both sides of the chest, on all four legs and underneath the tail. White markings appear on the head (blaze) and on the muzzle and can vary in length and width. Typically, white markings run from the throat to the chest, and can appear on all four feet and on the tip of the tail. The undercoat must be present, usually at the neck but also throughout the body; its color can range from dark gray (preferred) to light gray and tawny.

The breed is large, heavy-boned, well-muscled, and sturdy. The standard height at the shoulders is 25½ to 28½ inches (65 to 72 cm) for dogs, and 23½ to 27 inches (60 to 69 cm) for bitches. The body length to height proportion is approximately 10 to 9, thus appearing slightly longer than tall. A typical male weighs 115-140 pounds (52 to 64 kg), and a typical female weighs 85-110 pounds (38 to 50 kg).

The breed standard lists two disqualifications for the Greater Swiss Mountain Dog: any color other than the "Black, Red and White" tri-colored dog (such as "Blue/Charcoal, Red and White" or "Red and White"), and blue eye(s).

C. Temperament [2,4]

The Greater Swiss Mountain Dog is bold, faithful, and a willing worker; they are always alert and vigilant and are typically good with children. Shyness or aggressiveness are penalized. The breed requires moderate exercise, and their activity happens in short bursts.

II. Preliminary Results on Genetic Diversity of 34 Greater Swiss Mountain Dogs

A. Population genetics based on 33 STR loci on 25 canine 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 [5, 6]. Each STR locus contains 7 to 29 different alleles (average of 15.4 alleles/locus) in the breeds tested at the VGL so far. 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 can include phenomena such as popular sire effects, geographic isolation, catastrophes, outbreaks of disease, and ups and downs in popularity which can lead to increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies for the 34 Greater Swiss Mountain Dog individuals are listed in **Table 1**.

Table 1. Alleles and their frequencies for 33 STR markers in Greater Swiss Mountain Dogs (n=34). The allele that occurs at the highest frequency at each locus is bolded.

AHT121	AHT137	AHTH130	AHTTh171-A	AHTTh260	AHTk211
96 (0.03)	131 (0.03)	117 (0.38)	219 (0.25)	238 (0.19)	87 (0.60)
100 (0.09)	137 (0.57)	125 (0.16)	225 (0.49)	244 (0.09)	89 (0.25)
102 (0.44)	149 (0.07)	129 (0.46)	229 (0.01)	246 (0.57)	91 (0.15)
108 (0.43)	151 (0.29)		235 (0.25)	250 (0.10)	
114 (0.01)	153 (0.03)			254 (0.04)	
AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
286 (0.53)	116 (0.78)	132 (0.75)	148 (0.01)	230 (0.16)	95 (0.16)
288 (0.37)	118 (0.12)	140 (0.01)	152 (0.88)	232 (0.16)	101 (0.84)
292 (0.10)	120 (0.10)	148 (0.21)	156 (0.06)	238 (0.01)	
		152 (0.03)	164 (0.04)	240 (0.66)	
INU005	INU030	INU055	LEI004	REN105L03	REN162C04
124 (0.19)	144 (0.25)	210 (1.00)	85 (0.63)	233 (0.35)	204 (0.03)
126 (0.79)	150 (0.59)		95 (0.28)	237 (0.21)	206 (0.97)
128 (0.01)	156 (0.16)		97 (0.09)	241 (0.44)	
REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
216 (0.81)	164 (0.04)	268 (0.60)	226 (0.94)	147 (0.09)	12 (0.06)
220 (0.19)	168 (0.78)	274 (0.40)	234 (0.06)	149 (0.79)	13 (0.15)
	170 (0.18)			153 (0.12)	20.2 (0.43)
					23.2 (0.37)
VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
18.1 (0.78)	13 (0.01)	19 (0.44)	14 (0.03)	9 (0.13)	13 (0.01)
19.1 (0.03)	14 (0.79)	23 (0.03)	16 (0.01)	13 (0.50)	15 (0.24)
20.1 (0.16)	15 (0.06)	26 (0.04)	18 (0.01)	14 (0.34)	16 (0.12)
21.1 (0.03)	18 (0.09)	27 (0.01)	20 (0.15)	15 (0.03)	17 (0.10)
	19 (0.04)	28 (0.38)	21 (0.37)		18 (0.51)
		29 (0.09)	22 (0.41)		19 (0.01)
			23 (0.01)		
VGL2918	VGL3008	VGL3235			
12 (0.01)	13 (0.01)	13 (0.12)			
13 (0.44)	17 (0.07)	14 (0.72)			
14 (0.26)	18 (0.16)	15 (0.01)			
17.3 (0.28)	19 (0.41)	16 (0.06)			
	20 (0.25)	18 (0.09)			
	21 (0.09)				

Assessment of allelic diversity across the genome in the Greater Swiss Mountain Dog shows a low number of alleles per locus when compared to more popular dog breeds. This number ranged from one allele (INU055) to seven alleles (VGL1828) per STR locus in the study cohort. As expected for a purebred dog breed, one allele predominated (i.e., was found at a higher frequency) at most loci, with the remaining alleles being identified at lower frequencies. However, one allele was fixed (found in 100% of the dogs tested) at locus INU055 and in the case of five STR markers (FH2054, INRA21, REN162C04, REN169D01, and REN54P11), a single allele occurred at a high frequency (greater than 80%). This lack of allelic diversity suggests that the genomic regions harboring these six loci have been under strong positive selection since breed formation and are most likely associated with prominent breed-defining phenotypic traits. These loci also constitute a characteristic signature of an individual or closely related line that played an important role in the founding of the breed (i.e., a founder effect). Additional alleles for these 33 STR markers may be identified as more individuals are tested, but likely at low number and frequency.

