Genetic Diversity Testing for Alaskan Klee Kai

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 AKK breeders who wish to track and increase genetic diversity of their breed as a long term goal.

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 Alaskan Klee Kai (AKK) is a type of dog developed between the early 1970s and 1988 exclusively by Linda Spurlin and her family in Alaska. Apparently breeding this little dog was inspired by a mixed breed rescue named Curious that looked like a small Alaskan Husky. Curious was acquired on a visit to a brother-in-law in Oklahoma, and seemed to be the result of a mating between a purebred Alaskan Husky and a small dog of unknown ancestry. Due to the positive response to this dog once back in Alaska, Linda Spurlin decided to develop more of these dogs, and meanwhile her brother-in-law had the same idea. When he decided to stop, he sold his best dogs to Spurlin, who continued to refine these original crosses by carefully selecting only those individuals that met her standards for appearance, temperament, and health. When Spurlin retired, she sold her remaining stock to Eileen Gregory, who ultimately played a pivotal role in getting the breed more widely accepted.

Although the ancestry of the small dog that produced Curious was never determined, the AKK breed was purportedly the result of a limited number of crosses between only 9 dogs, several Siberian and Alaskan Huskies, a Schipperke and an American Eskimo Dogs. The new “breed” was first shown to the public in 1988 as the Klee Kai. It was later split into the Alaskan Klee Kai and Klee Kai in 1995, but consolidated again in 2002 under the name Alaskan Klee Kai. The Alaskan Klee Kai (AKK) was officially recognized by the American Rare Breed Association (ARBA) in 1995 and by the United Kennel Club (UKC) in 1997.

AKK occur in three size ranges: 1) Toy - ≤13 inches; 2) Miniature - >13 inches and ≤15 inches, and Standard - >15 inches and ≤17 inches. AKK of these various sizes may occur in the same litters. The temperament of AKK resembles that of their progenitors and they are usually described as extremely active, highly intelligent, easily trained, but with a tendency to be head strong. They require good socialization with other humans and dogs and a firm hand as to who is the boss. They are purportedly good chewers, diggers and jumpers and not always easily contained in a home or yard.

There is a need to obtain accurate information about potentially heritable disease problems in AKK, but it appears that most health problems have been acquired by descent from founding breeds. AKK suffer inordinately from hepato-portal shunts, cryptorchidism, umbilical hernias and patellar luxation. Liver shunts and luxating patella tend to be problems of many small breeds. Heart problems include mitral valve stenosis (more in larger breeds) and patent ductus arteriosus (more in small breeds). All of these conditions involve complex genetics (several genes) and the causative mutations appear to be ancient, as they are found in many pure breeds and occur with increasing frequency as a breed becomes more inbred. Severe pancreatitis also appears to affect the breed, as it does with Yorkshire Terriers and Schnauzers, and can be related to genetic problems such as post-prandial hyperlipidemia, which may involve a single gene mutation. The genetic basis for pancreatitis in AKK may have been inherited by descent or involve mutation(s) unique to the breed. Two conditions linked to the immune system appear to be on the rise in the breed. Autoimmune thyroiditis leading to hypothyroidism is usually the first and most common autoimmune disorders associated with excessive inbreeding. Allergies are related to autoimmunity and often increase in incidence with that of autoimmune disorders. Both conditions are related to a loss of genetic diversity, especially in the immune system, and affect

the ability of an individual to differentiate what is self from non-self (i.e., reacting to the thyroid gland as if it were foreign). Skin, food, and respiratory allergies occur when the immune system has trouble differentiating common parasites from things like pollen grains, molds and common house mites, or more ancient constituents of a dog's diet from modern ones.

The Canine Genetic Diversity Test

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. Individuals inherit one of these alleles from the sire and the other from the dam. Table 1 (link to table 1) lists the alleles recognized at each STR locus among the AKK tested to date, as well as listing the frequency of any given allele in the population.

1. Genetic assessment using allele and allele frequency data from individual STR loci

The allele and allele frequencies can also be used to do a standard genetic assessment of each STR locus (Table 2). Na is the number of alleles that are observed at each locus, while Ne is the number of effective alleles observed at each locus. Effective alleles are those alleles that make the greatest contribution to genetic diversity at each STR locus. The Na values for individual STR loci among the initial 370 AKK ranged from a low of 2 to a high of 10, while the Ne ranged from 1.144 to 4.872. Therefore, the AKK do not possess nearly as much allelic diversity at each locus as breeds that started with a much larger number of founder dogs. Some STR loci have only 2 or 3 alleles (i.e., AHTh171-A, AHTk253, C22.278, FH2848, REN169018, REN54P11 and INRA21), which is only a fraction of the known alleles at these loci. Therefore, AKK lack diversity in virtually all of the regions of the genome that are linked to an STR locus.

