Genetic Diversity Testing for Shiba Inu

Overview

The Veterinary Genetics Laboratory (VGL), in collaboration with Dr. Niels C. Pedersen and staff, has developed a panel of 40 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, rebalance or increase genetic diversity of their breed.

Genetic diversity testing of Shiba Inu is now in the preliminary results phase. During this phase, we will continue to test more registered dogs to provide breeders with an accurate assessment of genetic diversity in their breed. We are accepting Shiba Inu from all regions of the world. For this report we have tested 81 Shiba Inu originating from Japan and other countries, which should be adequate to determine 90% or more of existing genetic diversity. Additional dogs are tested the tables will be updated and genetic measurements re-calculated. The goal is to identify all existing diversity and to create a large DNA library for any future studies of Shiba Inu.

Results reported as:

Short tandem repeat (STR) loci: A total of 33 STR loci from across a representative portion of 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: Seven 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

The Shiba Inu is one of several spitz-type dogs originating in Japan around 300 BC and considered one of the world's oldest breeds [1-6]. The Akita Inu and Tosa Inu are the largest

native breeds, the Kishu Ken, Hokkaido, Kai Ken, Shikoku Inu are medium sized, while Shiba Inu are the smallest. Males are 14.5-16.5 inches (avg. 23 lbs) at the shoulder and females 13.5-15.5 inches (17 lbs). The Shiba Inu was bred to hunt and flush game such as birds and rabbit in areas of the Chūbu region, where their smaller size and agility were an advantage. Inu is the Japanese word for dog, but the origin of the prefix "Shiba" is less clear. The word shiba means "brushwood" in Japanese and refers to a type of tree or shrub whose leaves turn red in the fall [1-5]. The red coat common in Shiba Inu is like that of the shrubs. However, shiba also can mean "small", referring to the dog's diminutive stature compared to similar breeds. Therefore, Shiba Inu has also been translated to mean "Little Brushwood Dog".

Although Shiba Inu resemble several other Japanese Spitz-like breeds, they are assumed to be genetically distinct. Whether they are still the same as their ancient ancestors is another question. Western dog breeds were imported following the Meiji Restoration starting in 1868 and crosses with native Japanese breeds became increasingly popular in the following decades [1,2]. Almost no pure Shiba Inu remained by 1926 prompting Japanese lovers of this and other indigenous breeds to become active in their preservation. The first Japanese breed standard for the Shiba Inu was created by Nihon Ken Hozonkai, The Association for the Preservation of the Japanese Dog (Nippo) in 1934 [3]. The Shiba Inu was recognized as a Natural Monument of Japan through the Cultural Properties Act of December 1936 through the efforts of Nippo. It is one of 9 monument breeds of Japan [4].

Efforts to save the Shiba Inu were hampered during and after WWII by food shortages and a widespread canine distemper epizootic in the 1950s. Remnant dogs of three bloodlines survived this period [1-4]: 1) Shinshu Shiba from Nagano Prefecture, 2) Mino Shiba from the former Mino Province in the south of present-day Gifu Prefecture, and 3) San'in Shiba from Tottori and Shimane Prefectures. Survivors from these three lines differed somewhat in their phenotype. The Shinshu Shibas had thick double coats and were small and red in color. The Mino Shibas possessed pricked ears and a sickle-shaped rather than curved tail. The San'in Shibas were larger than contemporary shibas and were often solid black without the tan and white accents of contemporary Shiba Inu. Interbreeding of these three lines created the modern breed.

The first Shiba Inu were brought to the United States In 1954 by an armed forces family with more imports in following in the 1970s [4]. However, the first litter was not born in the US until 1979. The breed was recognized by the American Kennel Club in 1992 and added to the AKC Non-Sporting Group in 1993 [1,4]. The Shiba Inu was ranked 45/190 AKC breeds in 2017, while the Akita was ranked 47th [7]. The ranking of both breeds has remained relatively steady from 2013 to 2017. The Akita Inu, Shiba Inu, Shikoku Inu and Kai Ken are popular pure breeds of dogs in Japan, with the Shiba Inu being number one [8].

II. Genetic diversity studies of contemporary Shiba Inu

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

STR markers are highly polymorphic and have great power to determine genetic differences among individuals and breeds. The routine test panel contains 33 STRs consisting of those that are recommended for universal parentage determination for domestic dogs by the International

Society of Animal Genetics (ISAG) and additional markers developed by the VGL for forensic purposes. Each of these STR loci is known to contain from 7 to 27 different alleles when tested across many breeds of dogs. Each breed, having evolved from a small number of founders and having been exposed to artificial genetic bottlenecks will end up with only a portion of the total available diversity. Artificial genetic bottlenecks include such things as popular sire effects, geographic isolation, catastrophes, outbreaks of disease, and ups and downs in popularity and resulting increases and decreases in population size. The alleles identified at each of the 33 STR loci and their relative frequencies were determined for 81 Shiba Inu mainly from the USA and listed in Table 1.

Table 1. Allele designation and frequency at 33 STR loci for Shiba Inu. The allele that occurs at the highest frequency at each locus is highlighted.

