Genetic Diversity Testing for Havanese

Overview

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

Baseline genetic diversity testing of Havanese has been completed - please see Enrolling a Breed. We initially tested 86 dogs, but this has now been increased to 242 dogs. Testing of additional dogs has led to the identification of new genomic alleles and DLA haplotypes that were present at low frequency in the population. Therefore, we feel that allele and allele frequencies, and DLA class I and II haplotypes and haplotype frequencies will not change significantly with further baseline testing. Nevertheless, existing genetic diversity will continue to be adjusted if necessary as yet more Havanese are tested.

Price: $80
Price reduced to $70 when combined with a diagnostic test.

Havanese Coat Panel - $130
MC1R, Agouti, Brown, Dilute, Dominant Black, Piebald, Furnishings, Curl

Havanese Health Panel - $110
CDDY+CDPA, CMR1, vWF-I

ORDER TEST KITS
Allow 5-10 business days for results.

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

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

Internal Relatedness: The IR value is a measure of genetic diversity within an individual that takes into consideration both heterozygosity of alleles at each STR loci and their relative frequency in the population. Therefore, IR values heterozygosity over homozygosity and uncommon alleles over common alleles. IR values are unique to each dog and cannot be

compared between dogs. Two dogs may have identical IR values but with very different genetic makeups.

Introduction

The Havanese breed is based on the now extinct Blanquito de la Habana (little white dog of Havana), which descended in turn from the also extinct Spanish Bichon Tenerife. The Blanquito was bred with breeds of similar Bichon type and to Poodles to create the modern Havanese. There are also stories that the breed descended primarily from a small number of dogs that were brought with families fleeing Cuba in the 1950s and 60s, and that there have been some imports from Cuba since this time. The Havanese was officially recognized by the AKC in 1996 in 16 colors and 8 different markings. It ranked 25th in popularity among breeds in 2013, up from 55th in 2003. Havaneses are described as "happy little dogs with a spring in their step and gleam in their big brown eyes. A curled-over tail is a breed hallmark, as is the long silky and curly coat, making it look a little like a Puli. Some owners prefer to clip the coat to reduce grooming time." The results of the present study suggests that the modern Havanese is more likely a recreated breed resulting from crosses between a number of different small breeds, possibly including Blanquito, and other dogs of shared ancestry.

The Canine Genetic Diversity Test and What It Tells Us about Havanese

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

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

(link to table 1)

The allele and allele frequencies can also be used to do a standard genetic assessment of each STR locus (Table 2).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 most to genetic diversity at that STR locus. The Na values for individual STR loci ranged from a low of 4 to a high of 18 alleles/locus, while the Ne ranged from 2.100 to 8.474. Allele frequencies at each locus can be used to measure heterozygosity at each locus. Observed heterozygosity (Ho) is the actual heterozygosity based on the actual (observed) allele frequencies. The expected heterozygosity (He) at each STR loci is the value that would be predicted if the population was in Hardy-Weinberg equilibrium (HWE), a situation that occurs when mate selection has been totally random. The Ho for alleles at each STR locus ranged from 0.446 to 0.864, noting that the loci with the lowest and highest number of effective

alleles often have the lowest or highest mean Ho. The values for Ho and He are used to calculate what is known as F (or FIS, Fis, inbreeding coefficient), which is a measure of how near that locus is to Hardy-Weinberg equilibrium (HWE). HWE is zero when a population is randomly breeding, i.e., no human selection. Positive values of F indicate non-random selection (inbreeding), while negative values indicate outbreeding. Only seven of the 33 loci had negative F values, with the remainder being positive. However, only two loci had F values that were greater than +0.10, AHTk253 and VGL1165. Similar evidence for inbreeding in a small portion of the population is observed with certain DLA class I and II haplotypes (see section E below).