The number of alleles at each STR locus is a quantitative measure of genetic diversity, but it is also important to determine whether the existing alleles segregate in a random or non-random manner. Random segregation would limit the times that two identical alleles appear at each locus (homozygosity), while non-random segregation would limit this occurrence (heterozygosity). The frequency with which a specific allele occurs at each locus can used to measure heterozygosity or homozygosity at each locus. The less frequent an allele appears at a given STR locus, the less apt it will be associated with an identical allele and the greater the heterozygosity. Observed heterozygosity (Ho) is 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. Random mating limits the chances that identical alleles will occur at the same locus.

The Ho for alleles at each STR locus for the initial 370 AKK ranged from 0.407 to 0.860, noting that the loci with the lowest and highest number of effective alleles often have the lowest or highest mean Ho. The smaller the number of available alleles, the greater the chance will be for both alleles to be identical and the lower the Ho value. The values for Ho and He are used to calculate what is known as F, which is a measure of how near that locus is to Hardy-Weinberg equilibrium (HWE). HWE is zero when mate selection within a population is totally random. A value of -1.00 is observed when the alleles at each locus are totally different between individuals of the population, while a value of +1.00 would occur when all of the dogs shared the exact same alleles at each locus. Most of the STR loci for AKK had F values that were only slightly positive or negative, and therefore considered to be close to HWE. This means that AKK breeders are doing a very good job maintaining heterozygosity at each genomic STR locus, which can only occur when mate selection within the population is totally random. Non-random selection most commonly occurs from a popular sire effect, when one male contributes disproportionately to the genetics of a breed. It also occurs when a certain founder lineage is used more heavily than other bloodlines.

Table 2: Genetic assessments for individual STR loci. Na= mean alleles/locus; Ne= mean effective alleles/locus; Ho=observed heterozygosity; He=expected Heterozygosity; F=coefficient of inbreeding (deviation from HWE expectation)

#	Locus	N	Na	Ne	Ho	He	F
1	**AHT121**	540	7	3.169	0.696	0.684	-0.017
2	**AHT137**	540	6	4.908	0.819	0.796	-0.028
3	**AHTH130**	540	6	4.364	0.806	0.771	-0.045
4	**AHTh171-A**	540	4	2.142	0.524	0.533	0.017
5	**AHTh260**	540	5	3.052	0.698	0.672	-0.038
6	**AHTk211**	540	4	3.211	0.689	0.689	-0.000
7	**AHTk253**	540	4	2.976	0.669	0.664	-0.007
8	**C22.279**	540	4	2.763	0.622	0.638	0.025
9	**FH2001**	540	5	2.312	0.581	0.568	-0.025
10	**FH2054**	540	6	3.153	0.687	0.683	-0.006
11	**FH2848**	540	4	2.644	0.648	0.622	-0.042
12	**INRA21**	540	3	1.136	0.111	0.120	0.072
13	**INU005**	540	5	2.204	0.546	0.546	0.000
14	**INU030**	540	5	2.040	0.500	0.510	0.019
15	**INU055**	540	4	1.809	0.430	0.447	0.039
16	**LEI004**	540	4	2.063	0.504	0.515	0.023
17	**REN105L03**	540	5	2.189	0.530	0.543	0.025
18	**REN162C04**	540	3	1.961	0.485	0.490	0.010
19	**REN169D01**	540	6	2.791	0.639	0.642	0.004

20	**REN169O18**	540	5	1.767	0.420	0.434	0.032
21	**REN247M23**	540	4	2.290	0.515	0.563	0.086
22	**REN54P11**	540	4	2.009	0.530	0.502	-0.054
23	**REN64E19**	540	5	2.917	0.667	0.657	-0.014
24	**VGL0760**	540	9	4.785	0.800	0.791	-0.011
25	**VGL0910**	540	8	2.579	0.604	0.612	0.014
26	**VGL1063**	540	5	1.246	0.181	0.197	0.081
27	**VGL1165**	540	10	4.163	0.748	0.760	0.015
28	**VGL1828**	540	7	2.443	0.607	0.591	-0.028
29	**VGL2009**	540	6	3.672	0.731	0.728	-0.005
30	**VGL2409**	540	6	1.207	0.169	0.171	0.017
31	**VGL2918**	540	7	2.401	0.609	0.584	-0.044
32	**VGL3008**	540	9	3.251	0.687	0.692	0.008
33	**VGL3235**	540	6	1.830	0.456	0.454	-0.004