B. Assessment of population diversity using standard genetic parameters

Alleles for each of the 33 STR loci listed in Table 1 and their respective frequencies are used to determine basic genetic parameters for the population (**Table 2**). These parameters include the number of alleles found at each locus (**Na**); the number of effective alleles (**Ne**) per locus (i.e., the number of alleles that contribute most to genetic differences/heterozygosity); observed heterozygosity (**Ho**); expected heterozygosity (**He**) if the existing population was in Hardy-Weinberg equilibrium (i.e., random breeding); and the coefficient of inbreeding (**F**) derived from H_o and H_e values.

Table 2. Genetic Assessment of 34 Greater Swiss Mountain Dogs based on 33 autosomal STR loci. SE = standard error.

	Na	Ne	Ho	He	F
Mean	3.73	2.11	0.48	0.47	-0.004
SE	0.24	0.12	0.04	0.03	0.037

The average number of STR alleles (N_a) identified in this group of Greater Swiss Mountain Dogs was the lowest ever identified for a breed tested at the VGL, corresponding to only approximately 24% of the average number of alleles known to exist at each of these loci across breeds (3.73 out of 15.4 – see section IIA). This number is lower than that found in breeds with low genetic diversity such as the Berger Picard (25%), Polish Lowland Sheepdog (26%), and Irish Wolfhound (27%). When compared to its closely related breeds such as the Bernese Mountain Dog (38%) and Saint Bernard (36%), the lack of genetic diversity found across STR loci in the Greater Swiss Mountain Dog becomes even more apparent. The average number of effective alleles per locus in this cohort was also low ($N_e = 2.11$). Among the breeds tested at the VGL to date, it was only greater than the N_e value estimated for Berger Picard (1.98). The number of effective alleles (N_e) is a measure of effective population size; in the case of this cohort of Greater Swiss Mountain Dogs from the US and Canada, it indicates a low number of breed founders contributed to most of the diversity (heterozygosity), which is in line with the breed’s history (see section IA). *This finding highlights*

the need for breeders to maintain and further distribute currently existing heterozygosity by selecting the most unrelated mates whenever possible.

The mean observed heterozygosity (H_o) for this cohort was estimated at 0.48, while the expected heterozygosity (H_e) was slightly lower at 0.47. This yielded a coefficient of inbreeding (F) of -0.004 , which signifies that this group of dogs was, on average, 0.4% more outbred (or heterozygous) than expected for a random breeding population (Hardy-Weinberg equilibrium). Although the standard genetic assessment values indicate that the 34 Greater Swiss Mountain Dogs were as outbred as a random mating population, this conclusion is based on the cohort as a whole and not on individual dogs making up the population. Internal Relatedness (IR) scores provide a better picture of heterozygosity for each dog and should be used by breeders to select the most unrelated mates possible (see section E below).

C. Standard genetic assessment values for individual STR loci

Allele frequencies can be also used to perform a standard genetic assessment of heterozygosity at each of the 33 autosomal STR loci used in this study (**Table 3**). This provides an estimate of genetic similarities in the specific regions of the genome that are associated with each STR marker. The number of alleles (N_a) found in individual STR loci for this cohort ranged from 1 (INU055) to 7 alleles (VGL1828) per locus, while the number of effective alleles (N_e) ranged from 1 (INU055) to 3.68 alleles (VGL3008) per locus. The observed heterozygosity (H_o) for an individual STR locus ranged from 0 (INU055 and REN162C04) to 0.7 (VGL3008), while H_e ranged from 0 (INU055) to 0.72 (VGL3008) (**Table 3**).

Loci with the lowest H_o values contributed the least to heterozygosity levels across the breed; they are most likely associated with inherited traits that are important for the breed's phenotypic standard. Conversely, high H_o values for a particular locus signifies that it shows greater genetic diversity across the breed, and that these loci can be associated with phenotypic variation among individuals. High inbreeding coefficients ($F > 0.1$) were calculated for six of the 33 STR markers analyzed (18%, shaded in gray on **Table 3**), which suggests that these loci have been under strong positive selection for the period of breed development. However, the higher number of loci with negative F values (21 out of 33, or 64%) as well as the nearly zero F value estimated for the cohort as a whole (**Table 2**) suggest that this group of Greater Swiss Mountain Dogs was as unrelated as possible. Moreover, it can be concluded that breeders have kept a good balance in inbreeding levels across these genomic regions through mating practices.