Table 2: Standard genetic assessment of Havanese using alleles frequencies at each genomic STR locus. Na= alleles/locus; Ne= effective alleles/locus; Ho=observed heterozygosity; He=expected Heterozygosity if in HWE; F=coefficient of inbreeding (deviation from HWE expectation)

#	Locus	N	Na	Ne	Ho	He	F
1	**AHT121**	444	10	3.343	0.711	0.701	-0.015
2	**AHT137**	444	11	6.586	0.829	0.848	0.023
3	**AHTH130**	444	8	4.200	0.716	0.762	0.061
4	**AHTh171-A**	444	8	4.948	0.788	0.798	0.013
5	**AHTh260**	444	9	4.654	0.770	0.785	0.019
6	**AHTk211**	444	5	2.981	0.644	0.665	0.031
7	**AHTk253**	444	5	2.078	0.462	0.519	0.110
8	**C22.279**	444	7	4.445	0.740	0.775	0.045
9	**FH2001**	444	8	2.578	0.646	0.612	-0.055
10	**FH2054**	444	10	6.182	0.808	0.838	0.036
11	**FH2848**	444	9	4.698	0.775	0.787	0.016
12	**INRA21**	444	7	4.271	0.729	0.766	0.048
13	**INU005**	444	8	2.599	0.594	0.615	0.035
14	**INU030**	444	7	2.297	0.560	0.565	0.009
15	**INU055**	444	6	4.120	0.716	0.757	0.055
16	**LEI004**	444	9	2.152	0.533	0.535	0.005
17	**REN105L03**	444	9	3.291	0.662	0.696	0.049
18	**REN162C04**	444	6	4.276	0.765	0.766	0.001
19	**REN169D01**	444	7	3.887	0.732	0.743	0.014
20	**REN169O18**	444	7	5.304	0.784	0.811	0.034
21	**REN247M23**	444	6	3.824	0.730	0.738	0.012
22	**REN54P11**	444	7	5.678	0.795	0.824	0.036
23	**REN64E19**	444	8	3.544	0.704	0.718	0.019
24	**VGL0760**	444	14	6.744	0.822	0.852	0.035

25	**VGL0910**	444	15	6.249	0.808	0.840	0.038
26	**VGL1063**	444	13	8.557	0.856	0.883	0.031
27	**VGL1165**	444	19	5.435	0.718	0.816	0.120
28	**VGL1828**	444	11	4.266	0.790	0.766	-0.032
29	**VGL2009**	444	9	4.629	0.754	0.784	0.038
30	**VGL2409**	444	8	3.668	0.695	0.727	0.044
31	**VGL2918**	444	13	6.227	0.801	0.839	0.045
32	**VGL3008**	444	11	6.554	0.815	0.847	0.038
33	**VGL3235**	444	10	4.302	0.707	0.768	0.079

Allele frequencies across all 33 STR loci (Table 1) can also be used to calculate a mean observed heterozygosity (Ho) and expected heterozygosity (He) for the population as a whole (Table 3). The population of 242 Havanese which were tested had a mean Na of 8.58 alleles across all loci and a mean Ne of 4.48. Therefore, about one-half of the alleles across all 33 STR loci were contributing to the bulk of genetic diversity for the dogs tested. These values for mean Na and Ne are actually quite high when compared to most pure breeds that have been studied to date and comparable to those observed for Miniature Poodles. Mean values for Ho and He were also calculated using allele frequency data from all 33 STRs. A mean Ho of 0.723 for the 242 Havanese tested was high compared to other breeds that have been studied to date, again similar to that of Miniature Poodles. The mean He of 0.749 for the population was higher than expected for a random breeding population and the F value was therefore positive at 0.033, indicating that a small proportion of the Havanese were more inbred than the population as a whole.

