Genetic Diversity Testing for Magyar Agár

Overview

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

Genetic diversity testing of Magyar Agár is now in the data collection phase. During this phase, we will continue to test more registered dogs to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their breed. We are accepting dogs from all regions of the world. At the time this report was written we had tested 59 Magyar Agár, which should provide a preliminary picture of the genetic makeup of the breed. Thirty-nine dogs were from Finland, 14 from Hungary, and the origins of six dogs were not provided. The data derived from these 59 dogs is likely to change as more dogs are tested, but only a little and by additions of lower incidence alleles and haplotypes. The report will be updated until no further changes occur.

For **other breeds**, please see Enrolling a Breed

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

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

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

I. Introduction

A. Breed history (1-6)

The *Magyar Agár* is a type of sighthound originating in around 1,000 years ago in the Carpathian (Great) Basin of Central Europe, an area encompassed by modern Hungary. Oriental greyhound racing became popular during the 4th to the 6th century with the Great Migration of the Huns from the steppes of Central Asia, the Caucasus, and Eastern Europe. The Magyars, warlike horsemen from the Urals, invaded the region in 890 A.D. and brought with them Borzois, a large heavy coated greyhound type dog used for coursing game. Mingling of these two types of dogs resulted in what became known as the Hungarian Greyhound or Magyar Agár. Magyar Agár were used over the next centuries for hunting - a larger variety by the nobility and smaller versions by commoners. The smaller types later became extinct. Although Magyar Agár were found throughout the Great Basin, their hunting abilities were particularly known in the counties of Szabolcs-Szatmár-Bereg, Hajdú-Bihar and Somogy.

The Magyar Agár remained largely unchanged from the Medieval times to the early 1900's, when greyhounds from England were introduced to increase the speed of the breed. However, many aficionados of the Magyar Agár do not like to use the term "greyhound" when referring to the breed. As a result, the preference is to emphasize differences rather than similarities with European greyhounds. Magyar Agárs are longer in body relative to height and have a heavier bone structure and heads are more wedge-shaped with shorter snouts. They also have thicker skin with a short but denser smooth coat that is slightly longer during winter months. This is said to give them greater tolerance for cold temperatures better than other short-coated sighthounds. The Magyar Agár tend to have more rose-shaped ears and rounder eyes, giving them a somewhat more alert and gentle expression. They come in a greater variety of colors.

The sturdier frame of the Magyar Agár makes them ideal for coursing game over a rough terrain. They do not have the initial acceleration speed as Greyhounds on short sprints, but they possess greater endurance and stamina and can run longer distances over longer periods of time. This was essential in older times when they had to travel alongside their mounted masters. Hungarians claim that Magyar Agár were expected to travel 30-50 km per day. The sturdy frame of the Magyar Agár is also ideal for coursing game over a rugged terrain.

The Magyar Agár was approved by the FCI in 1966. The Magyar Agár has not been formally recognized by the AKC and belongs to the Sighthound and Pariah Group for the Kennel Club of the UK, and United Kennel Club. The North American Magyar Agár Association (NAMAA). NAMAA was founded in 2008 to serve as the breed parent club for the Magyar Agár in North America, and to preserve, protect and promote the interests of this wonderful breed. NAMAA maintains its own studbook and registers domestically-born and imported dogs. Membership in the NAMAA is world-wide.

II. Baseline genetic diversity testing and what it tells us about the Magyar Agár

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

1. Allele and allele frequencies for each of the 33 STR loci

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

Thirty-three STRs and their alleles were studied in 57 Magyar Agár from North America and Europe (including Hungary) (Table 1). The 33 STR loci chosen from 25/38 different canine autosomes were quite polymorphic with 3-9 alleles per locus (Table 1). This is a somewhat lower number of alleles than found in other breeds, but these numbers will likely increase as more dogs are tested. Although 2-3 alleles tended to dominate at each locus, only four alleles at four loci occurred at a frequency over 50%. The incidence of remaining alleles was reasonably dispersed with only a few occurring in ≤5% of dogs. This is another indication that more alleles have yet to be identified. However, most of these additional alleles will occur at a low incidence, because the common alleles are likely to be found among the 57 dogs tested.

Table 1. Allele designation and frequency at 33 STR loci for 59 Magyar Agár
Link to Table 1

AHT121	AHT137	AHTH130	AHTh171-A	AHTh260	AHTk211
94 (0.034)	131 (0.381)	119 (0.119)	219 (0.203)	236 (0.059)	87 (0.025)
96 (0.042)	137 (0.153)	125 (0.110)	223 (0.017)	240 (0.136)	89 (0.398)
98 (0.042)	141 (0.161)	127 (0.475)	225 (0.085)	244 (0.085)	91 (0.441)
100 (0.085)	145 (0.144)	129 (0.153)	227 (0.025)	246 (0.305)	93 (0.034)
102 (0.059)	147 (0.008)	131 (0.034)	229 (0.322)	248 (0.008)	95 (0.102)
104 (0.449)	149 (0.017)	133 (0.034)	231 (0.017)	250 (0.169)	
106 (0.195)	151 (0.017)	135 (0.059)	233 (0.076)	252 (0.186)	
110 (0.008)	153 (0.119)	137 (0.017)	235 (0.136)	254 (0.051)	
114 (0.017)			237 (0.119)		
118 (0.068)					