Table 3. Standard Genetic Assessment of individual STR loci for 34 Greater Swiss Mountain Dogs.

Locus	Na	Ne	Ho	He	F
AHT121	5	2.595	0.676	0.615	-0.101
AHT137	5	2.366	0.618	0.577	-0.07
AHTH130	3	2.63	0.529	0.62	0.146
AHTH171-A	4	2.772	0.647	0.639	-0.012
AHTH260	5	2.592	0.588	0.614	0.042
AHTk211	3	2.234	0.588	0.552	-0.065
AHTk253	3	2.347	0.618	0.574	-0.076
C22.279	3	1.582	0.412	0.368	-0.119
FH2001	4	1.65	0.324	0.394	0.179
FH2054	4	1.275	0.235	0.216	-0.09
FH2848	4	2.039	0.5	0.51	0.019
INRA21	2	1.372	0.324	0.271	-0.193
INU005	3	1.498	0.382	0.333	-0.15
INU030	3	2.3	0.676	0.565	-0.197
INU055	1	1	0	0	0
LEI004	3	2.059	0.588	0.514	-0.144
REN105L03	3	2.766	0.588	0.638	0.079
REN162C04	2	1.061	0	0.057	1
REN169D01	2	1.448	0.265	0.309	0.144
REN169O18	3	1.561	0.412	0.359	-0.146
REN247M23	2	1.919	0.559	0.479	-0.167
REN54P11	2	1.125	0.118	0.111	-0.063
REN64E19	3	1.533	0.382	0.348	-0.1
VGL0760	4	2.923	0.735	0.658	-0.118
VGL0910	4	1.574	0.382	0.365	-0.049
VGL1063	5	1.553	0.294	0.356	0.174
VGL1165	6	2.844	0.706	0.648	-0.089
VGL1828	7	3.05	0.647	0.672	0.037
VGL2009	4	2.612	0.647	0.617	-0.048
VGL2409	6	2.897	0.735	0.655	-0.123
VGL2918	4	2.916	0.5	0.657	0.239
VGL3008	6	3.682	0.706	0.728	0.031
VGL3235	5	1.836	0.5	0.455	-0.098

D. Differences in population structure as determined by Principal Coordinate Analysis (PCoA)

PCoA measures the genetic relatedness of individuals within a population. The data is computed in a spherical form, but often presented in the two dimensions that most closely represent its multi-dimensional form (usually coordinates 1 and 2). The closer individuals cluster together around the XY axis, the more closely related they are to each other. The 34 Greater Swiss Mountain Dogs in

this study clustered as expected for a population composed of genetically similar individuals (i.e., a breed) in the PCoA. Individual dogs were reasonably dispersed across all four quadrants of the graph with several dogs appearing as outliers (i.e., more genetically diverse), seen on the periphery of the plot (**Figure 3**). Therefore, it can be assumed that this group of 34 individuals are *reasonably unrelated* given the low amount of genetic diversity existing the breed. Moreover, individuals from different countries of origin are shown in different colors on the plot; based on the clustering pattern, they form a somewhat genetically homogeneous population, and not genetically distinct varieties based on country of origin. Again, individuals from different countries seen on the periphery of the plot can be considered more genetically diverse (**Figure 3**).

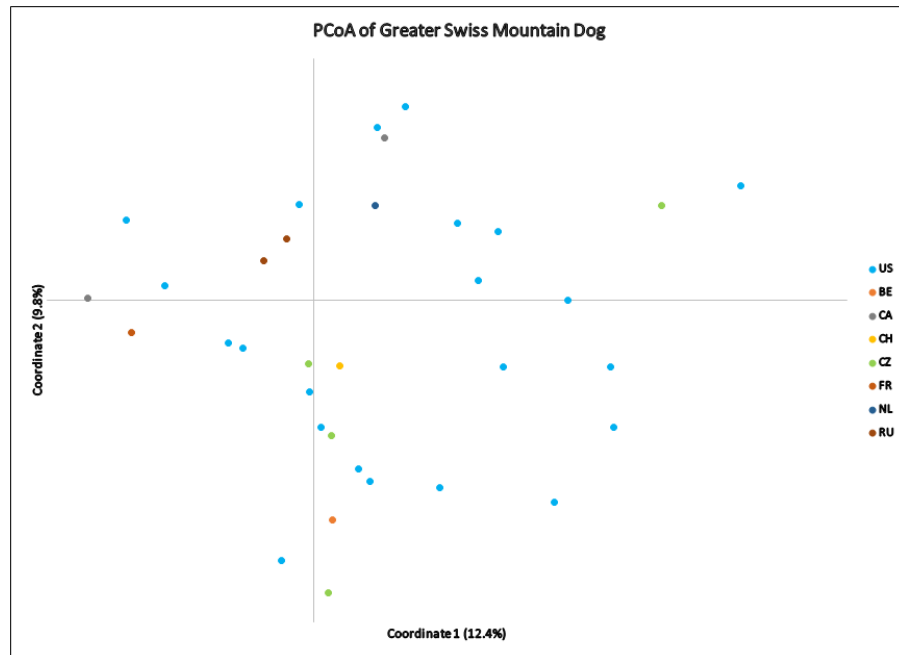


Figure 3. PCoA of Greater Swiss Mountain Dogs (n=34) based on alleles and allele frequencies at 33 autosomal STR loci. Individuals are color coded based on country of origin: US = United States; BE = Belgium; CA = Canada; CH = Switzerland; CZ = Czech Republic; FR = France; NL = Netherlands; RU = Russia.