Table 3: Standared genetic assessment of Havanese using allele frequencies at 33 genomic STR loci

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

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

Principal coordinate analysis (PCoA) uses genetic distance based on allele sharing to demonstrate genetic differentiation between individuals in related or unrelated populations (Fig. 1). An optimized two dimensional graph portrays the degree of genetic differentiation between the 242 Havanese tested. The more distant two points (dogs) are from each other, the greater the genetic differences and vice versa. This analysis shows that the 242 Havanese belong to a single breed (population), but that some individuals within the breed are not as genetically related to each other as one might suspect. Although the bulk of the 242 dogs cluster around the intersection of the two coordinates, there are a number of dogs that appear more distant. These are often referred to as genetic outliers. Because they are genotypically different, it is likely that these outliers also possess phenotypic traits somewhat distinct from the more tightly clustered

dogs. Having a diversity of phenotypes is good, because it infers that there is also a diversity of genotypes. Again, the Miniature Poodle would be the best example of this type of genetic diversity, as they also have a more diffuse PCoA pattern.

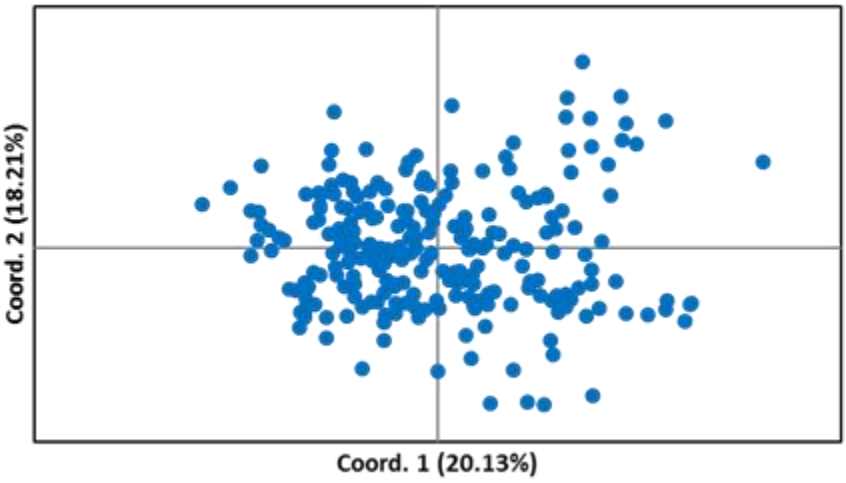


Figure 1: A principal coordinate analysis (PCoA) of allele frequency data from 33 genomic STRs and 242 Havanese dogs

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

Genetic assessments such as those presented in Table 3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity of individuals within the population. The genetic diversity of an individual is largely determined by the diversity inherited from each of the parents. Internal Relatedness (IR) is a calculation that has been used to determine the relative genetic contributions of both parents to an individual. The IR calculation evaluates homozygosity and uses allele frequencies to give more weight to rare and uncommon alleles. On average, the lower the IR, the more outbred the individual, and the higher the score, the more inbred. 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 are completely different at all 33 STR loci, while a dog with an IR value of +1.0 has parents that were genetically identical.

The IR values calculated for 242 Havanese ranged from -0.233 to +0.478 with the peak value for the population being 0.0227 (Table 4; Fig. 2). A value of +0.25 would be seen in offspring of parents that were full siblings, provided that the parents of the full siblings were randomly bred. IR values >0.25 occur when the parents of the full sibling parents were themselves highly inbred. Relatively few dogs had values >0.25 with the most inbred dog having a value of +0.478. This dog was a product of parents that were related to a greater degree than full siblings from a random breeding population. The IR curve supports the breed-wide standard genetic assessment values, i.e., Havanese are genetically heterogeneous and mostly random bred. The fact that the IR and IRVD curves are very similar to each other indicates that Havanese still retain a great deal of the genetic diversity present in ancestral village dogs. Nevertheless, there are still a number of mildly to extremely inbred dogs in the population.