Table 1 Link

B. Assessment of population heterozygosity using standard genetic parameters

Allele and allele frequencies were used to determine basic genetic parameters such as the number of alleles found at each STR locus (Na); the number of effective alleles (Ne) per locus; the observed or actual heterozygosity (Ho) that was found; and the heterozygosity that would be expected (He) if the existing population was in Hardy Weinberg equilibrium (HWE). HWE is achieved when the selection of mates is entirely random and subject to no positive or negative human selection pressure. The value F is a coefficient of inbreeding derived from the Ho and He values. A value of +1.0 would occur only if every individual were genetically indistinguishable at each of the 33 STR loci, while a value of -1.0 would be seen when all the dogs were completely different at each of the 33 loci.

The allele frequency data obtained from the 33 STR panels can also be used to assess heterozygosity within a population (Table 2). Using the 33-marker panel, the 81 Shiba Inu had an average of 6.879 alleles/loci (Na). However, the average number of alleles is less important than the number of alleles that have the greatest genetic influence on heterozygosity, a figure known as average effective alleles/loci or Ne. The Ne in this group of dogs averaged 3.432 effective alleles per locus, indicating that a most of the heterozygosity was determined by one-half of the alleles. The observed heterozygosity (Ho) across the 81 Shiba Inu was 0.658, while the expected heterozygosity (He) was 0.662. These values indicate that the population tested was randomly breeding. A calculation based on 1-He/Ho yieldied a measure of inbreeding (F) of 0.006. An F value of essentially zero also indicated that the study population was in Hardy-Weinberg (HWE) equilibrium, i.e., sires and dams were as unrelated as possible.

Table 2. Standard Genetic assessment (mean plus standard error) for Shiba Inu using allele frequencies of 33 STR loci (Updated October 09, 2019)

	N	Na	Ne	Ho	He	F
Mean	105	6.939	3.447	0.657	0.665	0.013
SE		0.318	0.210	0.025	0.025	0.008

C. Standard genetic assessment values for individual STR loci

The allele frequencies can be also used to do a standard genetic assessment of heterozygosity at each STR locus (Table 3). This provides an estimate of heterogeneity in in various regions of the genome that are in linkage with alleles at each STR marker. Higher values for observed heterozygosity (Ho) reflect more alleles (and more genetic diversity) at each locus, while low levels usually indicate a small level of heterozygosity. STR loci that have more than expected heterozygosity (He) will be under some positive non-random selection, while loci with less than expected heterozygosity will be under somewhat negative non-random selection. The number of alleles (Na) for an individual STR locus for this population of 81 Shiba Inu ranged from a low of 3 to a high of 11 alleles per locus, while the Ne ranged from 1.41 to 6.54 alleles per locus. The observed heterozygosity (Ho) for an individual STR locus ranged from 0.296 to 0.840, while He ranged from 0.289 to 0.847 (Table 3). Areas of the genome with less heterozygosity (or more homozygosity) are the most conserved and therefore likely associated with phenotypic traits that are strongly linked to the breed standard and shared by all individuals of the breed. Loci with high heterozygosity were more likely to be associated with subtle and variable phenotypic traits between individuals. The Ho and He values tended to be very close to each other, making the inbreeding coefficient (F) close to zero at most loci. The seventeen 33 STR loci with slightly positive F values were balanced by 16 loci that had slightly negative F values. These results indicated that each region of the genome represented by one of the 33 STR loci for these 81 dogs was under random selection.

Table 3. Standard Genetic Assessment for Shiba Inu using 33 STR loci (Updated October 09, 2019)

#	Locus	N	Na	Ne	Ho	He	F
1	**AHT121**	105	7	3.305	0.648	0.697	0.071
2	**AHT137**	105	7	6.512	0.829	0.846	0.021
3	**AHTH130**	105	8	1.408	0.276	0.290	0.047
4	**AHTh171-A**	105	6	1.453	0.333	0.312	-0.069
5	**AHTh260**	105	6	2.655	0.581	0.623	0.068
6	**AHTk211**	105	5	3.965	0.781	0.748	-0.044
7	**AHTk253**	105	6	4.379	0.781	0.772	-0.012
8	**C22.279**	105	8	5.029	0.819	0.801	-0.022
9	**FH2001**	105	8	4.261	0.771	0.765	-0.008
10	**FH2054**	105	7	3.072	0.648	0.675	0.040
11	**FH2848**	105	5	2.030	0.514	0.507	-0.014
12	**INRA21**	105	4	1.533	0.352	0.348	-0.013
13	**INU005**	105	5	3.476	0.752	0.712	-0.056
14	**INU030**	105	6	2.630	0.657	0.620	-0.060
15	**INU055**	105	6	3.942	0.743	0.746	0.005
16	**LEI004**	105	3	2.473	0.571	0.596	0.041

17	**REN105L03**	105	8	2.539	0.590	0.606	0.026
18	**REN162C04**	105	6	2.969	0.638	0.663	0.038
19	**REN169D01**	105	5	4.171	0.790	0.760	-0.040
20	**REN169O18**	105	5	3.212	0.648	0.689	0.060
21	**REN247M23**	105	5	2.556	0.543	0.609	0.108
22	**REN54P11**	105	8	5.466	0.762	0.817	0.067
23	**REN64E19**	105	9	4.829	0.838	0.793	-0.057
24	**VGL0760**	105	10	2.727	0.610	0.633	0.038
25	**VGL0910**	105	6	2.899	0.667	0.655	-0.018
26	**VGL1063**	105	9	1.857	0.438	0.461	0.051
27	**VGL1165**	105	9	3.824	0.752	0.739	-0.019
28	**VGL1828**	105	10	4.464	0.705	0.776	0.092
29	**VGL2009**	105	7	2.573	0.552	0.611	0.096
30	**VGL2409**	105	8	3.968	0.743	0.748	0.007
31	**VGL2918**	105	11	4.756	0.810	0.790	-0.025
32	**VGL3008**	105	8	4.115	0.733	0.757	0.031
33	**VGL3235**	105	8	4.717	0.790	0.788	-0.003

