Genetic Diversity Testing for ISSA Shiloh Shepherds

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 assess 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.

Genetic diversity testing of Shiloh Shepherds is now in the preliminary results phase. During this phase, we continue to test more registered dogs to build genetic data necessary to provide breeders with an accurate assessment of genetic diversity in their breed. This report is based on testing of 86 Shiloh Shepherds from North America and Europe. Allele and DLA haplotype frequencies are updated based on the current population of dogs tested.

Price: $80.

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.

I. Introduction

The Shiloh Shepherd is a new breed of German shepherd-type dogs created in the early 1990's and recognized by the International Shiloh Shepherd Alliance (ISSA) and the International Shiloh Shepherd Registry (ISSR), among others. They are straight-backed and larger than most modern German shepherd dogs (GSD). They have been described as being lower in energy and drive, intelligent, and with even temperaments. There are two coat types within the breed, and

their coats can be a variety of colors and color mixes. Details of the breed's origins were provided by Anne Becker and summarized below. Detailed descriptions of popular sires from region to region, average litter sizes, population data through the years, etc. are also available from Anne Becker.

The origin of the breed is remarkably similar to that of many other old and contemporary breeds. Tina Barber and her Shiloh kennel began a GSD breeding program in the mid-70's. The resultant lines solidified into an early bottleneck of eight dogs which would later become the foundation for her "Ursa" line and include one-half of breed founders.

The bloodlines of all contemporary Shiloh shepherds can be traced to 20 founders through pedigrees registered with the ISSA. Sixteen of these dogs were directly or indirectly related to the Shiloh kennel. Sixteen of the founders were in use around or just slightly prior to the time of breed creation in 1990-1, and four were outcrosses added later. Two of these 16 founders, Sabrina and Samson-Woo, had other breeds mixed into their GSD stock. The remaining 14 dogs were purebred GSD from Tina M. Barber's Shiloh Kennels, all of them related to each other in some degree. Two of these 16 founders that were indirectly related were "Kari" and "Boeg." These two dogs are considered to be the true foundation of the Shiloh shepherd dog, as they were used extensively at the breed's founding. Two additional dogs among the 16, Sabrina and Samson-Woo, were lines that also had less in common pedigree-wise with other GSD from Shiloh kennels. The former was roughly 10% European flock guardian lines and the latter roughly 40% Malamute by pedigree. There has been persistent rumor of Samson-Woo being part wolf, but this is not supported by pedigree data. Samson was the dog who finally took the Shiloh line officially out of the AKC as purebred GSD in 1991 and the impetus for creating a new breed and breed specific registries.

The four subsequent outcrosses mentioned above and added later to the initial 16 founders included:

1). Artus (Altdeutscher Schaeferhund or ADS) utilized from 1999-2001. The ADS is mostly composed of long-haired GSD or GSD-derived herding stock. Six of Artus' offspring had progeny also contributed to the Shiloh Shepherd gene pool. Artus was added to improve confidence, sturdiness, working ability, good tails and genetic diversity.
2). Orbit, a Canadian White Shepherd from the Hoofprint kennel lines, utilized for one litter in 2001. Five of his progeny still exist among our current population. The White Shepherd is completely derived from the German shepherd dog. Orbit was introduced to gain size and for genetic diversity.
3). Chani was utilized in 2002 and then again in 2006. One of her progeny from the first match and two from the second match contributed to the gene pool of today. She was an outbred German shepherd dog from some of Tina M. Barber's rarer old Shiloh lines, which had stayed in the AKC instead of splitting with the Shilohs. She was introduced to regain a bit more size, and to add in diversity from lines and dogs that Tina had otherwise lost.
4). Vlcak (also Vilk, Vlack) was a Czechoslovaky Vlcak (Czech Wolfdog) utilized in the mid 2000's. The Czech Wolfdog descends from German shepherd dog lines which were mixed with wolves at the time of their breed creation in the 1950's. This outcross was added in primarily for health and diversity. This last outcross was utilized very little; only three F1 dogs went on and

from each of them only one breeding F2 progeny was retained for breeding. A few F3's and F4's from this line still exist.