Table 4: Statistical breakdown of IR and IRVD values used to produce the graph in Fig. 2

	IR	IRVD
Min.	-0.23314	-0.14783
1st Qu	-0.06025	0.05508
Media	0.02268	0.14318
Mean	0.03504	0.14922
3rd Qu	0.10298	0.21927
Maxi.	0.47814	0.62769

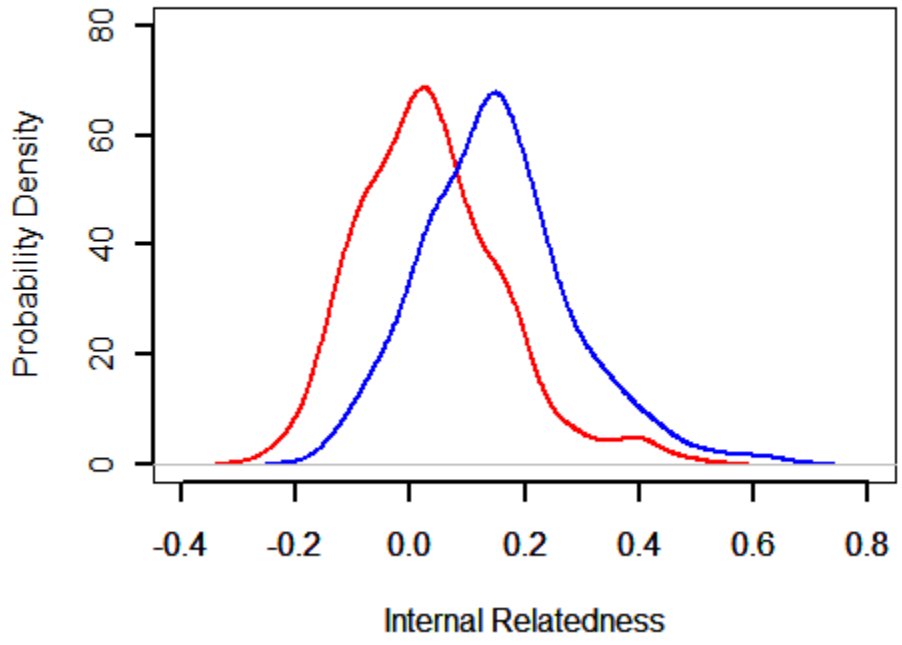


Fig. 2: Distribution of IR estimated in 242 Havanese based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). Lost diversity was determined by comparing allele frequencies at the same loci between Havanese and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population of dogs that we have studied.

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

The IR values can be adjusted in such a way as to provide an estimate of total genetic diversity lost from village dog ancestors of the breed to present time. This is done by using allele frequencies obtained from DNA of present day village dogs from the Middle East, SE Asia and Island Pacific nations, which closely reflect the ancestors of dogs before extensive human

directed genetic manipulation occurred. These dogs are the most random bred and genetically diverse population that has been studied to date. The adjusted IR value is known as IR-village dogs or IRVD.

The IRVD values for Havanese are shown in Fig. 2 (blue line). The mean IRVD was 0.149 for the Havanese population as a whole with individual IRVD values ranging from -0.119 to 0.428. The shift to the right in IRVD compared to IR values was not nearly as pronounced as it has been for other breeds we have studied and indicates that Havanese have retained a greater proportion of the diversity still present in domestic dogs. Nonetheless, like all pure breeds of dogs, some genetic diversity has been lost as a result of breed development and artificial genetic bottlenecks that may have occurred since the Havanese breed was officially registered and closed to outside blood.

E. DLA Class I and II Haplotype frequencies

The DLA consists of four gene rich regions making up a small part of canine chromosome 12. Two of these regions contain genes that help regulate normal cell- (Class I) and antibody-mediated (Class II) immunity. Polymorphisms in these regions have also been associated with abnormal immune responses responsible for autoimmune diseases. The Class I region contains several genes, but only one, DLA-88, is highly polymorphic (with many allelic forms) and is therefore most important for immune regulation. Specific alleles at the four STR loci associated with the DLA88 are linked together in various combinations, forming specific haplotypes (Table 4). Groups of genes and their alleles inherited as a block, rather than singly, are called haplotypes. The class II region also contains several genes, three of which are highly polymorphic, DLA-DRB1, DLA-DQB1 and DLA-DQA1. Specific alleles at STR loci associated with each of the three Class II genes are strongly linked and also inherited as a single block or haplotype (Table 5). One haplotype comes from each of the parents. The linkages between alleles within Class I or II regions are very strong; while linkages between regions of the DLA that are more distant from each other, such as Class I and II, are weaker. There are almost two million base pairs separating the class I and II regions, thus allowing for some genetic recombination to occur between DLA class I and II haplotypes. For example, the common DLA 1016 and 2066 haplotypes are inherited together in many Havanese, but may recombine in some dogs to form new combinations such as 1016 and 2003.