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

PCoA is a graphic portrayal of how closely individual dogs within a breed are related to each other (Fig. 1). The closer two dots (individual dogs) are to each other in the graph, the closer the two dogs are related. However, the actual plot is multi-dimensional (i.e., a sphere) and the graph shows the two planes (i.e., coordinates 1 and 2) of the sphere that most accurately portray existing relatedness in a two-dimensional manner. The population of 81 Shiba Inu form a single breed, but there is a tendency not to form a tight cluster, as would be seen if all the individuals were closely related. This tendency for individuals not to form a tight cluster is an indication of heterogeneity within the group of dogs tested. The fact that the two optimum coordinates only defined 10.9% of the individuals indicates that the 81 dogs were also dispersed in their spherical representation.

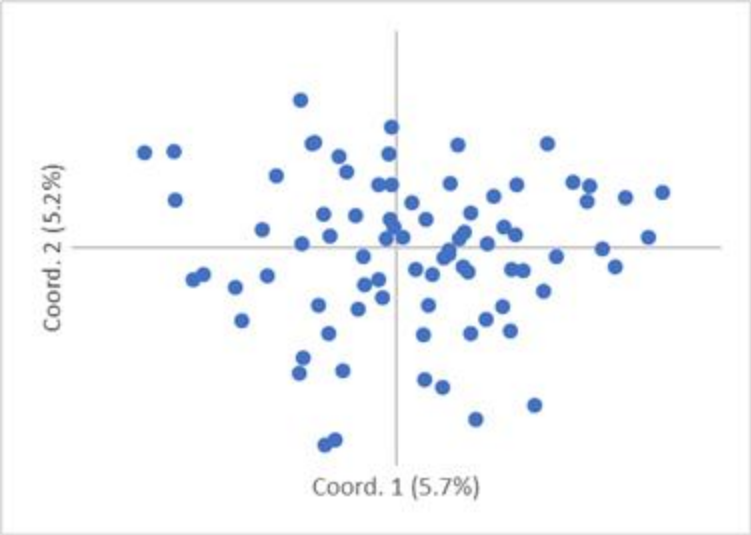

Fig. 1. PCoA of Shiba Inu (n=81) based on the 33 STRs

Although there is a tendency for individuals to disperse more widely in the graph, there is no evidence from the 81 dogs tested for the existence of subpopulations (bloodlines, varieties, geographic differences). The question is how much genetic difference must occur for a distinct bloodline or variety to become so distinct as to classify as a breed. The answer is "quite a bit." This can be demonstrated by doing a PCoA plot between three Japanese monument breeds (Fig. 3). There is clear segregation between the two varieties of Akita and Shiba Inu, although AKA and AKJ are much more closely related to each other than to Shiba Inu. Therefore, Shiba Inu are genetically distinct from the two varieties of Akita, as would be predicted by their significant phenotypic (and hence, genotypic) differences and breed histories. The Shiba Inu population in this comparison PCoA are also much more tightly clustered in this comparison than shown in Fig. 2. Such comparisons between genetically distinct breeds and varieties tend to minimize minor intra-population differences.

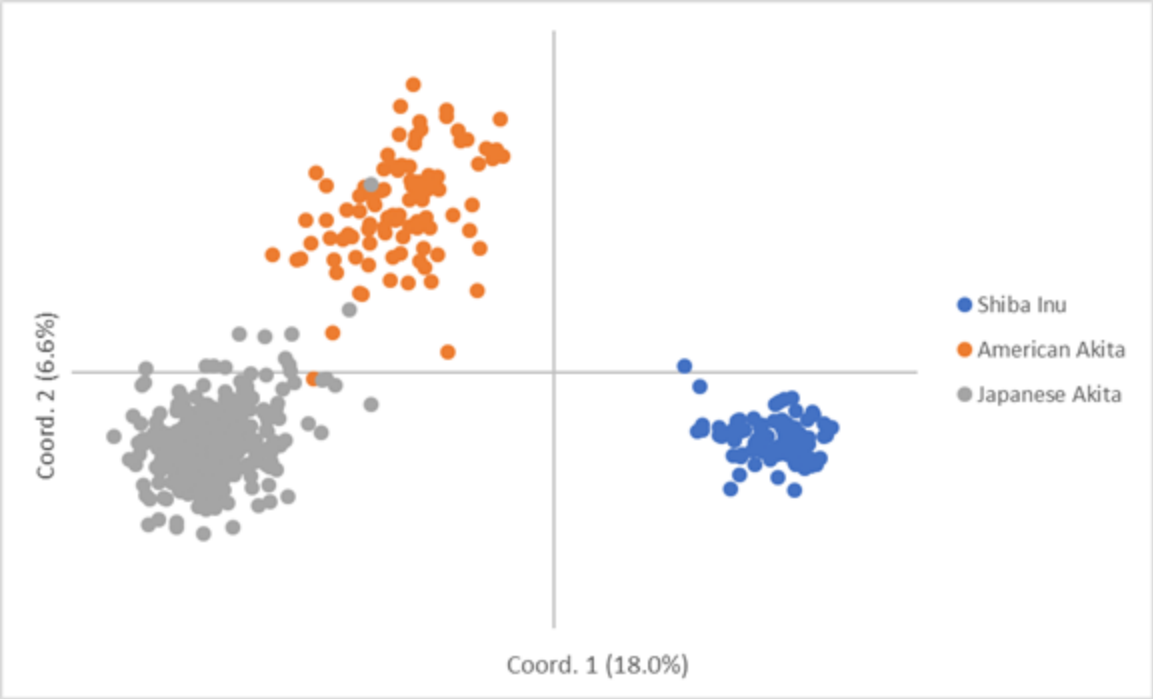

Fig. 2. PCoA of Shiba Inu (n=81), American Akita (n=97), and Japanese Akita (n=320) based on the 33 STRs. AKJ and AKA are genetically distinguishable but much more closely related to each other than to Shiba Inu.