AHTk253	C22.279	FH2001	FH2054	FH2848	INRA21
284 (0.042)	116 (0.034)	132 (0.042)	144 (0.076)	228 (0.034)	95 (0.263)
286 (0.034)	118 (0.492)	136 (0.127)	152 (0.119)	230 (0.093)	97 (0.161)
288 (0.331)	120 (0.017)	140 (0.017)	156 (0.305)	232 (0.059)	99 (0.483)
290 (0.576)	122 (0.008)	144 (0.746)	160 (0.229)	236 (0.432)	101 (0.085)
292 (0.017)	124 (0.195)	148 (0.068)	164 (0.025)	238 (0.153)	105 (0.008)
	126 (0.220)		168 (0.076)	240 (0.110)	
	130 (0.034)		172 (0.161)	242 (0.093)	
			176 (0.008)	244 (0.025)	

INU005	INU030	INU055	LEI004	REN105L03	REN162C04
124 (0.288)	144 (0.254)	208 (0.178)	85 (0.059)	227 (0.178)	198 (0.042)
126 (0.347)	150 (0.678)	210 (0.246)	95 (0.347)	229 (0.042)	200 (0.025)
128 (0.144)	152 (0.068)	212 (0.254)	97 (0.051)	231 (0.025)	202 (0.525)
130 (0.059)		214 (0.237)	105 (0.220)	233 (0.212)	204 (0.102)
132 (0.161)		218 (0.085)	107 (0.271)	235 (0.458)	206 (0.288)
			111 (0.051)	239 (0.085)	208 (0.017)

REN169D01	REN169O18	REN247M23	REN54P11	REN64E19	VGL0760
202 (0.008)	158 (0.085)	266 (0.254)	222 (0.017)	139 (0.042)	12 (0.042)
208 (0.025)	160 (0.059)	268 (0.280)	226 (0.220)	141 (0.025)	18.2 (0.424)
210 (0.008)	162 (0.398)	270 (0.153)	228 (0.153)	145 (0.517)	19.2 (0.008)
212 (0.212)	164 (0.212)	272 (0.246)	232 (0.042)	147 (0.331)	20.2 (0.364)
216 (0.534)	168 (0.153)	276 (0.017)	234 (0.068)	149 (0.059)	21.2 (0.025)
220 (0.212)	170 (0.093)	278 (0.051)	236 (0.212)	151 (0.017)	22.2 (0.093)
			238 (0.288)	153 (0.008)	23.2 (0.034)
					24.2 (0.008)

VGL0910	VGL1063	VGL1165	VGL1828	VGL2009	VGL2409
13 (0.119)	8 (0.068)	17 (0.017)	16 (0.102)	9 (0.390)	13 (0.373)
14 (0.008)	9 (0.119)	18 (0.034)	17 (0.127)	13 (0.186)	15 (0.136)
14.1 (0.034)	11 (0.085)	19 (0.102)	19 (0.136)	14 (0.339)	16 (0.068)
16.1 (0.178)	12 (0.076)	20 (0.034)	20 (0.500)	16 (0.085)	17 (0.220)
17 (0.017)	13 (0.280)	21 (0.093)	21 (0.008)		18 (0.169)
17.1 (0.034)	14 (0.076)	24 (0.008)	22 (0.110)		19 (0.025)
18 (0.373)	15 (0.169)	25 (0.186)	23 (0.017)		20 (0.008)
18.1 (0.034)	16 (0.127)	26 (0.093)			
19.1 (0.186)		28 (0.347)			
21.1 (0.017)		29 (0.042)			
		30 (0.017)			
		32 (0.025)			

VGL2918	VGL3008	VGL3235
12 (0.025)	14 (0.034)	12 (0.093)
13 (0.424)	15 (0.051)	13 (0.263)
14 (0.102)	16 (0.093)	14 (0.288)
15 (0.034)	17 (0.551)	15 (0.042)
18.3 (0.042)	18 (0.136)	16 (0.229)
19.3 (0.008)	19 (0.042)	17 (0.051)
20.3 (0.008)	20 (0.034)	19 (0.034)
21.3 (0.203)	21 (0.059)	
22.3 (0.153)		

2. Using allele frequency data to do standard genetic assessments of the entire population.

A standard genetic assessment of heterozygosity was made from allele frequency data for all 33 STR loci for all 59 Magyar Agár that were tested (Table 2). The average number of alleles (Na) per loci was 6.94 and the number of effective alleles (Ne) was 3.76. The effective alleles were those that contribute most to the heterozygosity. The observed (actual) heterozygosity (Ho) of alleles across the 33 STR loci was 0.72 and the heterozygosity expected (He) if the alleles were in Hardy-Weinberg equilibrium (HWE) was 0.71. HWE is achieved when a given population is in a state of random breeding. The fact that He and Ho were almost identical indicates that this population of 59 dogs is in HWE and that breeders are doing a good job in picking sires and dams from the population that are as unrelated as possible.

An inbreeding coefficient was calculated based on the differences in He and Ho and in this case, F was -0.017 (Table 2). A value of -1.0 would mean that no dog in the population shared alleles, while a value of +1.0 would mean that all the dogs were genetically the same. This F value was only slightly negative, indicating that the population contains slightly more outbred than inbred individuals. However, standard genetic assessment values are averages for the entire population and do not accurately measure the degree to which an individual dog is inbred or outbred. It is still possible to have a significant number of very inbred dogs in a population that appears to be in HWE if the contribution of these individuals is countered by an equal population of more outbred dogs. The actual heterozygosity status of individual dogs is better demonstrated by internal relatedness (IR) values (see section III).

Table 2. Summary of Standard Genetic Assessment for Magyar Agár using 33 STR loci (Updated February 22, 2019)

	N	Na	Ne	Ho	He	F
Mean	59	6.94	3.76	0.72	0.71	-0.02
SE		0.32	0.18	0.02	0.02	0.015

3. Standard genetic assessment values for individual STR loci

The allele and allele frequencies can be used to do a standard genetic assessment of heterozygosity at each STR locus (Table 3). The value Na is the number of alleles that are observed at each locus for a specific breed, while Ne is the number of effective alleles observed at each locus. Effective alleles are those alleles that contribute the bulk of the diversity. The Na values for individual STR loci for this population of 59 Magyar agár ranged from a low of 3 to a high of 12, while the Ne ranged from 1.73 (FH2001) to 6.23 (VGL1063).