Havanese possess considerable genetic diversity in both DLA class I and II regions, similar to that of the Miniature Poodle and much greater than breeds such as the Akita, Black Russian Terrier, Italian Greyhound, Flat-coated retrievers, and Alaska Klee Kai. This diversity involves 35 different STR-associated DLA Class I (Table 5) and 27 DLA Class II (Table 6) haplotypes. This was eight l class I and four class II haplotypes more than we identified in the original 86 Havanese that were tested. Eighteen DLA class I haplotypes (1084, 1115-1120, 1122-1127, 1132 and 1133, and 1140, 1147 and 1148) were unique to Havanese, when compared to other breeds that we have studied. However, except for 1115 (12.8%), 1116 (7.4%), and 1117 (3.1%), the unique haplotypes are all present at very low frequency. Unique DLA class II haplotypes include 2070-2073, 2075-2078, and 2087, all at very low frequency except for 2070 (7.2%). These unique DLA class I and II haplotypes may have originated from the original founders and some even from the original Blanquito de la Habana. There were three class I and class II haplotypes

that occurred with frequencies from 11 to 24%, which was noticeably higher than other haplotypes. The higher frequency of these six DLA class I and II haplotypes suggests that they originated from certain founding dogs that possessed phenotypic traits that help define the breed. They may also have resulted from some past artificial genetic bottleneck such as a popular sire effect. The latter explanation is more compatible with standard genetic assessments of the allele frequencies of the seven DLA class I and II associated STRs (Table 5, 6).

Tables 5 & 6: DLA Class I & II Haplotype Frequencies in Havanese.

DLA Class I Haplotype Frequencies (Updated Oct 9, 2019)		
DLA1 #	**STR types**	**Havanese (n=436)**
1003	387 375 277 186	0.042
1006	387 375 293 180	0.047
1011	376 365 281 180	0.001
1012	388 369 289 188	0.016
1014	375 373 287 178	0.033
1016	382 371 277 178	0.209
1018	375 373 287 186	0.003
1029	380 365 281 182	0.028
1030	380 373 293 178	0.003
1035	386 373 277 184	0.006
1040	380 371 277 186	0.021
1052	380 372 289 184	0.007
1054	382 379 277 184	0.126
1068	380 373 287 181	0.017
1084	376 373 277 184	0.002
1087	380 371 277 178	0.001
1092	376 379 277 181	0.087
1093	386 379 277 180	0.025
1105	382 379 277 178	0.001
1114	380 373 287 183	0.014
1115	386 371 277 182	0.111
1116	380 365 289 186	0.080
1117	376 373 277 180	0.039
1118	376 377 277 180	0.001
1119	376 378 277 180	0.001
1120	376 386 289 176	0.002

1121	380 371 277 183	0.006
1123	386 379 277 184	0.003
1124	388 373 289 178	0.008
1125	393 383 277 185	0.010
1126	387 373 287 182	0.001
1128	384 376 287 182	0.002
1129	382 371 277 181	0.001
1132	376 379 277 184	0.002
1133	378 365 287 172	0.014
1134	384 365 291 178	0.002
1136	382 371 277 182	0.001
1140	376 379 301 180	0.003
1142	376 379 277 180	0.002
1147	391 375 293 180	0.003
1148	376 375 277 180	0.005
1154	376 365 281 183	0.005
1155	388 369 287 184	0.001
1173	392 371 277 186	0.003