E. Internal relatedness (IR) of individuals and the population as a whole

1. IR testing

Genetic assessments such as those presented in Tables 1-3 are indicators of breed-wide (average) heterozygosity and do not reflect the genetic contributions that each parent gives to an individual. Internal Relatedness (IR) is a calculation that has been used to determine the degree to which the two parents of an individual dog were related. 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 found among offspring of full sibling parents from a random breeding population. IR values >0.25 occur when the parents of the full sibling parents were themselves highly inbred. The higher the IR value above 0.25 the more closely related were the parents and grandparents of the siblings.

A graph comparing IR values for 81 Shiba Inu from around the world (Fig. 3– red line) confirms that the population tested varies greatly in the degree of parental relatedness with individuals scoring as high as +0.306 (most inbred) and as low as -0.254 (most outbred) (Table 4). One half of the dogs had IR scores equal to or greater than 0.008 and one fourth of the dogs had IR scores of 0.069 or greater. An IR score of 0.25 would be seen in puppies resulting from the mating of full siblings from a randomly breeding and genetically diverse population. Less than 10% of the

dogs tested would be inbred to this level. This small inbred group of dogs is balanced by an equal sized group of strongly outbred dogs with IR scores from -.068 to -0. 254. Therefore, IR scores provide a more accurate representation of heterozygosity in individual dogs than the breed-wide averages obtained from the standard genetic assessment.

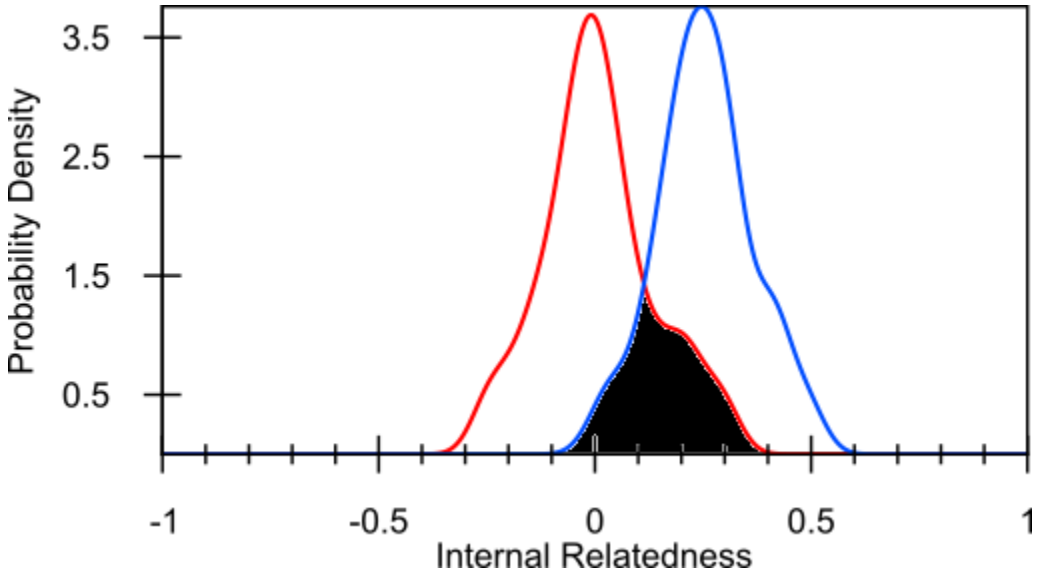

Fig. 3. Distribution of IR estimated in Shiba Inu (n=81) based on intra-breed diversity (red), compared with IR adjusted to diversity lost during breed development (blue). Lost diversity was determined by comparing allele frequencies at the same loci between Shiba Inu and village dogs from the Middle East, SE Asia, and the Islands Pacific. Village dogs were the most diverse population studied.

Table 4. Comparison of IR and IRVD values for 81 Shiba Inu

	IR	IRVD
Min	-0.254	-0.064
1st Qu	-0.068	0.125
Mean	0.008	0.226
Median	-0.006	0.219
3rd Qu	0.069	0.320
Max	0.306	0.511

2. Adjusted IR values (IRVD) as a measure of genetic diversity lost during breed evolution from time of origin to the present time.