The breed has a database that factors COI for thirty generations, well back into the ancestral German shepherd stock. A coefficient of inbreeding (COI) for every breeding dog within the existing gene pool has been calculated and the breed-wide COI averages 16.0%.

II. Genetic diversity studies of contemporary Shiloh Shepherds

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 86 Shiloh Shepherds from around the world and listed in Table 1.

Table 1: STR alleles from 33 genomic loci and their frequencies
Link to Table 1

B. Assessment of population diversity using standard genetic parameters

Allele and allele frequencies at each of the 33 STR loci are listed in Table 1 and used to determine basic genetic parameters (Table 2), such as the number of alleles found at each STR locus (Na); the number of effective alleles (Ne) per locus (i.e., the number of alleles that contribute most to genetic differences); the observed or actual heterozygosity (Ho) that was found; and the heterozygosity that would be expected (He) if the existing population is being randomly bred. 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 of the dogs were completely different at each of the 33 loci. A value of 0.00 would be seen if the selection of sires and dams was balanced in reference to the existing gene pool.

The allele frequency data obtained from the 33 STR panels can be used to assess heterozygosity within a population (Table 2). Using the 33 marker panel, the 86 Shiloh Shepherds had an average of 4.0 alleles/loci (Na). However, the average number of alleles is less important than the number of alleles that have the greatest genetic influence on the breed, a figure known as average effective alleles/loci or Ne. The Ne in this group of dogs averaged 2.63 effective alleles per locus, indicating that a small amount of the available genetic diversity was shared by a large

proportion of the population. The observed heterozygosity (Ho) across the 86 Shiloh Shepherds was 0.58, while the expected heterozygosity (He) was 0.55, yielding a coefficient of inbreeding (F) of -0.05. A population with an F value of 0.00 would be in state of random selection, which means that even though overall genetic diversity is low for the breed, breeders have been able to avoid inbreeding by careful mate selection using accurate pedigrees.

Table 2: Genetic assessment of 86 ISSA registered Shiloh Shepherds based on allele frequencies at 33 genomic STR loci on 25 chromosomes.

	Na	Ne	Ho	He	F
Mean	4	2.63	0.58	0.55	-0.05
SE		0.20	0.03	0.03	0.011

B. 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 genetic similarities in the specific regions of the genome that are associated with each STR marker. Phenotypic differences equate to genotypic differences. Therefore, alleles that are widely shared across the population are indicators that positive selection is occurring for certain desired traits. The Na values for an individual STR locus for this population of 86 Shiloh Shepherds ranged from a low of 2 to a high of 9 average alleles per locus, while the Ne ranged from 1.38 to 5.48 alleles per locus. It is important to remember that each STR locus can have from 7-27 different alleles when testing across all dogs. Therefore, the more alleles that are found for each locus in a breed, the larger the founder population and the greater the genetic diversity. Shiloh Shepherds have relatively few alleles at each locus, again demonstrating that the breed started with only a small number of related founders. The observed heterozygosity (Ho) for an individual STR locus ranged from 0.035 to 0.860, while He ranged from 0.034 to 0.822 (Table 3). Only seven loci had positive F values and 26 were negative. These findings also indicate that breeders are being careful to select sires and dams from within the existing breed that are least related (i.e., much closer to random selection).

Table 3: Genetic assessments for individual STR loci of ISSA Shiloh Shepherds. Na=alleles/locus; Ne=effective alleles/locus; Ho=observed heterozygosity; He=expected heterozygosity; F=coefficient of inbreeding (deviation from H-WE expectation).