Table 3. Genetic assessments for individual STR loci of Golden Retrievers. Na= alleles/locus; Ne= effective alleles/locus; Ho=observed heterozygosity; He=expected Heterozygosity; FIS=coefficient of inbreeding (deviation from HWE expectation).

Locus	N	Na	Ne	Ho	He	F
AHT121	59	10	3.84	0.66	0.74	0.107
AHT137	59	8	4.35	0.81	0.77	-0.057
AHTH130	59	8	3.56	0.60	0.72	0.081
AHTh171-A	59	9	5.22	0.83	0.81	-0.028
AHTh260	59	8	5.31	0.93	0.81	-0.148
AHTk211	59	5	2.74	0.76	0.64	-0.201
AHTk253	59	5	2.25	0.54	0.56	0.024
C22.279	59	7	3.02	0.66	0.67	0.01
FH2001	59	5	1.73	0.48	0.42	-0.13
FH2054	59	8	5.06	0.86	0.80	-0.08
FH2848	59	8	4.08	0.86	0.76	-0.15
INRA21	59	5	2.98	0.64	0.66	0.03
INU005	59	5	3.94	0.76	0.75	-0.02
INU030	59	3	1.89	0.37	0.47	0.21
INU055	59	5	4.54	0.85	0.78	-0.09
LEI004	59	6	3.98	0.80	0.75	-0.06
REN105L03	59	6	3.38	0.71	0.70	-0.01
REN162C04	59	6	2.69	0.63	0.63	0.001
REN169D01	59	6	2.66	0.58	0.62	0.08
REN169O18	59	6	4.06	0.76	0.75	-0.01
REN247M23	59	6	4.36	0.70	0.77	0.10
REN54P11	59	7	4.85	0.78	0.79	0.02
REN64E19	59	7	2.61	0.61	0.68	0.01
VGL0760	59	8	3.08	0.68	0.68	-0.004
VGL0910	59	10	4.47	0.88	0.78	-0.14
VGL1063	59	8	6.23	0.86	0.84	-0.03
VGL1165	59	12	5.30	0.80	0.81	0.02
VGL1828	59	7	3.25	0.75	0.69	-0.08
VGL2009	59	4	3.24	0.75	0.69	-0.08
VGL2409	59	7	4.17	0.66	0.76	0.13
VGL2918	59	9	3.87	0.71	0.74	0.04
VGL3008	59	8	2.94	0.63	0.66	0.05
VGL3235	59	7	4.57	0.86	0.78	-0.11

Ho ranged from 0.37 (INU030) to 0.93 (AHTh260) and He from 0.47 (INU030) to 0.81 (AHTh260)) between each of the 33 STR locus (Table 3). The Ho and He values were used to calculate the F value (1-Ho/He), a measure of deviation from HWE. Eighteen alleles had slightly to mildly negative FIS values and 15 slightly to mildly positive F values. The balancing of

positive and negative F values for alleles at each locus was further evidence that the population as a whole was in HEW.

3. Determining amount of known canid genetic diversity retained in contemporary breed

The number of known alleles at each of 33 loci for all the thousands of dogs and wolves tested to date by the VGL ranges from 7 to 28 (Table 4). The number of alleles identified among the 59 Maygar agár tested ranged from 3 to 12 per locus and the percent of known alleles occurring at each locus ranged from 29 % to 64% (average 40.4%) (Table 3). Therefore, approximately over 40% of
of known canid diversity at these 33 loci has been retained during breed evolution. Breed evolution not only includes the time period since the breed was officially registered, but also the much longer preceding period of human-directed selection. The percentage (40.4%) of distant ancestral genetic diversity for Llewellin setters is higher than that of related breeds such as the Irish red and white setter (34.8%) and Irish setter (30.3%). This value for retained genetic diversity will be validated by another method that compares this population of dogs to village dogs, a large and diverse random-breeding of dogs that share ancestry to most modern breeds (see section in IR/IRVD).

Table 4. Percent of all known alleles observed at each of the 33 STR loci in Llewellin setters

Locus	**# known alleles**	**% observed**
AHT121	24	10/24=42%
AHT137	17	8/17=47%
AHTH130	20	8/20=40%
AHTh171-A	14	9/14=64%
AHTh260	28	8/28=29%
AHTk211	7	5/7=71%
AHTk253	11	5/11=45%
C22.279	13	7/13=54%
FH2001	17	5/17=29%
FH2054	23	8/23=35%
FH2848	24	8/24=33%
INRA21	15	5/15=33%
INU005	14	5/14=36%
INU030	15	3/15=20%
INU055	11	5/11=45%
LEI004	15	6/15=60%
REN105L03	22	6/22=27%
REN162C04	14	6/14=43%
REN169D01	14	6/14=43%
REN169O18	14	6/14=43%
REN247M23	11	6/11=54%
AvREN54P11	14	7/14=50%
REN64E19	12	7/12=58%
VGL0760	26	8/26=31%

VGL0910	27	10/27=37%
VGL1063	17	8/17=47%
VGL1165	23	12/23=52%
VGL1828	22	7/22=32%
VGL2009	12	4/12=33%
VGL2409	13	7/13=54%
VGL2918	19	9/19=47%
VGL3008	18	8/18=44%
VGL3235	13	7/13=54%
		Average 40.4%

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

1. Genetic relationships of Magyar Agár to each other

Principal coordinate analysis (PCoA) uses genetic distance based on allele sharing to graph relatedness between individuals within and between various populations. The resulting graphic is multi-dimensional (spherical) but is usually portrayed in two dimensions by selecting two coordinates (planes of the sphere) that represent the greatest proportion of individuals. This usually includes coordinates 1 and 2, planes of the sphere that include most of the population being studied. We tested 59 Magyar Agár from several countries. All the dogs tested belonged to a single cluster (breed) but with a moderate degree of genetic drift between groupings of individual dogs (Fig. 1). This pattern is consistent with a population (breed) that has a noticeable degree of genotypic, and presumably phenotypic, diversity. Populations consisting of closely related individuals will form a much tighter cluster around the X/Y axis.