All breeds start with a relatively small population of dogs and after their registries are closed there are no further introgressions from outside the breed. Therefore, the goal of breeders is to maintain the original amount of genetic diversity by strict adherence to the breed standard and random mate selection. Most breeds have accomplished this to some extent, as one study showed that contemporary pure breeds of dogs have retained an average of 87% of their original diversity. Genetic diversity may be subsequently lost through genetic bottlenecks such as natural disasters, loss of popularity, popular sire effects, etc. It is possible to obtain an estimate of how much of the original dog diversity a breed possesses by adjusting the alleles and their frequency

to the frequency of those same alleles in a large population of village dogs from across the Middle East, SE Asia and Island Pacific. This is possible because village dogs contain far more of the original genetic diversity of dogs than any current breed and almost all modern breeds trace their ancestry to dogs from these regions. The IR values and IR values adjusted to village dogs (IRVD) can then be graphed and the graphs overlaid. If the IRVD peak overlays the IR peak, the breed has the same diversity as village dogs. If the IRVD and IR peaks are only somewhat overlapping, very little of the original genetic diversity has been retained.

The IRVD curve was shifted well to the right of the IR curve, reflecting the loss of available genetic diversity during breed development (Fig. 3). The peak (median) of the IRVD curve was +0.219, with some dogs as high as +0.511 and as low as -0.064 (Table 4). One half of the Shiba Inu tested had IRVD scores equal or greater than 0.219 and one fourth had scores of 0.320 or greater. Therefore, over one-half of the Shiba Inu have IRVD values equivalent to an offspring of a full sibling parents from among random bred village dogs. The area under the overlapping IR and IRVD curves (black) are a rough estimate of how much of the village dog genetic diversity has been retained in contemporary Shiba Inu (29.8%). This level of retained genetic diversity is higher than breeds such as the Swedish Vallhund (7%) and Doberman Pinscher (15%), comparable to Shiloh shepherd (27%), Japanese Akita (24.4%), Samoyed (35%) and Flat Coated Retriever (35.2%), and lower than genetically diverse breeds such as Golden Retrievers (50%), Alaskan Klee Kai (50%), Labrador Retrievers (54%) and Toy Poodle (60%).

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

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 5). 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. However, there is enough distance between these two regions to allow for a degree of recombination resulting in unusual class I/II combinations. 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.

1. DLA class I and II haplotypes existing in Shiba Inu

The 81 Shiba Inu tested to date possessed 15 DLA class I and 16 DLA class II haplotypes (Table 5). The DLA class I haplotypes 1191-1198 and DLA-II 2067, 2105-2011 haplotypes are unique to date for Shiba Inu. The other DLA class I and II haplotypes are found in several other breeds. The frequencies of the 1054 (40.7%), 1091 (22.2%) class I haplotypes, and 2018 (19.8%), 2067 (21.0%) and 2106 (42%) class II haplotypes were disproportionately high, occurring in over 60% of the dogs tested. These haplotypes were likely present in the founder population and inherited by descent for the last 25 centuries because of their close association with a trait or traits strongly associated with the breed.

The fact that DLA class I and II haplotypes are in strong linkage disequilibrium means that they are inherited by descent over many generations in a largely unchanged form as a block, with one copy from each parent. The number of haplotypes that exist in a breed is therefore a measure of the number of founders in the population and of genetic diversity. The numbers (16/15) of DLA class I and II haplotypes found in these 81 Shiba Inu is higher than Swedish Vallund (6,4) and Shiloh shepherd (7, 6); similar to Giant Schnauzer (14, 15) and Samoyed (13,12); and lower than Golden Retriever (26,23) and Miniature Poodle (33, 23).

Table 5. DLA class I and Class II haplotype and their frequencies

DLA Class I Haplotype Frequencies (Updated Oct 9, 2019)		
DLA1 #	**STR types**	**Shiba Inu (n=104)**
1007	380 372 281 182	0.005
1054	382 379 277 184	0.375
1081	395 379 289 178	0.010
1091	381 371 277 181	0.221
1109	381 379 291 186	0.024
1133	378 365 287 172	0.005
1160	386 369 289 176	0.014
1191	388 373 260 186	0.212
1192	376 373 281 182	0.005
1193	382 383 277 184	0.005
1194	385 369 291 178	0.014
1195	388 373 289 181	0.019
1196	390 372 291 180	0.024
1197	390 373 289 186	0.005
1198	392 371 277 184	0.063
DLA Class II Haplotype Frequencies (Updated Oct 9, 2019)		
DLA2 #	**STR types**	**Shiba Inu (n=104)**
2001	343 324 284	0.005
2006	339 325 280	0.005

2015	339 327 280	0.063
2018	339 324 284	0.202
2032	339 323 280	0.010
2067	343 322 284	0.212
2083	339 324 282	0.010
2097	343 327 276	0.014
2098	343 323 282	0.014
2105	341 325 276	0.024
2106	341 325 286	0.385
2107	343 322 286	0.005
2108	343 324 294	0.019
2109	345 322 276	0.024
2110	347 325 268	0.005
2111	351 324 276	0.005

2. Relatedness to other breeds based on DLA class I and II haplotypes

The DLA class I and II haplotypes are in strong linkage disequilibrium, less subject to recombination, and are inherited as extended haplotypes from each parent. Therefore, they can be useful in looking at how breeds might be related and at the founder population. Table 6 lists several breeds that share DLA class I and II haplotypes with the Shiba Inu. The major DLA class I 1054 is shared with the Labrador Retriever, Magyar Agar, Flat Coated Retriever and Havanese. The 1081 haplotype is present at high incidence in both Akita (AKJ and AKA) and Shiba Inu, while the 1091 haplotype is a major haplotype in both Shiba Inu and Black Russian Terrier. The 2018 DLA class II haplotype is also shared with the Flat Coated Retriever and the 2067 haplotype is found in 1% of Miniature Poodles. There is also minor haplotype sharing among several other breeds (Table 6).