The degree of relatedness among individuals within the breed can be further enhanced by comparing the 34 Greater Swiss Mountain Dogs with closely related breeds, such as Bernese Mountain Dog and Saint Bernard [7] (**Figure 4**).

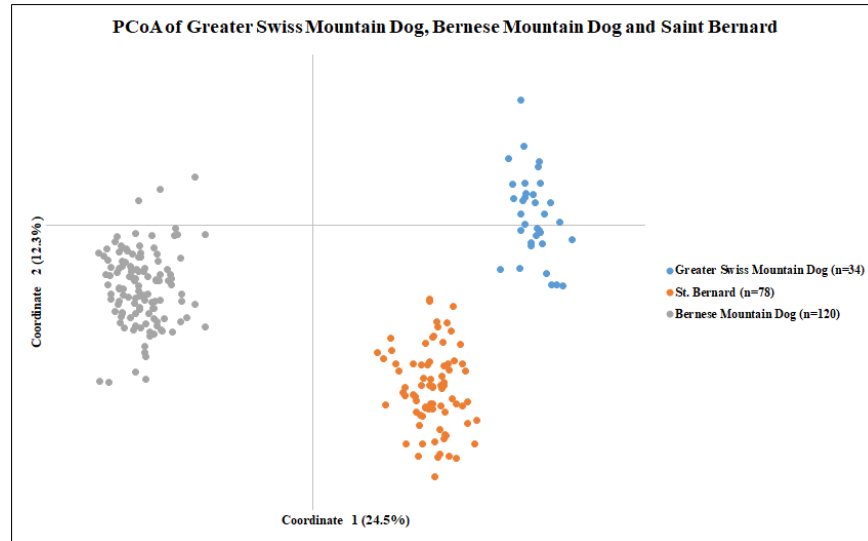


Figure 4. PCoA plot comparing intra- and inter-breed relatedness of Greater Swiss Mountain Dog (n=34) with Bernese Mountain Dog (n=120) and Saint Bernard (n=78).

PCoA shows that the three breeds are genetically distinguishable. Clustering patterns show that Greater Swiss Mountain Dogs are more closely related to Saint Bernard than to Bernese Mountain Dogs as the latter breed can be seen further away from the other two breeds, which cluster closer to each other on the same side of the plot along the X-axis (**Figure 4**).

E. Internal relatedness (IR) scores for Greater Swiss Mountain Dogs

1. IR testing and meaning

Genetic assessments such as those presented in Tables 1-3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity inherited by individuals from their parents. Internal Relatedness (IR) is a calculation that has been used to determine the degree of relatedness of parents of an individual dog. The IR calculation takes into consideration homozygosity at each of the 33 STR loci in this study and gives more weight to rare and uncommon alleles, which would presumably be identified 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 an IR value of -1.0 would have parents that are totally unrelated at all 33 STR loci, while a dog with an IR value of +1.0 has parents that are 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 are themselves highly inbred. *The higher the IR value is above 0.25 for a particular individual, the more closely related are the parents and grandparents of the sibling parents.* **Table 4** summarizes the IR values for the 34 Greater Swiss Mountain Dogs tested in this study.

Table 4. Internal relatedness (IR) and adjusted IR (IRVD) values calculated using allele numbers and frequencies for 33 STR loci in 34 Greater Swiss Mountain Dogs.

	IR	IRVD
Min	-0.2913	0.1675
1st Qu	-0.1277	0.3869
Mean	-0.0225	0.4477
Median	-0.0557	0.4536
3rd Qu	0.0882	0.5068
Max	0.3224	0.7171

The most outbred dog in the cohort had an IR score of -0.2913, while the most inbred dog had an IR score of 0.3224; the average IR score for this group was -0.0225. *This wide range of IR values shows that the degree of parental relatedness varies greatly in the study cohort, a typical finding for almost all pure breeds of dogs.* **Table 4** shows that approximately 75% of the dogs in this group had IR values between -0.2913 and 0.0882, and approximately 25% had IR values between 0.0882 and 0.3224. Therefore, although the standard genetic assessments made from allele frequencies indicated that this population of Greater Swiss Mountain Dogs was the product of random mating (**Tables 2 and 3**), IR values suggest that this assumption is misleading because more outbred dogs are cancelling out more inbred dogs. In reality, approximately one-fourth of Greater Swiss Mountain Dogs tested were products of closely related parents, some being equivalent to half-siblings or closer.

This separation of IR values in two groups (75% outbred; 25% inbred) can also be visualized in the IR curve for the study cohort (**Figure 5**, red line), which is bimodal (two peaks). Each peak represents a different degree of parental relatedness listed in **Table 4**: the first peak (left) represents the most outbred dogs (75%), while the second peak (IR > 0.0882) contains the most inbred individuals in the population (25%).