#	Locus Name	Na	Ne	Ho	He	F
1	**AHT121**	5	2.1	0.55	0.52	-0.04
2	**AHT137**	3	1.378	0.28	0.27	-0.02
3	**AHTH130**	4	1.913	0.5	0.48	-0.05
4	**AHTh171-A**	4	3.219	0.76	0.69	-0.1
5	**AHTh260**	4	1.562	0.36	0.36	-0
6	**AHTk211**	4	2.514	0.69	0.6	-0.14
7	**AHTk253**	2	1.302	0.22	0.23	0.046
8	**C22.279**	4	1.772	0.45	0.44	-0.04
9	**FH2001**	4	3.196	0.72	0.69	-0.05

10	**FH2054**	7	4.587	0.85	0.78	-0.09
11	**FH2848**	4	2.432	0.63	0.59	-0.07
12	**INRA21**	5	2.428	0.57	0.59	0.031
13	**INU005**	3	1.907	0.55	0.48	-0.15
14	**INU030**	4	2.447	0.54	0.59	0.095
15	**INU055**	4	2.363	0.64	0.58	-0.11
16	**LEI004**	2	1.897	0.56	0.47	-0.18
17	**REN105L03**	5	3.157	0.66	0.68	0.03
18	**REN162C04**	5	2.455	0.62	0.59	-0.04
19	**REN169D01**	2	1.525	0.37	0.34	-0.08
20	**REN169O18**	4	3.628	0.76	0.72	-0.04
21	**REN247M23**	4	3.088	0.76	0.68	-0.12
22	**REN54P11**	2	1.948	0.51	0.49	-0.05
23	**REN64E19**	4	2.395	0.58	0.58	0.002
24	**VGL0760**	9	4.85	0.85	0.79	-0.07
25	**VGL0910**	5	2.979	0.69	0.66	-0.03
26	**VGL1063**	3	1.621	0.41	0.38	-0.06
27	**VGL1165**	8	5.622	0.84	0.82	-0.02
28	**VGL1828**	2	1.035	0.04	0.03	-0.02
29	**VGL2009**	5	1.484	0.37	0.33	-0.14
30	**VGL2409**	4	2.187	0.51	0.54	0.057
31	**VGL2918**	6	3.991	0.72	0.75	0.038
32	**VGL3008**	9	5.479	0.86	0.82	-0.05
33	**VGL3235**	3	2.431	0.62	0.59	-0.05

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

1. IR testing

Genetic assessments such as those presented in Tables 1 to 3 are indicators of population-wide heterozygosity and do not reflect the genetic diversity being provided to individuals by their parents. 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 86 Shiloh Shepherds from around the world (Fig. 1– red line) confirms that the population tested varies in the degree of parental relatedness with individuals scoring as high as +0.40 and as low as -0.30. The peak IR score for the population was -0.10. An IR score of 0.25 would be equivalent to the relatedness of full siblings from a randomly breeding and genetically diverse population (e.g., village dogs). The IR curve for Shiloh Shepherds is somewhat biphasic, with the majority of dogs having IR scores <0.10 and a minority having scores >0.10. This second peak represents the most inbred dogs in the population (Table 3). Fortunately, only a small fraction of the dogs fall into this category.

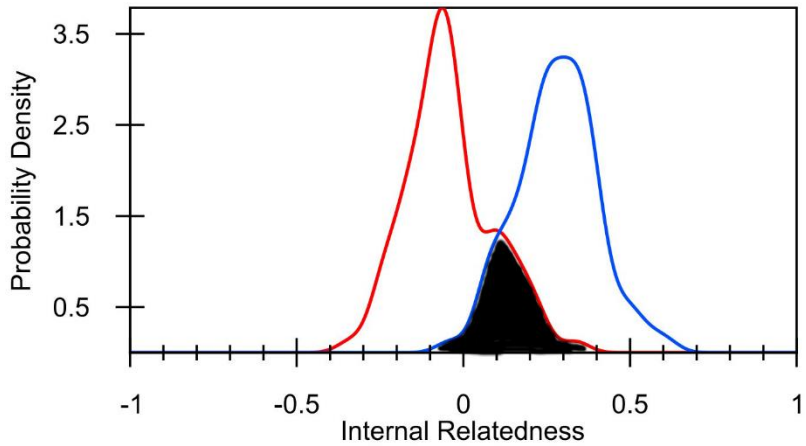


Figure 1: Distribution of IR estimates in 86 ISSA Shiloh Shepherds based on intra-breed diversity (Red line), compared with IR adjusted for diversity lost during breed development (Blue line). Diversity lost as a result of breed development was determined by comparing allele frequencies at the same loci between ISSA Shiloh Shepherds and randomly breeding village dogs from the Middle East, SE Asia, and the Pacific Islands. The blackened area shared by both IR and IRVD graphs is an estimate the available genetic diversity present in all dogs that was used to create the breed.