DLA Class I Haplotype Frequencies (Updated Sep 24, 2018)

DLA1 #	STR types	Black Russian Terrier (n=120)	Labrador Retriever (n=136)	Magyar Agar (n=42)	American Akita (n=87)	Japanese Akita (n=320)	Blend Akita (n=53)	Unknown Akita (n=4)	Golden Retriever (n=690)	Doberman Pinscher (n=476)	Flat Coated Retriever (n=409)	Havanese (n=389)	Samoyed (n=187)	Miniature Poodle (n=250)	Poodle (n=2299)	Shiba Inu (n=81)	Giant Schnauzer (n=179)	Polish Lowland Sheepdog (n=16)	Biewer (n=105)	Biewer Yorshire Terrier (n=53)	Yorkshire Terrier (n=16)	Biro Biewer (n=3)	Italian Greyhound (n=711)	Toy Poodle (n=111)
1007	380 372 281 182	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0322	0.006	--	--	0.005	0.009	0.03	--	--	--
1054	382 379 277 184	--	0.081	0.13	--	--	--	--	--	--	0.121	0.117	--	0.002	--	0.407	0.006	--	--	--	--	--	0.0162	--
1081	395 379 289 178	--	--	--	0.379	0.152	0.274	0.3	--	--	--	--	--	--	--	0.012	--	--	--	--	--	--	--	--
1091	381 371 277 181	0.508	--	--	--	--	--	--	--	0.001	--	--	--	--	--	0.222	0.053	--	--	--	--	--	--	--
1109	381 379 291 186	--	--	--	--	--	--	--	--	--	--	--	--	0.014	--	0.019	--	--	--	--	--	--	--	0.05
1133	378 365 287 172	--	--	--	--	--	--	--	--	--	--	0.022	--	--	--	0.006	--	--	--	--	--	--	--	--
1160	386 369 289 176	0.029	--	--	0.006	--	--	--	--	--	--	--	0.016	--	--	0.012	--	--	--	--	--	--	--	--
1191	388 373 260 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.21	--	--	--	--	--	--	--	--
1192	376 373 281 182	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--
1193	382 383 277 184	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--
1194	385 369 291 178	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.012	--	--	--	--	--	--	--	--
1195	388 373 289 181	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.012	--	--	--	--	--	--	--	--
1196	390 372 291 180	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.031	--	--	--	--	--	--	--	--
1197	390 373 289 186	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--
1198	392 371 277 184	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.031	--	--	--	--	--	--	--	--

DLA Class II Haplotype Frequencies (Updated Sep 24, 2018)

DLA2 #	STR types	Black Russian Terrier (n=120)	Labrador Retriever (n=136)	Magyar Agar (n=42)	American Akita (n=87)	Japanese Akita (n=320)	Blend Akita (n=53)	Unknown Akita (n=4)	Golden Retriever (n=690)	Doberman Pinscher (n=476)	Flat Coated Retriever (n=409)	Havanese (n=389)	Samoyed (n=187)	Miniature Poodle (n=250)	Poodle (n=2299)	Shiba Inu (n=81)	Giant Schnauzer (n=179)	Polish Lowland Sheepdog (n=16)	Biewer (n=105)	Biewer Yorshire Terrier (n=53)	Yorkshire Terrier (n=16)	Biro Biewer (n=3)	Italian Greyhound (n=711)	Toy Poodle (n=111)
2001	343 324 284	--	0.004	0.13	--	--	--	--	0.1406	--	--	0.04	--	0.016	0.605	0.006	0.008	--	--	--	--	--	--	0.005
2006	339 325 280	--	--	0.23	--	--	--	--	--	--	--	0.004	--	--	0.0322	0.006	0.156	--	--	--	--	--	--	--
2015	339 327 280	--	--	--	--	--	--	--	--	--	--	--	0.016	0.05	0.0063	0.031	--	--	--	--	--	--	0.0091	0.045
2018	339 324 284	--	--	--	--	--	--	--	--	--	0.149	0.021	--	--	0.0002	0.198	--	--	--	--	--	--	--	--
2032	339 323 280	0.083	--	--	--	--	--	--	--	--	--	0.03	--	0.014	--	0.012	--	--	--	--	--	--	0.0457	--
2067	343 322 284	--	--	--	--	--	--	--	--	--	--	--	--	0.01	--	0.21	--	--	--	--	--	--	--	--
2083	339 324 282	--	0.004	--	--	--	--	--	--	--	0.002	--	--	--	--	0.012	--	--	0.005	--	--	0.3	--	--
2097	343 327 276	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.012	--	--	--	--	--	--	--	--
2098	343 323 282	--	0.004	--	--	--	--	--	--	--	--	--	--	--	--	0.012	--	0.03	--	--	--	--	--	--
2105	341 325 276	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.031	--	--	--	--	--	--	--	--
2106	341 325 286	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.42	--	--	--	--	--	--	--	--
2107	343 322 286	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--
2108	343 324 294	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.012	--	--	--	--	--	--	--	--
2109	345 322 276	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.019	--	--	--	--	--	--	--	--
2110	347 325 268	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--
2111	351 324 276	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.006	--	--	--	--	--	--	--	--

Table 6. A comparison of recognized DLA class I and II haplotypes in several different breeds. There is considerable haplotype sharing between breeds, reflecting the common evolution of modern breeds from indigenous dog populations that were greatly expanded during the Neolithic period.