2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed development

The IR values obtained from known STR alleles and their frequencies can be used to approximate the amount of genetic diversity that has been lost as a breed evolves from its oldest common ancestors to the present day. Village dogs that exist throughout the SE Asia, the Middle East and the Island Pacific region are randomly breeding descendants of dogs from which most modern breeds evolved. The known STR alleles and their frequencies of a given breed can be compared with the same alleles and their frequency in modern village dogs to yield an adjusted IR score (IR-village dog or IRVD) (**Table 4; Figure 5**, blue line). Therefore, the IRVD score approximates how the IR score for a Greater Swiss Mountain Dog would compare to other village dogs if its parents were also village dogs. The curve representing IRVD scores for this cohort (blue line) is shifted well to the right of their actual IR scores (red line), which emphasizes the lack of genetic diversity among Greater Swiss Mountain Dogs when compared to randomly breeding village dogs. Roughly 94% of this cohort (32 out of 34 dogs) have IRVD values of 0.25 or greater (**Table 4, Figure 5**).

This signifies that if they were picked from among from village dogs, all of them would be considered inbred to the level of offspring of full sibling parents or even more closely related than that.

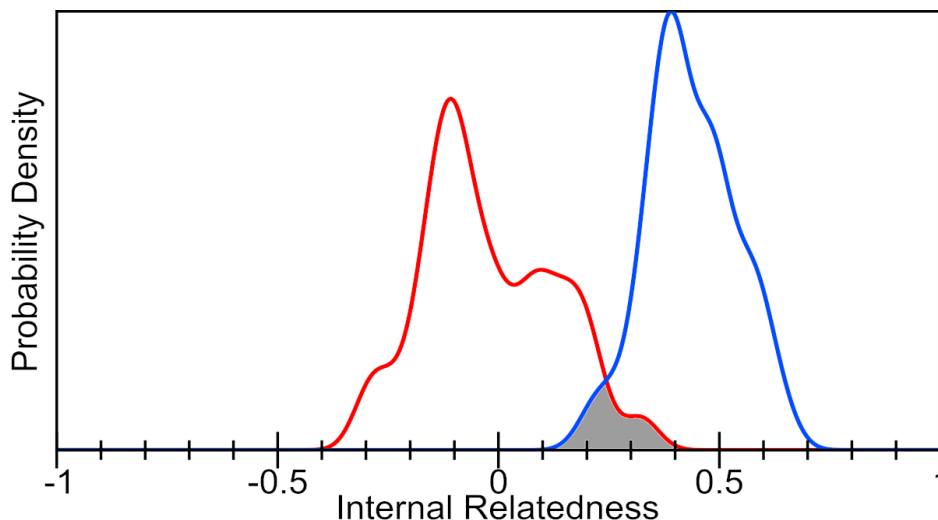


Figure 5. Distribution of IR (red line) and IR-village dog (IRVD) (blue line) values for Greater Swiss Mountain Dogs (n=34). The overlap between the curves (gray) represents the degree of allele sharing (7.2%) between this breed and village dogs.

The gray area in **Figure 5** indicates that Greater Swiss Mountain Dogs tested in this study only retain 7.2% of the genetic diversity existing in present-day randomly breeding village dogs. This is similar to Swedish Vallhund (7% retained diversity), another breed with low genetic diversity. This low amount of retained genetic diversity observed in Greater Swiss Mountain Dogs becomes apparent when compared to the approximately 30% retained genetic diversity calculated from comparisons with known alleles at the 33 STR loci of all canids tested at VGL (**section IIB**). The Bernese Mountain Dog, another Sennenhund breed previously tested at the VGL, retains 16.8% of the genetic diversity of village dogs.

All purebred dog breeds originated from relatively small founder populations, which consequently leads to limited genetic diversity from the time registries were created and studbooks were closed. In the case of Greater Swiss Mountain Dogs, this could have happened after it was recognized as a breed in 1909 following Dr. Albert Heim's and Franz Schertenlieb's efforts. Subsequently, more genetic diversity may have been lost due to other genetic bottlenecks introduced throughout breed development (for example, when the breed was first imported into the United States).

F. DLA class I and II haplotype frequencies and genetic diversity

The DLA consists of four gene-rich regions that make up a small portion 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, which can cause autoimmune diseases, allergies, and resistance/susceptibility to infectious diseases.

The Class I region contains several genes, but only one, DLA88, is highly polymorphic (i.e., with many allelic forms) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with the DLA88 gene are linked in various combinations, forming specific haplotypes (**Table 5**). Groups of genes (and consequently 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 these three STR loci associated with the three class II genes are strongly linked, and often inherited as a single haplotype (**Table 6**). An individual inherits one haplotype from each of the parents. The STR-based haplotype nomenclature used in this breed diversity analysis is based on numerical ranking: class I haplotypes are named 1001, 1002, and so on; class II haplotypes are named 2001, 2002, etc. It is common for different dog breeds to share common and even rare haplotypes for these loci, depending on common ancestry.