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 registries are closed there are theoretically no further introgressions from outside the breed. Therefore, the goal of breeders is to start with as much of the potential genetics among all dogs as possible and to maintain that diversity by strict adherence to random or judicious mate selection.This has been largely achieved for most existing breeds of dogs, as one study showed that pure breeds as a whole have retained an average of 87% of their starting diversity.

It is possible to determine the amount of potential genetic diversity a breed has by adjusting their allele frequencies to the frequencies of those same alleles found in a large population of village dogs from across the Middle East, SE Asia and Island Pacific. While village dogs do not contain all known genetic diversity of dogs, they maintain a significant proportion of it. Village dogs possess 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 similar genetic diversity as village dogs. If the IRVD and IR

peaks are only somewhat overlapping, only a fraction of potential genetic diversity exists in the breed.

The IRVD curve for Shiloh Shepherds was shifted well to the right of the IR curve, reflecting a relatively small founder population and subsequent artificial genetic bottlenecks (Fig. 1). All pure breeds of dogs have come from relatively small founder populations and have therefore had limited genetic diversity from the time registries were created and closed. More genetic diversity may have been lost in subsequent breed evolution through artificial genetic bottlenecks such as cataclysmic events (e.g., world wars) or inbreeding (e.g., popular sire effects).

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. The more closely individuals cluster together around the XY axis, the more related they are to each other. Figure 2 shows the inter-relationship of 86 Shiloh Shepherds from the USA, Canada, The Netherlands, and Europe outside of the Netherlands. Although individuals within the breed are relatively scattered (i.e., less interrelated) compared to longer established breeds, the relationship of the 86 dogs is close enough to constitute a single, but genetically diverse, breed. Dogs from the USA show less genetic relationship to each other and Shiloh Shepherds from the other regions of the world. There also tends to be more genetic overlap of dogs from the USA with dogs from other geographic regions. The Canadian, European and Dutch populations are starting to show a degree of genetic separation from each other, which is typical of other world-wide breeds. One interpretation is that dogs from the USA are more widely travelled, while dogs from these other regions are more likely to stay put.

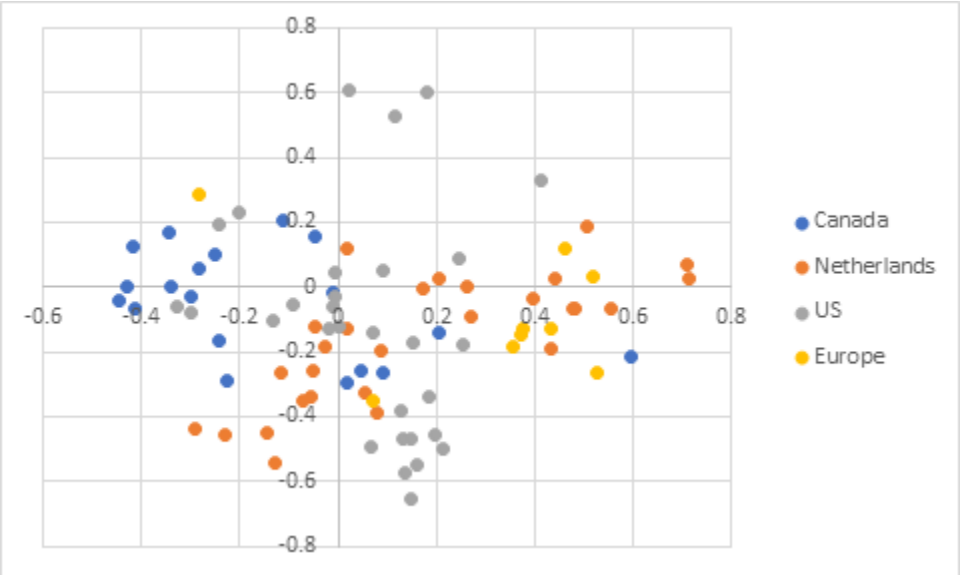


Figure 2: PCoA plot of 86 ISSA Shiloh Shepherds.