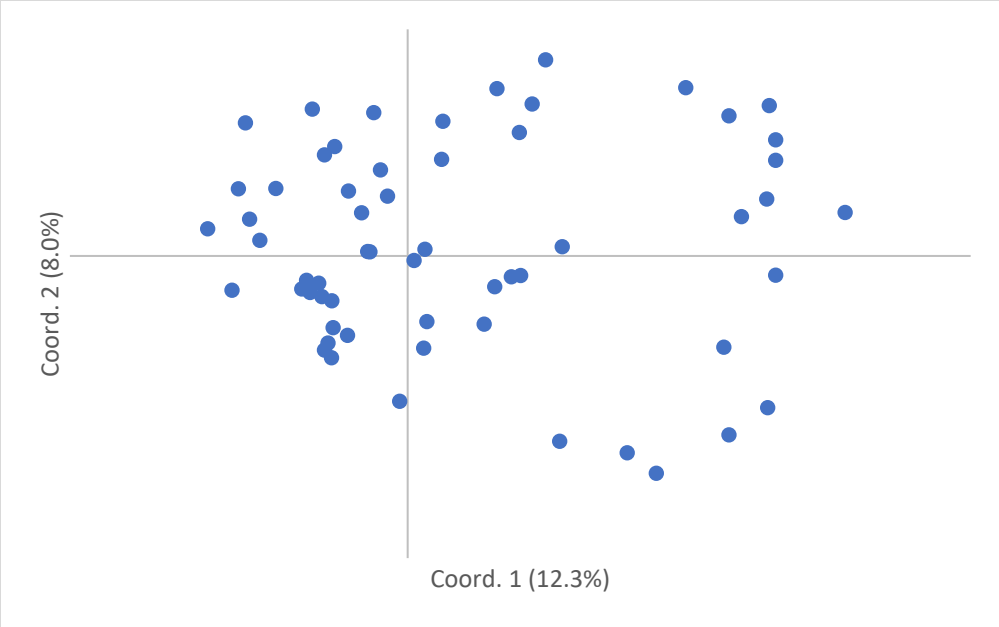


Figure 1. PCoA of Magyar Agár (n=59) based on the 33 STRs

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

Standard genetic assessments such as the one presented in Table 2 are indicators of population-wide heterozygosity (i.e. a mean or average) and do not show heterozygosity for individual dogs. The heterozygosity of an individual dog is determined by each of its parents, which is usually unknown. Internal Relatedness (IR) is a calculation that has been used to estimate the degree to which the two parents were related by looking only at their offspring. 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 equivalent to offspring of full sibling parents from a random breeding population. IR values >0.25 occur only if the parents of the full sibling parents were also highly related.

IR scores can be provided for the population (Table 5) or graphed for individual dogs (Fig. 2). Mean IR scores for the 59 Magyar Agár ranged from a low of -0.195 (most outbred or parents least related) to a high of 0.222 (most inbred or parents most related), with a mean (average) value of -0.017. One-fourth of the dogs had IR scores from -0.089 to -0.195 and were more outbred than the average. Conversely, one-fourth ranged from 0.050 to 0.222 and were more

inbred. Therefore, even though the standard genetic assessment indicated that this group of dogs was from a randomly breeding population, one quarter of the individuals were more outbred than indicated and one-fourth more inbred. It is noteworthy that very few dogs in this group had IR scores near 0.25. This is unusual because most other breeds have contained a higher proportion of even more inbred individuals. This is a very positive finding and bodes well for what will be found when more dogs are tested.

Table 5. IR vs IRVD comparison for Magyar Agár (n=59)

	IR	IRVD
Min	-0.1951	-0.0685
1st Qu	-0.0890	0.0738
Mean	-0.0172	0.1358
Median	-0.0213	0.1268
3rd Qu	0.0502	0.1999
Max	0.2224	0.3774