2. Genetic assessment of the population as a whole by combining allele and allele frequency data from all 33 STR loci

Allele frequencies across all 33 STR loci 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 the initial 370 AKK tested had a mean Na of 4.788 alleles per locus and a mean Ne of 2.620 alleles per locus. Therefore, only about one-half of the alleles across all 33 STR loci were contributing to the bulk of genetic diversity for the dogs tested. Mean values for Ho and He were also calculated using allele frequency data from all 33 STRs. A mean Ho of 0.573 for the 370 AKK tested was somewhat low, similar to a breed such as the Akita, which also started from a small founder populations and is therefore less genetically diverse than breeds such as the Standard Poodle and Miniature Poodle. The mean He of 0.573 for the population was nearly identical to the Ho, giving an F value of zero. This meant that the AKK population as a whole was in HWE, i.e, breeders have been doing an excellent job of avoiding inbreeding.

Table 3: Genetic assessment based allele frequencies at 33 STRs for the population as a whole.

	N	Na	Ne	Ho	He	F
Mean	540	5.485	2.650	0.573	0.572	0.003
SE		0.294	0.162	0.029	0.028	0.006

3. 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. An optimized two dimensional graph portrays the degree of genetic differentiation between the 460 AKK tested. The more distant two points (dogs) are from each other, the greater the genetic differences and vice versa. This analysis shows that the 460 AKK belong to a single breed (populations). The bulk of the 460 dogs cluster around the intersection of the two coordinates, with relative few genetic outliers. This indicates that most individuals in the breed are closely related. The tightness of the cluster is somewhat unusual, given the phenotypic diversity of the breed. This indicates that the genes responsible for differences in the phenotype are widely distributed within and between individual AKK.

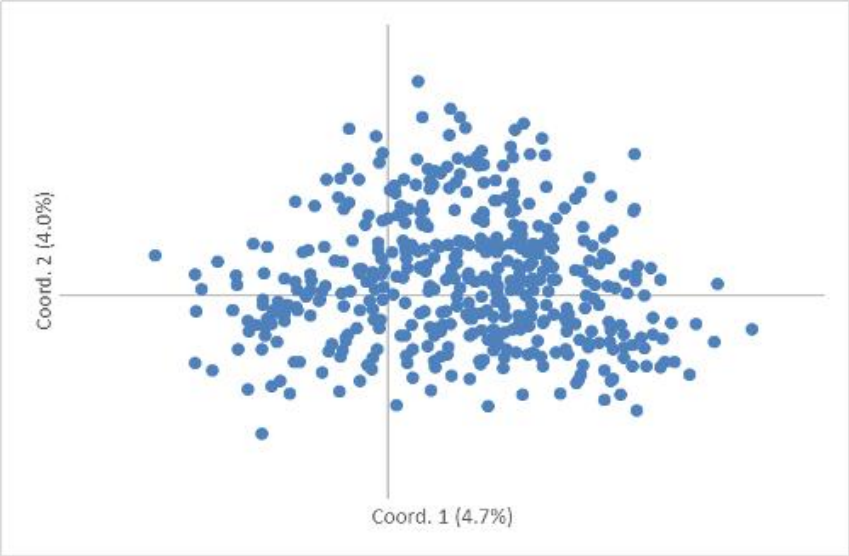


Figure 1: A principal coordinate analysis (PCoA) of allele frequency data from 33 genomic STRs and 460 Alaskan Klee Kai dogs

B. The use of genomic allele frequencies to determine internal relatedness values

1. A comparison of internal relatedness of individuals in a population

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 (identical alleles at a locus) and gives 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 received totally different alleles from both parents at each of the 33 STR loci (i.e., parents are totally unrelated), while a dog with an IR value of +1.0 has parents that contributed identical alleles at every locus (i.e., these parents were extremely related).