E. 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 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 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 Shiloh Shepherds

The 86 Shiloh Shepherds in this study possessed only seven DLA class I and five DLA class II haplotypes (Table 4). This is the lowest number of DLA class I and II haplotypes identified in any other breed to date and is a reflection of the small number of founders that contribute to DLA diversity in contemporary Shiloh Shepherds. It is possible that a few additional haplotypes will be identified as more dogs are tested, but they are likely to occur at low frequency. Three DLA class I (1052, 1065, 1165) and II haplotypes (2017, 2053, 2080) are found in 88% (DLA class I) and 93% (DLA class II) individuals. Since the DLA is genetically conserved compared to most other regions of the genome and each DLA region inherited as a block of linked genes from each parent and passed on by descent, this indicates that two closely related founder lines are the genetic backbone of the breed. This is consistent with the breed history.

Table 4: DLA class I and II haplotypes identified to date in ISSA Shiloh Shepherd

DLA Class I Haplotype Frequencies (Updated Oct 17, 2019)			
DLA1 #	**STR types**	**Shiloh Shepherd (n=4)**	**Shiloh Shepherd, ISSA (n=182)**
1001	380 373 281 182	--	0.005
1035	386 373 277 184	--	0.005
1045	376 371 277 186	--	0.003
1052	380 372 289 184	0.3	0.363

1068	380 373 287 181	0.5	0.247
1165	392 369 281 182	0.1	0.245
1166	388 379 277 184	--	0.063
1167	397 381 277 184	0.1	0.060
1172	390 369 289 188	--	0.008

DLA Class II Haplotype Frequencies (Updated Oct 17, 2019)

DLA2 #	STR types	Shiloh Shepherd (n=4)	Shiloh Shepherd, ISSA (n=182)
2001	343 324 284	--	0.005
2003	343 324 282	--	0.008
2017	343 322 280	0.3	0.363
2022	339 327 282	--	0.063
2026	351 324 284	--	0.005
2039	345 327 276	--	0.003
2053	343 324 280	0.6	0.308
2080	339 325 276	0.1	0.245

The DLA class I and II regions are frequently shared between breeds, reflecting common distant ancestry and inheritance by descent (Table 5). Only the DLA class I 1165 (major), 1166 (minor), and 1167 (minor) haplotypes have not been seen before in other breeds tested by the VGL. All of the DLA class II haplotypes are extensively shared with other breeds.

Table 5. The occurrence and incidence of specific DLA class I and II haplotypes in various breeds.

DLA Class I Haplotype Frequencies (Updated Aug 3, 2017)

	1035	1045	1052	1068	1165	1166	1167
American Akita (n=79)	--	0.01	--	--	--	--	--
Japanese Akita (n=264)	--	--	--	--	--	--	--
Blend Akita (n=19)	--	--	--	--	--	--	--
Biewer (n=67)	--	--	--	--	--	--	--
Biewer Yorshire Terrier (n=50)	0.01	--	--	0.01	--	--	--
Yorkshire Terrier (n=16)	--	--	--	--	--	--	--
Doberman Pinscher (n=292)	--	--	0.00	--	--	--	--
English Bulldog (n=160)	--	--	--	--	--	--	--
Bulldog (n=1)	--	--	--	--	--	--	--
Flat Coated Retriever (n=252)	0.00	--	--	0.26	--	--	--
Golden Retriever (n=676)	--	--	--	0.05	--	--	--
Havanese (n=297)	0.01	--	0.01	0.02	--	--	--

Italian Greyhound (n=615)	--	--	0.19	--	--	--	--
Magyar Agar (n=26)	--	--	0.06	--	--	--	--
Polish Lowland Sheepdog (n=15)	--	--	--	--	--	--	--
Poodle (n=1646)	0.00	0.00	--	--	--	--	--
Miniature Poodle (n=219)	--	0.00	--	0.02	--	--	--
Samoyed (n=278)	--	--	--	0.03	--	--	--
Shiloh Shepherd, ISSA (n=86)	0.01	0.01	0.36	0.27	0.25	0.06	0.05