1. DLA class I and II haplotypes existing in the Greater Swiss Mountain Dog

Only two DLA class I and two DLA class II haplotypes were identified in this study cohort (**Table 5**). This number is the lowest found for any breed tested at the VGL, and lower than breeds with equally limited genetic diversity such as the Swedish Vallhund (6 and 4, respectively) and Shiloh Shepherd (7 and 6, respectively). DLA-I haplotype 1012 was hugely predominant, being identified in 94% of the 34 dogs tested; the most frequent DLA-II haplotype was 2003, also at the high frequency of 94%. *This suggests a strong genetic and phenotypic influence of a single founder or founder line on both the breed and DLA haplotype diversity.* Equal allele frequency distributions identified between DLA-I/DLA-II haplotypes 1012/2003 (94% each) and 1016/2066 (6% each) indicate that they are linked, and thus inherited as larger extended haplotypes (**Table 5**).

Table 5. DLA class I and II haplotypes identified in Greater Swiss Mountain Dogs (n = 34) with their respective frequencies. The haplotype with the highest frequency is bolded.

DLA-I Haplotype	STR types	Frequency (%)
1012	388 369 289 188	94
1016	382 371 277 178	6
DLA-II Haplotype	STR types	Frequency (%)
2003	343 324 282	94
2066	339 324 280	6

Further analysis showed that the 2 DLA-I/DLA-II haplotypes identified in Greater Swiss Mountain Dogs are shared with 35 other dog breeds/varieties (**Table 6**), and none of these are unique to the breed. Recombination within and between these blocks of genes tends to be low, allowing them to remain mostly unchanged over the generations. Therefore, the DLA haplotypes found in a breed can be used to estimate the founder or founder lines that were used to create a breed and the influence of these various founders in subsequent breed evolution.

None of the DLA-I/DLA-II haplotypes found in Greater Swiss Mountain Dog were found in Saint Bernard, and only one DLA-I haplotype (1016) was found in Bernese Mountain Dogs (closely related breeds). Interestingly, the DLA-I haplotype 1012, occurring in 94% of the study cohort, was also found in relatively high frequency in Terrier breeds (Lakeland Terrier – 76%; Yorkshire Terrier – 28%; Biewer Terrier – 23%), Cardigan Welsh Corgi (26%), and English Bulldog (41%). Strong DLA class II haplotype sharing was also observed with all the aforementioned breeds, plus Miniature and Toy Poodle (**Table 6**).

Table 6. Sharing of DLA class I and II haplotypes between Greater Swiss Mountain Dogs (highlighted in blue) and other dog breeds/varieties tested at the VGL (n=35).

DLA-I Haplotype	STR types	Greater Swiss Mountain Dog (n=34)	American Eskimo, Standard (n=62)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	Border Collie (n=61)	Bernese Mountain Dog (n=120)	Black Russian Terrier (n=148)	Biewer (n=123)	Biewer Yorkshire Terrier (n=53)	Biewer Terrier (n=197)	Yorkshire Terrier (n=16)	Borzoi (n=144)	Cardigan Welsh Corgi (n=39)	Collie (n=49)	Doberman Pinscher (n=974)	English Bulldog (n=163)	Bulldog (n=1)	English Mastiff (n=30)	Whippet (n=95)
1012	388 369 289 188	0.94	0.016	0.04	--	0.057	--	--	0.199	0.151	0.226	0.28	--	0.26	--	0.001	0.414	--	--	0.042
1016	382 371 277 178	0.06	0.008	0.04	0.11	--	0.033	0.01	0.024	0.019	0.013	0.03	--	--	0.04	0.0185	0.095	0.5	0.15	0.121
DLA-I Haplotype	STR types	Greater Swiss Mountain Dog (n=34)	Mastiff (n=13)	Flat Coated Retriever (n=738)	Giant Schnauzer (n=303)	Golden Retriever (n=828)	Havanese (n=746)	Italian Greyhound (n=1169)	Lakeland Terrier (n=124)	Labrador Retriever (n=211)	Llewellyn Setter (n=107)	Polish Lowland Sheepdog (n=51)	Toy Poodle (n=197)	Miniature Poodle (n=354)	Poodle (n=4249)	Rat Terrier (n=39)	Samoyed (n=191)	Scottish Collie (n=111)	Shiloh Shepherd, ISSA (n=239)	
1012	388 369 289 188	0.94	--	--	--	0.0012	0.0141	0.0081	0.762	--	--	--	0.02	0.059	0.0142	0.05	0.013	0.009	--	
1016	382 371 277 178	0.06	0.19	--	0.046	0.0048	0.1991	0.0599	0.008	0.009	0.023	0.039	0.025	0.027	0.0214	0.05	--	0.099	--	
DLA-II Haplotype	STR types	Greater Swiss Mountain Dog (n=34)	American Eskimo, Standard (n=62)	American Eskimo, Miniature (n=38)	American Eskimo, Toy (n=14)	Border Collie (n=61)	Bernese Mountain Dog (n=120)	Black Russian Terrier (n=148)	Biewer (n=123)	Biewer Yorkshire Terrier (n=53)	Biewer Terrier (n=197)	Yorkshire Terrier (n=16)	Borzoi (n=144)	Cardigan Welsh Corgi (n=39)	Collie (n=49)	Doberman Pinscher (n=974)	English Bulldog (n=163)	Bulldog (n=1)	English Mastiff (n=30)	Whippet (n=95)
2003	343 324 282	0.94	0.016	0.04	--	0.057	--	--	0.24	0.208	0.244	0.38	--	0.26	--	0.0005	0.598	--	--	0.095
2066	339 324 280	0.06	0.008	--	--	--	--	--	--	--	--	--	0.007	--	--	--	--	--	--	--
DLA-II Haplotype	STR types	Greater Swiss Mountain Dog (n=34)	Mastiff (n=13)	Flat Coated Retriever (n=738)	Giant Schnauzer (n=303)	Golden Retriever (n=828)	Havanese (n=746)	Italian Greyhound (n=1169)	Lakeland Terrier (n=124)	Labrador Retriever (n=211)	Llewellyn Setter (n=107)	Polish Lowland Sheepdog (n=51)	Toy Poodle (n=197)	Miniature Poodle (n=354)	Poodle (n=4249)	Rat Terrier (n=39)	Samoyed (n=191)	Scottish Collie (n=111)	Shiloh Shepherd, ISSA (n=239)	
2003	343 324 282	0.94	--	0.1396	0.031	0.0205	0.2111	0.0073	0.762	0.014	--	--	0.419	0.513	0.1018	0.1	0.013	0.018	0.006	
2066	339 324 280	0.06	--	0.0007	--	--	0.1763	--	--	--	--	--	--	0.028	0.0001	--	--	--	--	