The red line in figure 1 graphs the frequency of IR scores for 460 AKK. The peak value for the population is around -0.1, with very few individuals having IR scores greater than 0.20. An IR score of 0.25 would be the mean value observed in a litter of puppies born to full sibling parents that came from a normal randomly breeding cross-section of dogs. Values increasingly greater than 0.25 would only occur in situations where the full sibling parents were from populations that were not randomly bred, i.e., progressively more inbred. The relatively low peak IR values for the AKK are another indication that their parents are genetically different from each other.

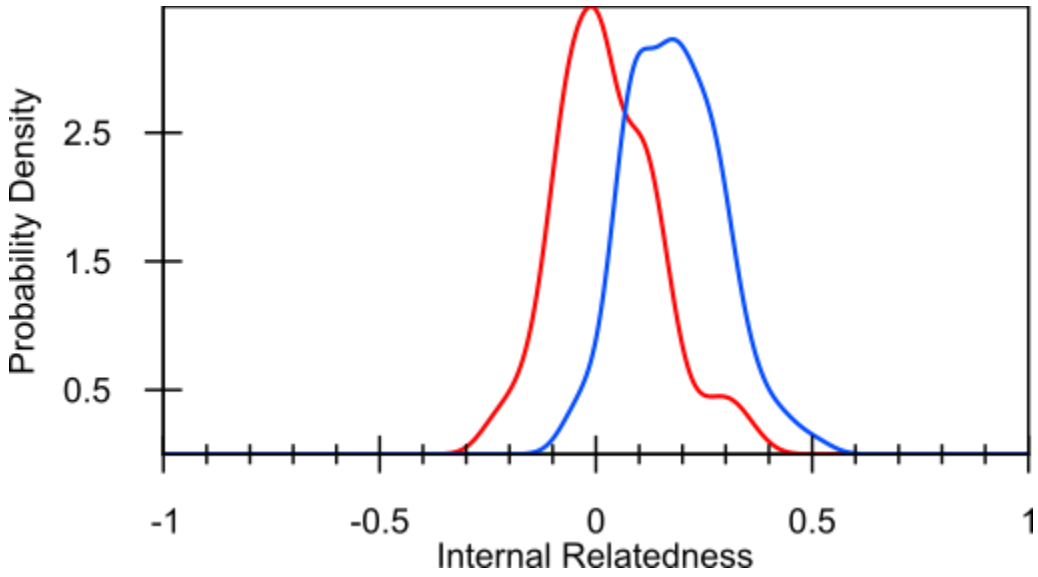


Figure 2: Distribution of IR estimated in Alaska Klee Kai (n=460) based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). Lost diversity was determined by comparing allele frequencies at the same loci between Alaska Klee Kai and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied

2. 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 AKK are shown in Fig. 2 (blue line). The mean IRVD is around 0.30 for the AKK with individual IRVD values ranging from 0.00 to 0.50. The shift of the peak to the right in IRVD compared to IR values was pronounced and the bulk of AKK would now be considered products of full-sibling matings if they were village dogs. Therefore, it is obvious that the breed has lost considerable genetic diversity as they evolved from their village dog

predecessors to modern day AKK. However, this loss of diversity is not dissimilar to other breeds with small founder populations, such as the Akita or breeds with small numbers and less phenotypic diversity such as the Black Russian Terrier.

C. DLA Class I and II Haplotype frequencies

1. DLA class I and II haplotypes and 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.

We have identified 11 different STR-associated DLA Class I (Table 4) and 8 DLA Class II (Table 5) haplotypes among 365 AKK. All of these haplotypes are shared with other breeds of dogs. The number of DLA class I and II haplotypes is low compared to larger breeds such as the Standard and Miniature Poodles and Italian Greyhound, but are more typical of breeds that started with a small founder population such as the Akita, Biewer Terrier and Black Russian Terrier. There is an imbalance in the frequency of certain DLA class I and II haplotypes. The 1014 and 1040 class I haplotypes and the 2021, 2037 and 2043 class II haplotypes make 50-70% of the available haplotypes. Evidence obtained from other genetic assessments using existing DLA class I and II haplotype frequencies (Table 6) suggest that this imbalance was created at the level of the initial founders and at a time that required extensive inbreeding. However, after an initial period of inbreeding, mate selection from within the founder population eventually became quite random. Therefore, these particular haplotypes are ancestral to the breed, and it would be interesting to see if any of the heritable deleterious traits can be associated with these haplotypes.