DLA Class II Haplotype Frequencies (Updated Oct 9, 2019)

DLA2 #	STR types	Havanese (n=436)
2001	343 324 284	0.047
2003	343 324 282	0.219
2005	339 322 280	0.002
2006	339 325 280	0.003
2007	351 327 280	0.050
2012	345 322 280	0.006
2014	339 322 284	0.008
2016	339 323 284	0.014
2017	343 322 280	0.008
2018	339 324 284	0.018
2021	339 324 268	0.002
2022	339 327 282	0.120
2023	341 323 282	0.003
2024	343 323 280	0.003
2032	339 323 280	0.028

2033	339 323 282	0.002
2035	341 323 280	0.001
2037	341 327 280	0.033
2040	345 327 280	0.003
2053	343 324 280	0.038
2066	339 324 280	0.182
2070	347 324 282	0.079
2071	339 322 286	0.006
2072	339 325 282	0.014
2073	339 327 286	0.008
2074	341 324 284	0.041
2075	341 327 282	0.028
2076	345 322 282	0.008
2077	347 325 286	0.014
2079	343 323 278	0.002
2082	339 325 268	0.006
2087	347 324 280	0.001

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. Interestingly, about one-half of DLA class I and II haplotypes are also found in Standard Poodles. Havanese do not possess any DLA class I haplotype found only in Miniature Poodles, but there were five class II haplotype in Havanese that were shared by Miniature and Standard Poodles. However, the 2066 class II haplotype, which occurred with a frequency of 18.6% in Havanese, was previously found only in Miniature Poodles.

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

Genetic diversity can also be assessed by studying the frequency of the DLA class I and II alleles of the four DLA class I and three DLA class II STR loci in the same manner as employed with the 33 genomic STR loci (Tables 7, 8). Although these STRs are associated only with the DLA class I and II regions on chromosome 12, the numerous genes and their alleles that make the entire DLA is in strong linkage disequilibrium, meaning that it is inherited as a large block of genes that are less subject to recombination.

Table 7 is a standard genetic assessment of each STR locus associated with the DLA class I and II region of 242 Havanese. All but one of the 7 loci have positive F values, which also supports the conclusion that dogs with certain haplotypes are under some degree of positive selection.

Table 7: Standard genetic assessment of 242 Havanese based on allele and allele frequencies at seven individual DLA class I and II associated STR loci

#	Locus	N	Na	Ne	Ho	He	F
1	**DLA I-3CCA**	444	12	5.050	0.765	0.802	0.046
2	**DLA I-4ACA**	444	12	4.348	0.718	0.770	0.068
3	**DLA I-4BCT**	444	7	1.929	0.460	0.482	0.044
4	**DLA1131**	444	11	6.307	0.819	0.841	0.026
5	**5ACA**	444	6	3.422	0.639	0.708	0.097
6	**5ACT**	444	5	2.194	0.485	0.544	0.108
7	**5BCA**	444	6	2.726	0.605	0.633	0.045

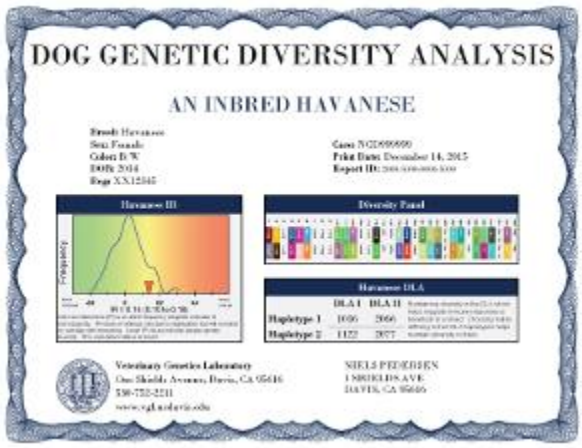
Havanese have a large number of DLA class I and II associated alleles at each of the 7 STR loci (mean Na=8.143), but somewhat less than one half of them (mean Ne=3.526) contribute to most of the diversity (Table 8), reflecting the disproportionately high frequency of the three most common DLA class I and II haplotypes (Tables 5, 6). The He is higher than Ho, leading to a slightly positive value for F (0.069), which was slightly higher than F=0.033 for the 33 genomic markers (Tables 3, 8). Therefore, the DLA region of Havanese is less heterogeneous (more inbred) than other regions of the genome, again suggesting a degree of positive selection for the most common DLA haplotypes.