2. Heterozygosity in the DLA region

Due to their physical proximity in canine chromosome 12, the seven loci that define the DLA class I and II haplotypes are in stronger linkage disequilibrium (i.e., have a higher probability of being inherited together) when compared to other parts of the genome as measured by the 33 autosomal STR markers. However, the expectation is that these loci have achieved an equilibrium with other loci in the genome over time, and thus will be inherited randomly as well. This assumption can be tested through a standard genetic assessment of each locus (**Table 7**) and averaged across all loci (**Table 8**).

The highest number of alleles (N_a) at each DLA locus for Greater Swiss Mountain Dogs was two, and like the 33 STR markers across the genome, the number of effective alleles (N_e) per locus within this region was low (1.13). The lower N_a and N_e values for alleles in the DLA class I and II regions is a result of the high frequency of the 1012/2003 extended haplotypes due to a strong founder effect. Due to this, estimation of observed and expected heterozygosity (H_o and H_e , respectively) in the DLA region yielded in extremely low values (0.1 for each). We expect that additional alleles for the seven DLA class I and II loci will be identified with testing of more individuals from this breed, which will allow for a more accurate estimation of heterozygosity in this region.

Table 7. Standard genetic assessment for Greater Swiss Mountain Dog using each of the 7 STRs in the DLA class I and II regions (n=34).

CA
CA
CT

Table 8. Summary of standard genetic assessment for Greater Swiss Mountain Dog using 7 STRs in the DLA class I and II regions (n=34).

III. What does this assessment of genetic diversity tell us about the Greater Swiss Mountain Dogs.

Greater Swiss Mountain Dogs have a **low** level of genetic diversity. This lack of genetic diversity is even greater in the DLA region than across the genome. These findings indicate that the breed was developed from a relatively small number of founders, which have contributed significantly to the measures of genetic diversity shown here. Outliers that are more genetically diverse exist in the study cohort, which suggests that more dogs need to be tested to better capture the diversity that exists in the breed. This lack of genetic diversity is likely a consequence of various genetic bottlenecks that happened during early stages of breed development. According to the Greater Swiss Mountain Dog Club of America (GSMDCA), this breed is still quite rare both in the US and in Europe, so even though it is expected that additional alleles might be found with testing of more dogs, these will probably be found at lower frequencies.

However, a lack of genetic diversity is not inherently bad if the original founding stock lacked heritable diseases and if a further loss or imbalance in the existing diversity has been avoided, which is the case in the cohort represented in this study. Nonetheless, it is more important for less populous breeds, even if relatively healthy, to maintain existing genetic diversity by breeding the least related parents possible. Breeders should be aware of this when selecting mates for their breeding programs, in order to redistribute the diversity that currently exists in the breed. The goal is to produce dogs with IR scores lower than zero.

IV. Health of the Greater Swiss Mountain Dog

A. Lifespan

The breed's average lifespan is 8 to 11 years [2], which is similar to that of other dog breeds of the same size.

B. Diseases [2,4]

1. Urinary Incontinence

It has been reported that Greater Swiss Mountain Dogs may suffer from urinary incontinence, which is involuntary urination while sleeping due to a weakened bladder sphincter. This condition is usually more frequently found in spayed females, although males can also suffer from urinary incontinence.

2. Hip Dysplasia (HD)

As is the case of most medium and large sized dog breeds, Greater Swiss Mountain Dogs can also suffer from hip dysplasia. HD results from an unstable hip socket and leads to a degenerative, sometimes crippling, arthritis in mature dogs.