Tables 4 & 5: DLA Class I & II Haplotype Frequencies

DLA Class I Haplotype Frequencies (Updated Oct 8, 2019)		
DLA1 #	**STR types**	**Alaskan Klee Kai (n=540)**
1008	386 373 289 182	0.0611
1011	376 365 281 180	0.0565
1014	375 373 287 178	0.3731
1040	380 371 277 186	0.2157
1060	380 365 277 183	0.0074
1061	380 365 281 183	0.0435
1062	382 371 277 183	0.2157
1063	382 373 289 182	0.0102
1064	389 375 293 180	0.0139
1072	376 365 277 183	0.0019
1113	382 371 289 182	0.0009
DLA Class II Haplotype Frequencies (Updated Oct 8, 2019)		
DLA2 #	**STR types**	**Alaskan Klee Kai (n=540)**
2007	351 327 280	0.0139
2012	345 322 280	0.0602
2014	339 322 284	0.0722
2021	339 324 268	0.2139
2037	341 327 280	0.3731
2042	341 324 286	0.0435
2043	343 324 296	0.2231

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

Heterozygosity within the DLA region 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 (Tables 4, 5), in the same manner as employed with the 33 genomic STR loci. As for genomic STR, low numbers of alleles (or haplotypes in this case) at each STR locus decreases diversity, but it does not necessarily affect heterozygosity. Although the strong linkage that occurs between the 200+ genes and numerous alleles makes heterozygosity assessments with haplotype frequencies less accurate than for genomic STR alleles, they do provide a measure of heterozygosity within the DLA region between individual AKK.

Table 6 shows the standard genetic assessment values using alleles found at each of the 7 STR loci that correlate with the regions of the DLA containing the class I and II genes. There is a

tendency for Ne to be closer to Na, suggesting that there is a more balanced usage of DLA alleles. The observed heterozygosity (Ho) is reasonably high (0.655), but the expected heterozygosity is even higher (0.680). This means that alleles in the seven DLA class I and II STR loci are not as close to HWE as were the alleles for the 33 genomic STR loci. This is reflected by a slightly positive F value of 0.025. However, this value is very near to zero, and it appears that the DLA is reasonably heterogenic.

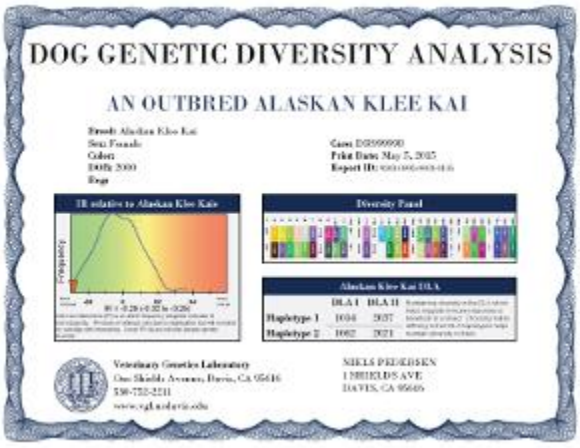
Table 6: Standard genetic assessment of the DLA regions using 7 STRs associated with the DLA class I and II regions.

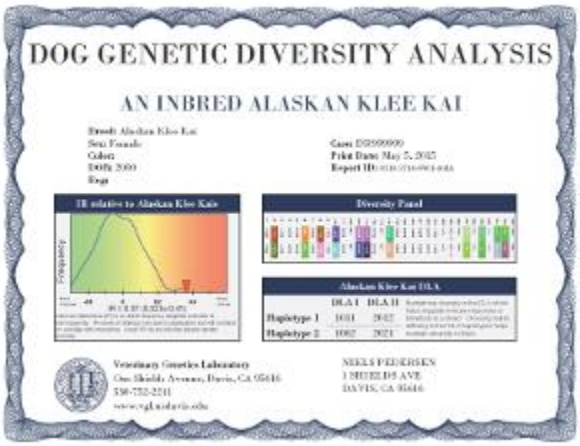
	N	Na	Ne	Ho	He	F
Mean	540	4.714	3.126	0.659	0.672	0.021
SE		0.333	0.179	0.022	0.019	0.006

D. How will you be given the results of DNA-based genetic diversity testing on your dog and how to use it.

1. Certificates listing basic genetic information in pictorial format

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 beingtested is related to the population as a whole.