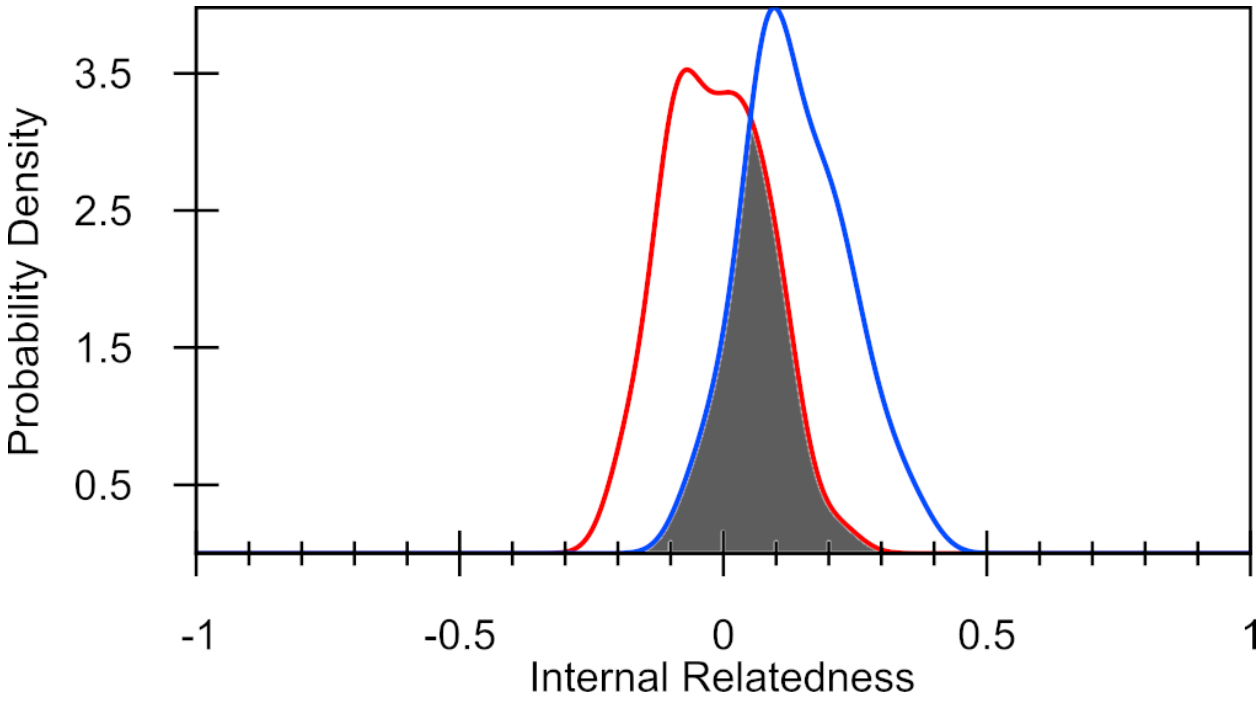


Figure 2**.** Distribution of IR estimated in Magyar Agár (n=59) based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). Lost diversity was determined by comparing allele frequencies at the same loci with village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.

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

The IR values can also be used to approximate how much genetic diversity was lost breed's evolution. This is done by comparing the frequency of a given allele in Magyar Agár 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 still randomly breeding and largely unchanged from the ancestors of almost all modern dog breeds. The adjusted frequencies are then used to calculate the IRVD and presented tabular form for the total population (Table 5) or graphed for individual dogs (Fig. 2).

The blue line in Fig. 2 is a graphic representation of how modern Magyar Agár would compare to village dogs rather than other members of their breed. The blue curve is shifted to the right with one-fourth of the dogs now having values ranging from 0.150 to 0.344. Therefore, some of the 59 Magyar Agár would now have IR values compatible with offspring of sibling parents if they were considered as village dogs.

A comparison of IR values (red curve) and IRVD values (blue curve) can be used as to estimate how much of ancestral canine diversity has been maintained in Magyar Agár through all the genetic bottlenecks associated with breed evolution. A rough estimate based on areas under the curve (black), indicates that Magyar Agár possess 48.7% of the genetic diversity still present in all dogs. This is close to the 40.4% retained canid genetic diversity calculated from allele frequencies at the 33 STR loci (Table 4). The retention of 48.7% of known dog genetic diversity is above average when compared to other breeds. For instance, the Swedish Vallhund has retained only 7% of canine genetic diversity, the Doberman Pinscher 15% and the Shiloh Shepherd 27%, while at the highest level the Golden Retriever 50.4%, the Miniature Poodle 51%, the Labrador retriever 54% and the Toy Poodle 60%.

IV. DLA Class I and II Haplotypes

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 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 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.

Table 6 lists the DLA class I and II haplotypes that were identified among the 59 Magyar Agár that were tested. Eighteen class I and 7 class II haplotypes were found. Class I haplotypes 1161-1209 are unique to this point to Magyar Agár and were found in 29% of the dogs, while the rest of the class I haplotypes and all of the class II haplotypes have been recognized in other breeds. The presence of this number (n=9) of unique DLA class I haplotypes is in itself noteworthy, but it the fact that these unique class I haplotypes are not associated with unique class II haplotypes is equally noteworthy. These findings indicate that Magyar Agár share ancestry with a group of dogs that were isolated from the major trade route between SE Asia, the Middle East and Europe. Dogs from this region are ancestors for almost all of the common breeds. It is known that other ancestral dog populations existed further north than these regions, which could be the origin of these unique DLA class I haplotypes. Dogs that were ancestral to Magyar Agár were brought by the Huns from Central Asia, the Caucasus, and Eastern Europe.

Most breeds tested to date possess one or more DLA class I haplotypes that occur in 25-50% of individuals, indicating the strong influence of a small number of founders. This pattern was not seen with the Magyar agar, which appear to have many class I haplotypes occurring at an incidence of 5-14%. This finding coupled with the unusually large number of unique low incidence haplotypes indicates a much more diverse founder base than other pure breeds.

Magyar Agár, unlike all other breeds studied to date, also have a marked imbalance in the number of DLA type I over type II haplotypes. Other breeds have similar numbers of type I and type I haplotypes, while the Magyar agar has over twice as many class I as II haplotypes. Moreover, one of the class II haplotypes (2017), occurred in 41% of the dogs tested. The lack of dominant DLA class I and II haplotypes and lower than expected numbers of class II relative to class I haplotypes is unlikely to be an artifact caused by the relatively small number of dogs tested. It is more likely due to some selection pressure that occurred during breed evolution that inadvertently favored retention of the 2017 haplotype.

Table 6. DLA class I and Class II haplotypes and their frequencies (Updated April 4, 2019). The haplotypes with the highest incidence are highlighted.