3. A genetic assessment of allele and allele frequencies of STRs associated with DLA I and II haplotypes

The DLA region is extremely important because of its role in immune regulation and the linkage of certain haplotypes to autoimmune disorders in dogs. The numerous genes in this region tend to be in strong linkage disequilibrium (LD) and are inherited as a block from sire and dam. This strong LD allows them to be under artificial selection pressures separate from other regions of the genome. However, the expectation is that DLA haplotypes will be inherited randomly. This can be tested by treating the 7 DLA loci and their alleles in the same manner as the 33 genomic STR markers. The most common DLA class I 1054 haplotype is made up of the alleles 382 379, 277 and 184, while the most common class II 2106 haplotype is made up of alleles 341 325 286 (Table 7). You will notice that some of the alleles for the DLA class I haplotypes are shared between haplotypes, such as 381, 379, 289, and 186. Table 8 provides a standard genetic assessment of heterozygosity among alleles of the seven DLA-associated STR loci. The average number of alleles for all loci was 7.143, but only 2.97 of these alleles affects most of the diversity. However, the observed and expected heterozygosity (Ho and He) values were similar (0.603 vs. 0.654) and yielded an inbreeding coefficient F of 0.077. This is higher than the F value for the 33 genomic markers (0.077 vs. 0.006, meaning that there is a small subpopulation of Shiba Inu are more inbred in the DLA region than in other parts of the genome covered by the 33

STR loci. This degree of positive artificial selection is most likely inadvertent, caused either by the relatively small number of dogs tested to date and/or the presence of a highly desired trait(s) in dogs possessing certain haplotypes.

Table 7. Standard Genetic Assessment for Shiba Inu using 7 STRs in the DLA region (Updated October 09, 2019)

#	Locus	N	Na	Ne	Ho	He	F
1	**DLA I-3CCA**	105	11	3.841	0.676	0.740	0.086
2	**DLA I-4ACA**	105	7	3.291	0.648	0.696	0.070
3	**DLA I-4BCT**	105	6	2.048	0.524	0.512	-0.024
4	**DLA1131**	105	8	3.235	0.686	0.691	0.007
5	**5ACA**	105	6	3.127	0.638	0.680	0.062
6	**5ACT**	105	5	3.360	0.648	0.702	0.078
7	**5BCA**	105	7	2.979	0.600	0.664	0.097

Table 8. Summary of Standard Genetic Assessment for Shiba Inu using 7 STRs in the DLA region (Updated October 09, 2019)

	N	Na	Ne	Ho	He	F
Mean	105	7.143	3.126	0.631	0.669	0.054
SE		0.683	0.191	0.019	0.026	0.016

III. Heritable health problems of Shiba Inu

Overall, the Shiba Inu is a healthy breed [5,6]. Their average life expectancy is from 12 to 15 years, typical for dogs of this size. However, a Shiba Inu named Pusuke died at the age of 26 in early December 2011 without any prior health problems, becoming the world's the longest living dog of his time and the 8th longest dog lifespan ever recorded [9]. Although this should not be taken as evidence that the breed is long lived or exceptionally healthy, Pusuke's life brought much positive attention to the breed.

1. Orthopedic problems

Orthopedic disorders are a problem in the breed. Like other smaller-sized breeds, Shiba Inu suffer from patellar luxation, varying in severity from grade 1(patella can be manually luxated with some exertion but readily returns to normal position) to grade 4 (permanent luxation that cannot be manually corrected) [6]. Most affected Shiba are grade 1. Hip dysplasia is a problem in the breed with 1998 Orthopedic Foundation of America (OFA) figures showing 79% of 889 dogs tested to have excellent (15%) or good (63.8%) hips, with 3.5% mildly dysplastic, 3.5% moderately dysplastic, and 0.6% severely dysplastic [6]. Joint problems usually become apparent by two years of age. Orthopedic problems of this type have a complex pattern of

heritability and polymorphisms predisposing to them occur in many breeds and have apparently existed in dogs for a long period of time and have been inherited by descent at some point in breed evolution.

2. Ocular disorders

Eye problems presumed to be of a heritable nature are seen in the breed [6]. A study of 553 Shiba done from 1991-97 by the Canine Eye Registry Foundation (CERF) found 17.9% of dogs to have one or more eye problems. Eye problems tended to occur somewhat more frequently in females and in dogs from 6 months to five years of age. Distichiasis is one of the most common eye problems in Shiba Inu world-wide. Persistent pupillary membranes and entropion are also seen in puppies.

Cataracts appearing around two years of age and often progressing to blindness are the most serious eye problem in the breed, occurring in 65/553 (11.8%) Shiba examined. Progressive retinal atrophy has been recognized in the breed but is uncommon (4/553 = 0.7%) and has been largely excluded from countries outside Japan by careful selection and testing of imports.

Glaucoma has also been listed as an important disease of Shiba Inu and can lead to blindness if not diagnosed and properly treated. The heritability of these conditions is not entirely known, but cataracts, glaucoma and progressive retinal atrophy are inherited as simple recessive mutations other breeds where they have been studied.

3. Allergies, autoimmunity, and miscellaneous problems

Food and inhalant allergies causing runny eyes, loss of hair on the face and intense itching around the muzzle, ears and between the toes have been recognized in the breed [6]. Pollen is a common cause and can be diagnosed by its seasonal occurrence. Indoor allergens such as dust mites tend to be year-around will improve when the dog is put outdoors. Shiba Inu are prone to hypothyroidism, a disorder of autoimmune origin, and common in many pure breeds.