3. Elbow Dysplasia (ED)

This potentially crippling condition affects the elbow joint. Elbow dysplasia can result in lameness and affect puppies as young as 5 months. The only way to confirm and evaluate ED is by x-ray. This condition is believed to be inherited, since ED is less likely to be present in puppies born to parents that do not possess the condition. Further studies are needed to evaluate this hypothesis.

4. Osteochondritis Dissecans (OCD)

This disorder can lead to crippling arthritis when a lack of blood supply to the bone underneath the cartilage of a joint leads to its death. Like some dysplasias, there are genetic components to this disease, although no scientific studies have been conducted in Greater Swiss Mountain Dogs to determine the heritability of OCD. Reputable breeders will certify breeding stock through the OFA (Orthopedic Foundation for Animals) before breeding.

5. Epilepsy

A debilitating disease found in the Greater Swiss Mountain Dog is epilepsy. Seizures can range from very mild to severe. This disorder is believed to have a genetic component, although no genetic tests are available. Breeders are advised to use pedigree analysis to avoid the condition in their stock. The first signs of epilepsy generally occur between 1 and 5 years of age.

6. Gastric and Splenic Torsion

Twisting of the stomach (gastric) and the spleen (splenic) are common life-threatening issues seen in Greater Swiss Mountain Dogs. The torsion causes blood flow to these organs to be interrupted, usually leading to death if not treated immediately. Symptoms can include distended abdomen, excessive salivation, lethargy, and pale mucous membranes.

7. Entropion and Distichiasis

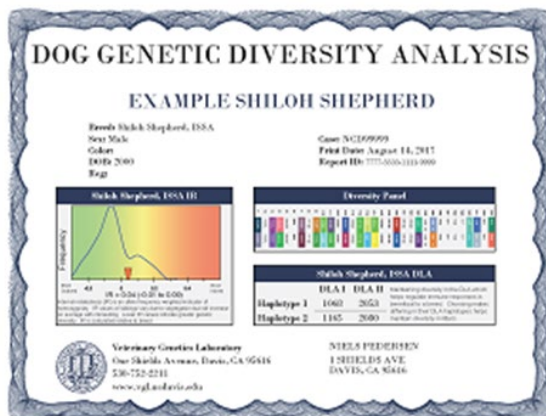
These conditions affect the eyelids, usually resulting in damage to the dog's eye. Entropion is a condition in which the eyelid rolls inward, causing irritation to the surface of the eye; distichiasis is characterized by the presence of extra eyelashes along the eyelid, which grow inward. Eye examinations by a boarded ACVO Ophthalmologist are recommended for breeding dogs to prevent the use of individuals with such ocular disorders in breeding programs. Entropion and distichiasis are believed to be inherited disorders, but further studies are needed to evaluate this hypothesis.

V. Results of VGL Canine 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 reported in relation to others in the population. 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 can be different (heterozygous) or the same (homozygous). Each allele is inherited from one of the parents. Dogs from closely related parents will be homozygous for more alleles at each locus, or in regions of the genome that are under strong positive selection for phenotypic trait or traits mostly favored in the breed. Dogs with a predominance of rare (i.e., low frequency) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high frequency) alleles. A sample genetic diversity report is shown below.



B. What should you do with this information?

DNA testing for genetic diversity in the Greater Swiss Mountain Dog shows a low level of genome-wide genetic diversity and inbreeding in a fraction of the study cohort. The number of DLA-I and DLA-II haplotypes identified in this cohort was extremely low when compared to other breeds. This is an indication of a founder effect followed by possible subsequent genetic bottlenecks. Therefore, the breed needs to be carefully managed with the goal of redistributing the existing diversity more evenly across the population. It is important to monitor existing diversity into the future both across the genome and in the DLA region. We believe that this can be most accurately done with DNA testing and the use of interrelatedness scores and DLA-I/II haplotypes to better balance and maintain genetic diversity and as a supplement to in-depth pedigrees.

If the breed were to consider increasing genetic diversity by further genetic introgressions or outcrossing, DNA testing of dogs intended for such practices would also be essential in order to avoid deleterious mutations and to ensure that the added DNA is properly incorporated into the existing population.

The goal for breeders should be to continue to produce puppies with IR scores close to zero, and as informed breeding decisions are made, even lower scores. Mates should be preferably selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype; moreover, mating of dogs with less frequent genomic alleles or DLA haplotypes is encouraged. 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. However, because IR values reflect the unique genetics of individuals, they cannot be used as the primary criterion for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, breeding dogs with high IR values (providing they are genetically different) may produce puppies with much lower IR scores than either parent. A mating between a dog with a high IR value and one with 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 could 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 of the mates. You want to avoid breeding dogs that will produce puppies homozygous for the same haplotypes; once again, less common haplotypes may increase breed diversity in relation to common ones.

Breeders who would like to predict the genetic outcome of puppies of certain sires and dams should screen them for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Rare alleles should be favored over common ones. This information is included on all certificates and on the breed-wide data found on the VGL website.

VI. References

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