DLA-I	STR types	Incidence (n=59)
1003	387 375 277 186	0.08
1015	380 373 291 186	0.05
1033	382 379 277 181	0.13
1052	380 372 289 184	0.05
1054	382 379 277 184	0.14
1058	387 378 287 186	0.05
1066	376 375 277 178	0.08
1067	376 373 277 178	0.01

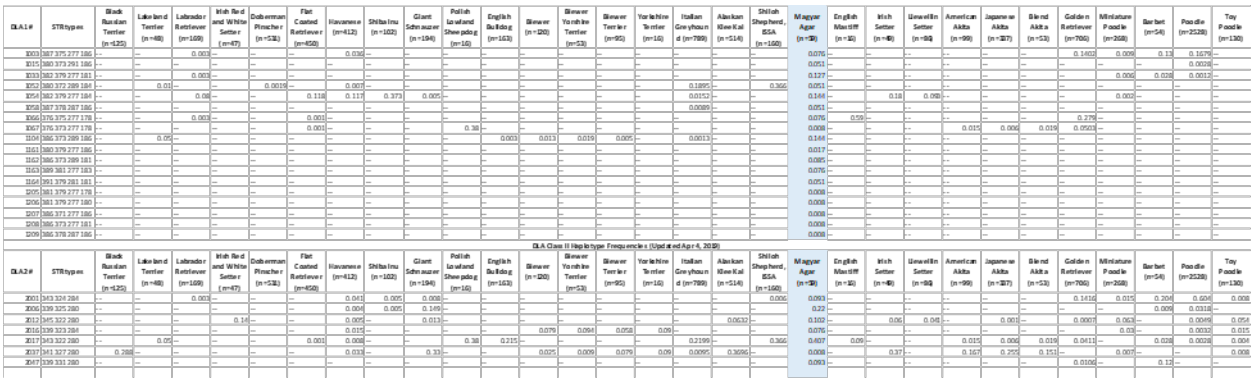
1104	386 373 289 186	0.14
1161	380 379 277 186	0.02
1162	386 373 289 181	0.09
1163	389 381 277 183	0.08
1164	391 379 281 181	0.05
1205	381 379 277 178	0.01
1206	381 379 277 180	0.01
1207	386 371 277 186	0.01
1208	386 373 277 181	0.01
1209	386 378 287 186	0.01
DLA-II		
2001	343 324 284	0.09
2006	339 325 280	0.22
2012	345 322 280	0.10
2016	339 323 284	0.08
2017	343 322 280	0.41
2037	341 327 280	0.01
2047	339 331 280	0.09

2. DLA class I and II haplotype sharing between Magyar Agár and other breeds

DLA haplotypes are in strong linkage disequilibrium and represent a large block of genes that undergo limited recombination and inherited by descent from one generation to another. Therefore, they have some value in determining breed evolution. All but three of the DLA class I and all class II haplotypes observed in this group of Magyar Agár have been found in other breeds (Table 7). DLA class I haplotype sharing based on both numbers and frequency of occurrence was seen between Labrador, Golden and Flat Coated Retrievers; Shiloh shepherd, Miniature and Standard Poodles, Havanese, Shiba Inu and Italian greyhound. DLA class II haplotype sharing was also with Miniature and Standard Poodles, Havanese, Shiloh Shepherds, Golden retriever and Shiba Inu, in addition to the Toy Poodle, Japanese and American Akita, and Giant Schnauzer. This extensive sharing of DLA haplotypes with other breeds indicates that Magyar Agár were derived from many common ancestors.

Table 7 shows DLA class I and II haplotypes present in Magyar Agár that have been identified in many dog breeds. These haplotypes are limited in number among all dogs, wolves and coyotes and are inherited by descent through generations. Various breeds will inherit a portion of these haplotypes from their founders. Some haplotypes will be unique to a breed, but most are shared at different incidences between breeds.

Table 7. DLA class I and II haplotype sharing between Magyar Agár and several other pure breeds of dogs



DLA1 #	STR types	Black Russian Terrier (n=125)	Lakeland Terrier (n=48)	Labrador Retriever (n=169)	Irish Red and White Setter (n=47)	Doberman Pinscher (n=531)	Flat Coated Retriever (n=650)	Havanese (n=412)	Shiba Inu (n=102)	Giant Schnauzer (n=194)	Polish Lowland Sheepdog (n=16)	English Bulldog (n=163)	Bichon (n=120)	Bichon Yorkshire Terrier (n=53)	Bichon Terrier (n=95)	Yorkshire Terrier (n=16)	Italian Greyhound (n=799)	Alaskan Klee Kai (n=514)	Shiloh Shepherd, ISSA (n=160)	Magyar Agar (n=39)	English Mastiff (n=10)	Irish Setter (n=49)	Llewellin Setter (n=93)	American Akita (n=99)	Japanese Akita (n=127)	Blend Akita (n=53)	Golden Retriever (n=706)	Miniature Poodle (n=268)	Barbet (n=54)	Poodle (n=2528)	Toy Poodle (n=130)
1003	387 375 277 186	--	--	0.003	--	--	--	0.036	--	--	--	--	--	--	--	--	--	--	--	0.076	--	--	--	--	--	--	0.1402	0.009	0.13	0.1679	--
1015	380 373 291 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.051	--	--	--	--	--	--	--	--	--	0.0028	--
1033	382 379 277 181	--	--	0.003	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.127	--	--	--	--	--	--	--	0.006	0.028	0.0013	--
1052	380 372 289 184	--	0.01	--	--	0.0019	--	0.007	--	--	--	--	--	--	--	--	0.1895	--	0.366	0.051	--	--	--	--	--	--	--	--	--	--	--
1054	382 379 277 184	--	--	0.08	--	--	0.118	0.117	0.373	0.005	--	--	--	--	--	--	0.0152	--	--	0.146	--	0.18	0.08	--	--	--	--	0.002	--	--	--
1058	387 378 287 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0089	--	--	0.051	--	--	--	--	--	--	--	--	--	--	--
1065	376 375 277 178	--	--	0.003	--	--	0.001	--	--	--	--	--	--	--	--	--	--	--	--	0.076	0.59	--	--	--	--	--	0.276	--	--	--	--
1067	376 373 277 178	--	--	--	--	--	0.001	--	--	--	0.38	--	--	--	--	--	--	--	--	0.038	--	--	--	0.015	0.006	0.019	0.0503	--	--	--	--
1104	386 373 289 186	--	0.05	--	--	--	--	--	--	--	--	0.003	0.013	0.019	0.005	--	0.0013	--	--	0.166	--	--	--	--	--	--	--	--	--	--	--
1161	380 379 277 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.017	--	--	--	--	--	--	--	--	--	--	--
1162	386 373 289 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.085	--	--	--	--	--	--	--	--	--	--	--
1163	389 381 277 183	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.076	--	--	--	--	--	--	--	--	--	--	--
1164	391 379 281 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.051	--	--	--	--	--	--	--	--	--	--	--
1205	381 379 277 178	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--	--	--	--
1206	381 379 277 180	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--	--	--	--
1207	386 373 277 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--	--	--	--
1208	386 373 277 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--	--	--	--
1209	386 378 287 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.038	--	--	--	--	--	--	--	--	--	--	--