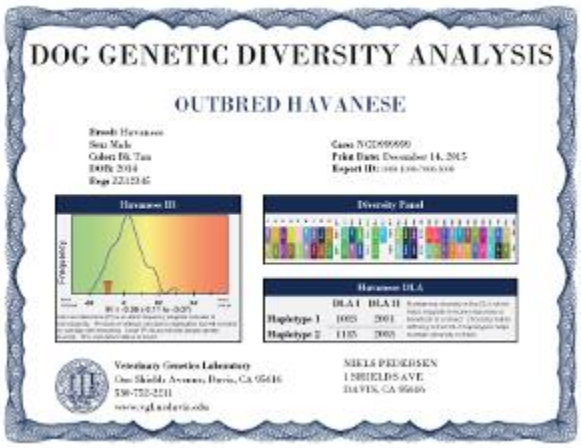
Table 8: Standard genetic assessment of the DLA regions using 7 STRs associated with the DLA class I and II regions.

	N	Na	Ne	Ho	He	F
Mean	444	8.429	3.711	0.642	0.683	0.062
SE		1.085	0.562	0.048	0.047	0.011

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

After a sample is submitted for genetic testing, the identity of the dog and owner will be replaced by a laboratory barcode identifier. This identifier will be used for all subsequent activities and each owner will be provided with a certificate that reports the internal relatedness, genomic STR genotypes and DLA class I and II haplotypes for the dog(s) tested. The internal relatedness value for the dog being tested is related to the population as a whole.





What should you do with this information?

Increasing the number of Havanese tested from 86 to 242 has led to the discovery of additional genetic diversity in both the 33 genomic STR loci and in the DLA class I and II regions. The breed has great genetic diversity, largely due to the many founders that have been used to establish the breed and the wide range of phenotypic diversity that has been allowed. However, there is still some indication that inbreeding is occurring, albeit at low level. The goal for Havanese breeders should be to continue to produce puppies with IR scores less than 0, and with time even lower scores. This should be easily obtainable given the large amount of genetic diversity that exists both in the genome and in the DLA region for the 242 dogs tested. Therefore, Havanese breeders should breed both to maintain their existing diversity and to reverse any imbalances that are now apparent, such as in the DLA class I and II regions. Mates should be selected to avoid homozygosity at any genomic loci or DLA class I and II haplotype and encourage the use of dogs with less common genomic alleles or DLA haplotypes. Maintaining existing genomic diversity will require using IR values of potential mates based on the 33 STR loci to assure puppies of equal or greater overall diversity, similar to what is being done by many Standard Poodle breeders. However, IR values, because they reflect the unique genetics of each individual, cannot be used as the criteria for selecting ideal mates. Mates with identical IR values may produce puppies significantly more or less diverse than their parents. Conversely, a mating between dogs with high IR values, providing they are genetically different, may produce puppies having much lower IR scores than either parent. A mating between a dog with a high IR value and a low IR value, providing the latter has few alleles and DLA haplotypes in common, will produce puppies much more diverse than the highly inbred parent. Breeders should also realize that a litter of puppies may have a wide range of IR values, depending on the comparative contributions of each of the parents. The more genetically diverse and different the parents, the greater the range of IR values in their offspring.

Breeders who do not have access to computer programs to predict the outcome of matings based on IR values of sire and dam can also compare values by manual screening. Potential sires and dams should be first screened for genetic differences in alleles and allele frequencies for the 33 genomic STR loci. Some extra weight should be given to rare vs common alleles. This information is included on all certificates and on the breed-wide data on the VGL website.