DLA Class II Haplotype Frequencies **(Updated Aug 3, 2017)**

	2017	**2022**	**2026**	**2039**	**2053**	**2080**
American Akita (n=79)	0.02	--	--	0.08	--	--
Japanese Akita (n=264)	0.01	--	--	0.09	--	--
Blend Akita (n=19)	--	--	--	0.26	--	--
Biewer (n=67)	--	--	--	--	--	0.01
Biewer Yorshire Terrier (n=50)	--	--	--	--	0.01	0.01
Yorkshire Terrier (n=16)	--	--	--	--	--	0.03
Doberman Pinscher (n=292)	--	0.00	--	0.01	--	--
English Bulldog (n=160)	0.22	0.02	--	--	--	--
Bulldog (n=1)	0.50	--	--	--	--	--
Flat Coated Retriever (n=252)	0.00	0.12	--	--	0.14	--
Golden Retriever (n=676)	0.04	0.00	--	--	0.03	--
Havanese (n=297)	0.01	0.12	--	--	0.04	--
Italian Greyhound (n=615)	0.22	--	--	0.11	--	--
Magyar Agar (n=26)	0.29	--	--	--	--	--
Polish Lowland Sheepdog (n=15)	0.40	--	--	--	--	--
Poodle (n=1646)	0.00	0.00	0.00	0.00	--	--
Miniature Poodle (n=219)	--	0.00	--	--	0.02	--
Samoyed (n=278)	--	0.09	--	--	0.61	--
Shiloh Shepherd, ISSA (n=86)	0.36	0.06	0.01	0.01	0.32	0.25

III. What does this assessment of genetic diversity tell us about contemporary Shiloh Shepherds

This study confirmed that Shiloh Shepherds from around the world constitute a single breed, albeit with more genetic variation than other breeds and with some tendency towards geographic differentiation. The breed originated from a common founder population that appears to be relatively small and this has been possibly compounded by artificial genetic bottlenecks that have occurred since the breed was closed to outside blood. Therefore, DNA based genetic diversity testing confirms what is known from pedigrees.

Although the breed lacks genetic diversity, it is obvious from DNA testing that breeders have been careful in breeding across the full range of genetic diversity that exists. A lack of genetic diversity is not in itself bad, providing the founder population was relatively free of deleterious genetic traits and breeders have been judicious in avoiding any artificial genetic bottlenecks that may cause either a loss or imbalance of that original diversity.

The lack of genetic diversity in the DLA class I and II region of these 86 Shiloh Shepherds is troublesome, but it is uncertain what it means. Certain DLA class I and II haplotypes have been associated with specific autoimmune diseases in certain breeds, but autoimmune disorders have not been documented as serious problems in the breed. Exocrine pancreatic insufficiency is an autoimmune disorder recognized in Shiloh Shepherds, and it is known to have association with certain DLA class II types.

Breeds that lack genetic diversity must be managed much more closely to avoid further loss of genetic diversity and have less leeway in dealing with simple recessive or complex polygenic disorders that might arise. Elimination of deleterious traits may result in loss of genetic diversity, especially when diversity is limited.

IV. Heritable disorders of Shiloh Shepherds

There is not a lot of information on heritable diseases in Shiloh Shepherds. They do suffer from hip dysplasia, a complex genetic disorder that has been undoubtedly inherited by descent from their German shepherd founders. They also suffer from gastric torsion, which is more of a conformational disorder associated with large deep-chested breeds.

Degenerative myelopathy, associated with homozygosity to certain mutations in the SOD1 gene, has been identified in some Shiloh Shepherds. One study found 24% of Shiloh Shepherds to be carriers of the SOD1 mutation and 3.2% at risk for the disease. Elimination of the causative mutations by testing dogs prior to breeding is possible, but genetic studies would be required to determine what effect eliminating carriers would have on breed-wide genetic diversity. This mutation also is common in German Shepherds, which are heavily involved in the breed. A propensity for exocrine pancreatic insufficiency and ventricular arrhythmias have also been inherited by descent from German shepherd dogs. Panosteitis is seen in juvenile Shiloh Shepherds and is also commonly seen in other breeds. The disorder appears to be due to over-production of certain inflammatory cytokines and how young dogs with this propensity respond to immune stimuli.