Dental problems occur in Shiba as in many other breeds, especially of smaller size. Small dogs are more apt to have poorly aligned teeth and often do not exercise their teeth and gums by chewing bones and toys as larger dogs. Dental problems ultimately lead to gingival and periodontal disease and premature tooth loss.

Cancer occurs in Shiba Inu as it does in all pure breeds, but the incidence appears to be low compared to several breeds considered to be at high risk. These types of conditions are also of complex heritability and probably also acquired by descent from distant ancestors.

4. Simple autosomal recessive disorders

GM1 gangliosidosis (shiba inu type) is an autosomal recessive Lysosomal Storage Disorder that has been found mainly in Shiba Inu dogs from the Honshu districts and presumed to be from a popular sire effect. Affected dogs typically present with symptoms of neurologic disease around 5 to 6 months of age with clinical signs of vision loss, difficulties walking, loss of balance, head

tremors, lethargy and weight loss. Affected dogs usually die by 15 months of age. The disorder is caused by an autosomal recessive mutation of the GLB1 gene [12]. About 2.9% out of 68 Shiba Inu tested from northern Japan and 1.0% of 590 from across Japan carry the mutation. Based on these carrier rates, the actual disease incidence in Japanese dogs would be 61/659 x61/659 x 0.25= 0.09 x 0.09 x 0.25=0.002 or **2/1000** dogs.

IV. What does DNA-based testing tell us about contemporary Shiba Inu

The 81 Shiba Inu tested were of average genetic diversity compared to other breeds based on amount of IR/IRVD curve overlap and DLA haplotype numbers. However, this may increase somewhat with the testing of additional dogs. Dogs from Japan, as well as other regions of Asia, are also required to better define the total amount of genetic diversity that still exists in the breed worldwide. The Korean Jindo dog is phenotypically like the Shibu Inu [10] and although it is thought to have originated entirely in Korea, a recent study indicates a close genetic relationship with Chinese breeds [11]. Although some relationship to Akita would be expected, it was only apparent from a single shared DLA class I haplotype (1081) that was shared at high incidence only between AKJ/AKA and Shiba Inu.

Data on the number and incidence of specific DLA class I and II haplotypes indicated that three or four founder lines or individuals were strong contributors to contemporary genetic diversity. Some of these haplotypes were shared with several other breeds, as would be expected given the common origin of most breeds from village dogs of the Middle East and SE Asia. However, a several DLA class I and II haplotypes appear to be unique to Shiba Inu indicating a contribution from ancestors either no longer in the wild or not yet sampled. There was also evidence that certain DLA haplotypes were not in HWE because of inadvertent positive selection. This could be easily corrected as there are many low frequency haplotypes in the breed.

Heterozygosity appears to have been well maintained by Shiba Inu breeders based on population-wide averages. Only a small proportion of the individuals tested appeared to be offspring of closely related parents when tested by internal relatedness (IR) and these were balanced by a comparable population of dogs whose parents were more outbred than the population average.

The breed has not suffered as much from disorders associated with simple autosomal recessive mutations, although disorders such as progressive retinal atrophy and cataracts occur in the breed. These types of disorders have been closely linked with several different recessive mutations in other breeds and the low incidence of these diseases reflects the positive efforts of breeders to maintain genetic diversity and avoid bouts of inbreeding. This could change if the breed were to ever become extremely popular and demand for puppies exceed the ability of dedicated breeders to meet demand.

The major heritable disorders of Shiba Inu appear to be orthopedic in nature. Hip dysplasia is problem in the breed, which is often unexpected in smaller statured non-chondrodystrophic dogs. Patella luxation is a problem in many smaller statured breeds. Both conditions have a complex (polygenic) origin that most likely entered the breed through the original foundation animals. Dogs have been undergoing positive human-directed selection for thousands of years and this

selection has apparently allowed deleterious traits to slowly accumulate in the ancestral stock of modern breeds and be amplified by descent from specific founders. Dogs that have suffered hip dysplasia and/or patella luxation are more apt to pass on the genetic polymorphism that increase disease risk. Therefore, the best way to combat these types of disorders is to make sure that potential parents are free of clinical markers for such disorders.

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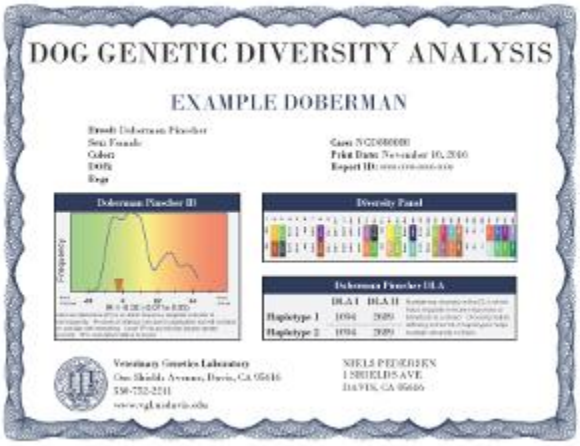
VI. Results of Diversity testing

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 related to the population as a whole.


DOG GENETIC DIVERSITY ANALYSIS

EXAMPLE DOBERMAN

Doberman Pinscher (I)

Diversity Panel

Doberman Pinscher DLA

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

Veterinary Genetics Laboratory
One Shields Avenue, Davis, CA 95616
530-752-2211

SHELS PEDERSEN
1 SHIELDS AVE
DAVIS, CA 95616

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 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.

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.