DLA Class II Haplotype Frequencies (Updated Apr 4, 2019)

DLA2 #	STR types	Black Russian Terrier (n=125)	Lakeland Terrier (n=48)	Labrador Retriever (n=169)	Irish Red and White Setter (n=47)	Doberman Pinscher (n=531)	Flat Coated Retriever (n=650)	Havanese (n=412)	Shiba Inu (n=102)	Giant Schnauzer (n=194)	Polish Lowland Sheepdog (n=16)	English Bulldog (n=163)	Bichon (n=120)	Bichon Yorkshire Terrier (n=53)	Bichon Terrier (n=95)	Yorkshire Terrier (n=16)	Italian Greyhound (n=799)	Alaskan Klee Kai (n=514)	Shiloh Shepherd, ISSA (n=160)	Magyar Agar (n=39)	English Mastiff (n=10)	Irish Setter (n=49)	Llewellin Setter (n=93)	American Akita (n=99)	Japanese Akita (n=127)	Blend Akita (n=53)	Golden Retriever (n=706)	Miniature Poodle (n=268)	Barbet (n=54)	Poodle (n=2528)	Toy Poodle (n=130)
2001	343 324 284	--	--	--	0.003	--	--	--	0.041	0.005	0.008	--	--	--	--	--	--	--	0.006	0.093	--	--	--	--	--	--	0.1416	0.015	0.204	0.604	0.008
2006	339 325 280	--	--	--	--	--	--	--	0.004	0.005	0.149	--	--	--	--	--	--	--	--	0.22	--	--	--	--	--	--	--	--	0.009	0.0318	--
2012	345 322 280	--	--	--	--	0.14	--	--	0.005	--	0.013	--	--	--	--	--	--	0.0032	--	0.102	--	0.06	0.04	--	--	0.001	--	0.0007	0.063	--	0.0049	0.054
2016	339 323 284	--	--	--	--	--	--	--	0.015	--	--	--	--	0.079	0.084	0.058	0.09	--	--	0.076	--	--	--	--	--	--	--	--	0.03	--	0.0032	0.015
2017	343 322 280	--	0.05	--	--	--	0.001	--	0.006	--	0.38	0.215	--	--	--	--	0.2199	--	0.366	0.407	0.09	--	--	0.015	0.006	0.019	0.0411	--	0.028	0.0028	0.004
2037	341 327 280	0.288	--	--	--	--	--	0.033	--	0.33	--	--	0.025	0.009	0.079	0.09	0.0095	0.3696	--	0.008	--	0.37	--	0.167	0.259	0.151	--	0.007	--	--	0.008
2047	339 331 280	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.093	--	--	--	--	--	--	0.0166	--	0.13	--	--

V. Health problems of Magyar Agár (4, 7-10)

The lifespan of Magyar Agar's is 12-14 years, which is longer than the 10-12 years for European-type greyhounds. The breed is generally healthy, but several health problems have been recognized at a relatively low incidence and they should be monitored in breeding individuals and lines of related dogs so that the incidence does not increase. Most of the health problems that occur in the breed are heritable and either involve complex genetic polymorphisms that appear to have accumulated in dogs over hundreds and even thousands of years and concentrated by descent in various breeds. Complex genetic disorders include hip and elbow dysplasia, autoimmune diseases, and epilepsy. Disorders caused by simple (Mendelian) genetic mutations can occur spontaneously in a breed or inherited by descent from certain founders. Progressive retinal atrophy is an example of this type of heritable disease.

A. Orthopedic problems

1. Hip dysplasia

2. Elbow dysplasia

B. Eye problems

1. Progressive Retinal Atrophy –not common in this breed but has been associated with a several autosomal recessive and sex-linked mutations in other breeds. PRA-causing mutations tend to be breed specific and the genetic basis of PRA in Magyar Agár is unknown. Breeding stock, especially for affected lines, should be screened for the disorder.

2. Entropion and ectropion

C. Autoimmune disease

1. Hypothyroidism – caused by immune destruction of the thyroid gland has been noted in Magyar Agárs starting around 2 to 3 years of age or older. As for autoimmune disorders in general, females are affected more frequently than males. Hypothyroidism (autoimmune

thyroiditis) is the most common autoimmune condition of dogs and occurs in many pure breeds and some random-bred dogs. Autoimmune disorders become more common as genetic diversity is lost and individuals become more inbred. The propensity for autoimmunity is polygenic and is thought to result from the gradual accumulation of positive risk factors.