Anne Becker writes:
The ISSA registry performed an informal health survey in the summer of 2017, in which all breeders were asked to consult their records of disorders reported to them by their puppy owners between January of 2012 and July 2017. As this relies on owner communication, it is possible that some data went unreported. 567 pups were produced during this period of time by the breeders within the ISSA registry.

The findings showed that the most frequent disorders seen in this group of dogs are hip dysplasia, ventricular arrhythmia, cryptorchidism, subaortic stenosis, exocrine pancreatic

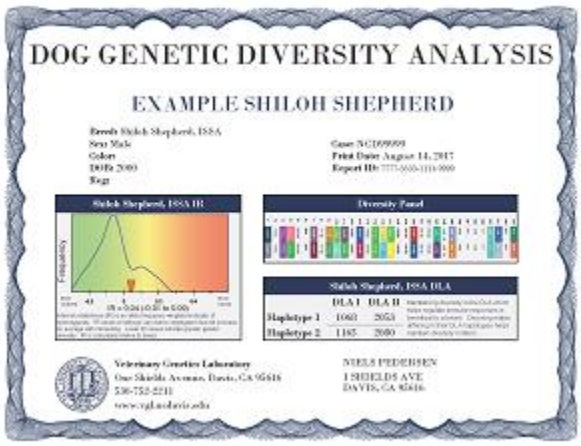
insufficiency, genetic mega-esophagus, and allergies (both food and environmental). Of these seven top disorders, the last four have a below 2% incidence rate in this pool of dogs. SAS and cryptorchids are between 2 and 3%. Arrythmia and hip dysplasia findings are still being analyzed as they cannot be compared to the total number of dogs born, but only against those dogs in the pool who were tested. This data is being gathered.

Panosteitis does occur but appears to have a very low incidence rate (or has remained unreported). Bloat (gastric torsion) also has a low incidence rate as reported, around 1%. The ISSA breeders have experienced zero cases of degenerative myelopathy over this 5.6 year period. DNA testing for degenerative myelopathy is mandated for all breeding dogs in the ISSA and the number of carriers currently in the gene pool is less than five.

V. 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. The alleles at each of the 33 STR loci are presented as numbers that correspond to those found in Table 1. Each loci will have two alleles, which are either different (heterozygous) or the same (homozygous). Each allele is inherited from each of the parents. More of the alleles at each locus will be homozygous in dogs from closely related parents or that in regions of the genome that are under strong positive selection for some favored phenotypic trait or traits. Dogs with a predominance of rarer (i.e.,low incidence) alleles will be more distantly related to the bulk of the population than dogs that have a predominance of common (i.e., high incidence) alleles.



B. What should you do with this information?

The use of DNA for testing genetic diversity in Shiloh Shepherds has confirmed what has already been anticipated, i.e., that the breed suffers from a lack of genetic diversity due to its

origins from a small number of founder individuals/lines. The fact that breeders have maintained accurate and deep pedigrees lessens the need for DNA testing for activities such as ideal mate selection. However, if the breed were to consider increasing genetic diversity by further genetic introgressions, DNA testing of dogs intended for such introgressions would be extremely helpful. DNA testing would also be useful in monitoring the effect of genetic outcrossing on the breed.

The use of genetic diversity testing of this type may be less helpful for Shiloh Shepherds than for other breeds. The IR graph for the breed as a whole has a sharp peak, it is relatively narrow, and most dogs appear to be products of mating's that are as genetically unrelated as possible given the limited diversity that exists in the breed. Nevertheless, 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. 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.

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.

The VGL provides a simple online mating evaluation tool that accepts two VGL case numbers, one from a female and another from a male.

A more effective use of this study is to contribute the genetic information to a web repository, hopefully under the control of the registry. The best format for such a repository and testing has been provided by Standard Poodle breeders. This information could be incorporated into a mate selection service that will allow a breeder to identify, among all of the dogs tested, potential mates that would be most ideal for increasing genetic diversity in their litters- https://www.betterbred.com/.