2. What should you do with this information?

The AKK is a breed that started with a single breeder and gained a personal following. This following has eventually led to breed recognition and a growing public acceptance. Like other such breeds, genetic diversity started at a low level, but due to the diligence of breeders, that existing diversity has been well maintained through random mate selection. The low level of diversity, both in the genome and in the DLA class I and II regions makes it more difficult to increase breed-wide diversity from within. Nevertheless, DNA testing will identify parents that will yield the most diverse puppies that are possible. Mates should be selected to avoid homozygosity at any genomic loci or DLA class I and II

haplotype whenever possible. IR values based on the 33 STR loci, coupled with knowledge of DLA haplotypes, can be used to assist in optimum mate selection. 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.

E. Health problems in AKK that are of a heritable nature

Limited genetic diversity does not necessarily equate with ill-health, although it certainly makes it more difficult to manage heritable disorders when they start to reach troublesome levels. It is possible to start a breed with a small number of founders, providing that those founders are free of heritable disease traits, either of a complex (polygenic) or simple (usually recessive mutation) genetic basis. The problem is that many of the heritable traits identified in dogs are of a complex nature and are ancestral in even random bred dog populations. Pure breeds are created from random bred populations of dogs that often inhabit the regions from which they evolved. Therefore, they inevitably inherit by descent many of these heritable ancestral disorders, and it is subsequent inbreeding that causes them to be more than an occasional occurrence.

Unfortunately, it does not appear that AKK are free from health problems. As expected, many of these health problems are not unique to AKK, but rather result from heritable traits that are ancestral in dogs and have been inherited by descent from distant ancestors of most modern pure breeds. These deleterious heritable traits occur at relatively low incidence even in genetically diverse populations of dogs but are concentrated by inbreeding. AKK, like many small pure breed dogs, suffer inordinately from hepato-portal shunts, cryptorchidism, umbilical hernias and patellar luxation. Patent ductus arteriosus is a congenital disorder of the heart and is more commonly seen in small breeds. Some congenital heart problems such as mitral valve stenosis are more common in larger dogs. Pyometra is reportedly also problem in the breed.

Several genetic disorders that involve simple mutations, usually of a recessive nature, have been recognized in AKK. These also appear to have been acquired by descent from founding dogs. Severe pancreatitis occurs in AKK, as it does with Yorkshire Terriers and Schnauzers, and may be related to genetic problems such as post-prandial hyperlipidemia, which may involve a single gene mutation. Factor VII deficiency, a form of hemophilia, has been recognized in the breed and is due to a simple genetic mutation that is also common in dogs.

Two conditions linked to the immune system are increasingly recognized in AKK. Autoimmune thyroiditis leading to hypothyroidism is usually the first and most common autoimmune disorders associated with excessive inbreeding. Allergies are related to autoimmunity and often increase in incidence with that of autoimmune disorders. Both conditions are related to a loss of genetic diversity, especially in the immune system, and affect the ability of an individual to differentiate what is self from non-self (i.e., reacting to the thyroid gland as if it were foreign). Skin, food, and respiratory allergies occur when the immune system has trouble differentiating common parasites from things like pollen grains, molds and common house mites, or more ancient constituents of a dog's diet from modern ones.

The usual solution for both complex and simple heritable diseases is to avoid them in the first place. However, there are no genetic tests for complex genetic traits and there are consequences of trying to eliminate simple recessive genes from a population where genetic diversity is low and the incidence of carriers is high. Elimination of the deleterious gene can cause a considerable loss of existing diversity and potentially increase the incidence of heritable problems. This situation is seen in Pug dogs and necrotizing meningoencephalitis (Pug dog encephalitis or PDE). Elimination of the high risk region of DLA class II would eliminate up to one-third of the genetic diversity in a breed already lacking diversity. The highest incidence of PDE is in individuals carrying two identical copies of the risk haplotype, while the risk is much lower for individuals with only one copy. Therefore, Pug dog breeders can greatly reduce risk of PDE by selecting only for heterozygotes, which does not cause a loss of genetic diversity. If avoiding homozygosity whenever possible, at either genomic STR loci or at DLA class II haplotypes, will reduce disease problems in the breed to acceptable levels, this would be the best possible outcome. However, if genetic diversity needs to be increased, AKK breeders would need to consider a careful program of outcrossing and backcrossing.