D. Miscellaneous disorders

1. Epilepsy –characterized by aberrant activity in a group of cells in the brain, causing seizures affecting only a portion of the body (focal) or can be generalized (tonic/clonic). Epilepsy is usually controllable with medication. Like autoimmunity, epilepsy is seen in many pure breeds of dogs and the incidence increases with loss of genetic diversity and level of inbreeding. Therefore, it is an indicator of genetic soundness. There is no effective screening test for this condition and affected dogs should not be bred.
2. gastric dilatation/torsion – This is a problem in all larger and deep-chested dogs
3.Sensitivity to anesthetics and other drugs (10). Research has shown that Greyhounds, and presumably closely related breeds, do not metabolize drugs as other dogs do. A major reason is their low concentration of hepatic cytochrome P-450 enzymes (CYP). Therefore, the dosage of drugs such as barbiturates that are metabolized by the CYP pathway must be given at a lower dosage and more carefully monitored. Greyhounds also have high glomerular filtration rate (GFR) and volume of distribution and may have differences in intestinal drug absorption.

IV. What does preliminary DNA-based testing tell us about contemporary Magyar Agár

The results obtained for 59 Magyar Agár are still preliminary and more dogs need to be tested before a final genetic assessment can be made. However, the results are adequate to provide a snap-shot of breed genetics. The dogs that were tested were very heterogeneous based on standard genetic calculations. The heterogeneity of the 59 dogs was also shown by the PCoA plot. Heterogeneity usually translates to subtle, yet noticeable, phenotypic differences between individual Magyar Agár. Phenotypic differences also translate to genotypic differences. Internal relatedness values confirmed that only a very small proportion of dogs were highly inbred and none to anywhere near IR scores $\geq$0.25 expected for offspring from a full-sibling or equivalent mating. The IR curve for the breed was also among the most favorable that has been observed among the many pure breeds tested to date. These findings indicate that the breeders of the first 59 dogs have done an excellent job in selecting the most unrelated parents. This will hopefully continue to hold true as more dogs are added to the study.

Genetic diversity based on comparisons with village dogs as well as thousands of other dogs in the VGL database indicated that Magyar agar have retained 40-48% of the genetic diversity that still exists across a world-wide swath of indigenous village dogs. This degree of retained genetic diversity is only surpassed by very large and popular breeds such as the Labrador and Golden Retrievers and the various Poodles.

The findings that Magyar Agár have retained a fair amount of genetic diversity during breed evolution, and breeder's success in avoiding inbreeding, may explain the comparatively good health of the breed. Only one genetic disorder (i.e., PRA) of presumed Mendelian inheritance has been recognized in the breed at low frequency, which also reflects the both the favorable genetic

diversity and health of the founding population and strict adherence to random breeding and avoidance of artificial genetic bottlenecks such as popular sire effect. However, the occurrence of hypothyroidism and epilepsy in the breed are warning signs of low genetic diversity and excessive inbreeding. Therefore, it will be critical for breeders to maintain existing genetic diversity, continually breed away from heritable disorders whether simple or complex and avoid artificial genetic bottlenecks.

What is equally noteworthy is how much of the genetic diversity in Magyar Agár has been shared with other major dog breeds. This was demonstrated by the DLA class I and II haplotypes found in Magyar Agár and other breeds. Although many of the DLA class I and all the class II haplotypes found in Magyar Agár are shared with many other breeds, about one-third of the dogs tested had DLA class I haplotypes that have only been found in Magyar Agár to date. This demonstrates the importance of unique ancestors in the founding of the breed a thousand or more years ago. These unique ancestors were likely to have lived in the Great Basin of Central Europe for a very long time and in isolation from dogs originating during the Neolithic expansion in the fertile crescent, SE Asia and Island pacific regions (11). Unfortunately, data on Greyhound was not available for comparison. However, there was strong sharing of DLA class I and II haplotypes with the Italian greyhound.

V. References

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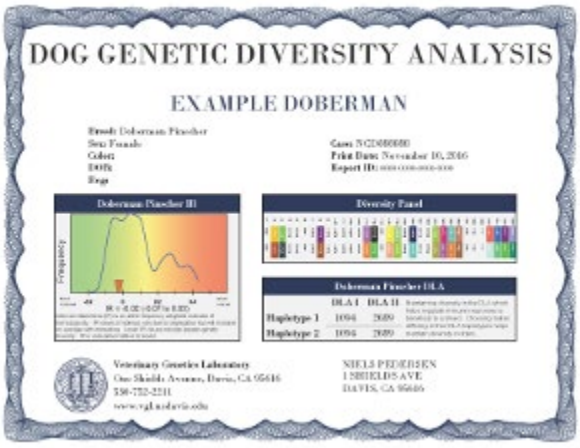
VI. Results of Diversity testing

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 compared to the results for every dog that has been tested.


DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE DOBERMAN

Doberman Finder III

Diversity Panel

Doberman Finder DLA

	DLA I	DLA II
Haplotype 1	1094	2089
Haplotype 2	1094	2089

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B. What should you do with this information?

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. <u>However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates.</u> 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 pairings based on IR values of sire and dam can also compare values by manual screening. Potential sires and dams should be first screened for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Some extra weight should be given to rare vs common alleles. This information is included on all certificates and on the breed-wide data on the VGL website.

Puppies, once born, should be tested for their actual IR values, which will reflect the actual genetic impact of each parent on internal diversity. Considerations of mate choices for genetic diversity should be balanced with other breeding goals but maintaining and/or improving genetic diversity in puppies should be paramount.

An additional goal of this study is to contribute this genetic information to a web repository, hopefully under the control of a registry such as NAMAA (4). This information could be also incorporated into a mate selection service that will allow a breeder to identify, among all of the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters.