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DNA Test as a Basis of Identifying Illegal Dogs in Denmark

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Abstract

In 2010, possession and breeding of American Staffordshire Terrier (Amstaff) and 12 other dog breeds became illegal in Denmark when the Danish breed specific legislation was introduced. If the police suspect a dog to be included by this law because of its phenotypical appearance, the owner is required to prove that the dog is legal. Presently, the owner can meet the burden of proof by documenting the dog to be an offspring of legal breeds. Today, this is not possible by the use of a DNA test to identify a dog's breed. The current study investigates the possibility to use such DNA test in Denmark to detect purebred Amstaff and mixed-breeds containing Amstaff. An American company has developed a DNA test, Wisdom Panel, based on breed specific SNP markers and used to identify the composition of dog breeds to a limit of 12.5% in a dog. The test is mainly based on American DNA samples and therefore, the usability in Denmark is uncertain.

To investigate if Wisdom Panel is usable in Denmark, DNA material from 20 Swedish Amstaffs (representing the Danish population) and six American Amstaffs were analyzed with Wisdom Panel 4.0. In addition, DNA material from 192 Danish dogs was analyzed. In total, 55 different dog breeds were represented in the study.

The results revealed that Wisdom Panel was able to correctly detect all samples from 46 out of the 55 analyzed dog breeds including Amstaff. The 46 detected dog breeds are all included in the Wisdom Panel database whereas the remaining nine are not included. It is concluded that a DNA test is usable in Denmark as Wisdom Panel is able to detect Amstaff and other breeds included in the database to a limit of 12.5%. Regarding the breed-specific legislation the implementation of a DNA test could improve the legal rights, as the test improve the ability to prove a dog's breed composition and is more accurate compared to visual breed identification.

Resume

I 2010 blev besiddelse og avl af Amerikansk Staffordshire Terrier (Amstaff) og 12 andre hunderacer ulovliggjort i Danmark, da hundelovens forbudsordning trådte i kraft. Formoder politiet på baggrund af en hunds udseende, at der kan være tale om en hund der er omfattet af forbuddet, stilles der krav om, at ejeren kan bevise, at hunden er lovlig. I dag kan denne omvendte bevisbyrde kun løftes såfremt ejeren kan bevise, at hunden stammer fra lovlige racer, og det er ikke muligt at identificere hundens racemæssige sammensætning ved hjælp af en DNA- test. Dette studie undersøger muligheden for brug af en sådan DNA-test i Danmark til at identificere renracede Amstaff og blandingshunde, hvor Amstaff indgår. Et amerikansk firma har udviklet en DNA-test, Wisdom Panel, der er baseret på racespecifikke SNP markører som kan bruges til at identificere tilstedeværelsen af hunderacer i en hund ned til 12,5%. Testen er hovedsageligt baseret på DNA-prøver fra amerikanske hunde, og det er derfor usikkert, om testen kan bruges i Danmark.

For at undersøge om Wisdom Panel kan bruges i Danmark, blev DNA-materiale fra 20 svenske Amstaffere (repræsentative for den danske population) og 6 amerikanske Amstaffere testet med Wisdom Panel 4.0. Yderligere blev DNA-materiale fra 192 danske hunde testet. I alt er 55 forskellige racer repræsenterede i dette studie.

Resultaterne viste, at Wisdom Panel kunne detektere alle prøver fra 46 ud af de 55 indsendte hunderacer, heriblandt Amstaff, korrekt. De 46 hunderacer optræder alle i Wisdom Panels database, hvorimod de resterende ni ikke gør. Det konkluderes, at en DNA-test kan bruges i Danmark, da Wisdom Panel kan detektere racen Amstaff og andre hunderacer, som er registrerede i databasen, ned til 12,5%. I relation til hundeloven vil implementering af en DNA-test øge retssikkerheden, da denne test forbedrer mulighederne for at bevise en hunds racesammensætning og er mere præcis end visuel bedømmelse.

Foreword and Acknowledgement

This veterinary master's thesis was written as a part of the Veterinary Medicine master program at the Faculty of Health and Medical Sciences at the University of Copenhagen. The project was carried out as the genetic part of a project supported by the Ministry of Environment and Food of Denmark. This thesis targets the politicians of Denmark, veterinarians, dog owners, shelters and people with special interest in the breed-specific legislation.

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We would also like to thank the Danish Kennel Club for establishing contact to the Swedish Kennel Club, and lastly, a special thanks to the 20 Swedish American Staffordshire Terriers and their owners.

List of Abbreviations

Amstaff:	American Staffordshire Terrier
bp:	Base pair
F1:	First filial (offspring as a result of breeding of two purebred dogs)
FCI:	Fédération Cynologique Internationale
LD:	Linkage Disequilibrium
PCA:	Principal Component Analysis
PCR:	Polymerase Chain Reaction
SNP:	Single Nucleotide Polymorphism
U.S.:	United States
USDA:	United States Department of Agriculture

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1. Background

1.1 The Danish Breed-Specific Legislation

After several reports of episodes with Pit Bull Terriers attacking humans in the late 1980's, the Danish Ministry of Justice passed in 1991 a breed-specific legislation prohibiting the two dog breeds Pit Bull Terrier and Tosa Inu, along the same lines as seen in United Kingdom at that time (Parliament of the United Kingdom, 1991; Betænkning om farlige hunde, 2010). However, the implementation of the breed-specific legislation did not stop the discussion about dangerous dogs in Denmark as an increase in acquiring dogs from legal breeds with some of the same characteristics as the Pit Bull Terriers was seen in the following years (Betænkning om farlige hunde, 2010). In 2009, the Danish Kennel Club estimated that there were around 20,000 dogs in Denmark, which could be referred to as muscle or fighting dogs. A committee was set to investigate the need for additions to the law from 1991 regarding dangerous dogs (Betænkning om farlige hunde, 2010). In June 2010, the Danish Government introduced a new breed-specific legislation (§1a and §1b in the Danish Dogs Act "Bekendtgørelse af lov om hunde") prohibiting an additional 11 dog breeds giving a total of 13 prohibited dog breeds and mixed-breed dogs where one or more of these breeds were included. These dogs were all classified as "dangerous dogs" (Bekendtgørelse af lov om hunde, 2017). The American Staffordshire Terrier (Amstaff) was one of the prohibited breeds and was in 2009, with 6,769 registrations in the Danish Dog Register, by far the most popular breed of the 13 prohibited breeds (Betænkning om farlige hunde, 2010).

The breed-specific legislation relies on reversed burden of proof (Bekendtgørelse af lov om hunde, 2017). This means that when the police suspect a dog to be of one of the illegal dog breeds or a mixed-breed including at least one of these breeds, based on its phenotypical appearance, it is the dog owner's responsibility to present evidence that their dog is of a legal breed. Currently, the ways to prove a dog's origin are through trustworthy pedigrees, statements from breeders or a paternity test that proves a dog to be offspring from parents of legal breeds. According to the breed-specific legislation it is not possible to prove a dog's breed from the phenotypic appearance or behavior of the dog. At present, it is not even possible in Denmark to identify a dog's breed from a DNA sample, which makes it difficult for dog owners of dogs without a studbook to provide sufficient documentation proving that the dog consists of legal breeds (Vejledning om hundelovens forbudsordning, 2016). According to the Danish Dog Registry Denmark has approximately 580,000 registered dogs (personal communication, Dansk

Hunderegister 2017). Of these, 18% are registered as mixed-breeds and 82% are registered as purebreds. The purebreds can be further divided into two groups: 33% registered as purebred dogs with a pedigree in Danish Kennel Club and 49% registered as purebreds with only verification of breed by owner and vet and no pedigree (Proschowsky, 2017). In the Danish breed-specific legislation the definition of a cross- or a mixed-breed dog is not specified and there is no percentage limit of how much of an illegal dog breed is allowed to be present before a dog is included by this law (Bekendtgørelse af lov om hunde, 2017).

If the dog owner fails to prove the dog's legality, euthanasia of the dog can be demanded by the police (Bekendtgørelse af lov om hunde, 2017). Dog owners can appeal the decision and one of the most recent cases concerning illegal dogs in Denmark were brought to the Supreme Court in October 2017 as a matter of principle. The case concerned two dogs who in 2014 were suspected to be of the breed Amstaff or a mixed-breed including this breed and were placed in a shelter for three years while the case was being processed. Based on visual assessment by the police and a veterinarian and the fact that the owners could not prove the dogs' descent, the Supreme Court upheld the decision from the High Court to euthanize the dogs (Højesteret 2017; DR, Emil Søndergård Ingvorsen, 2017).

A recently published record from the Danish Ministry of Environment and Food shows that in the period from June 2010, when the law was passed, to August 2017, 552 dogs have been euthanized due to the Danish breed-specific legislation. This number only includes the cases that have been reported to the Danish National Police (Miljø-og Fødevareministeriet, 2017). A report from a committee formed by the former Danish Ministry of Food, Agriculture and Fisheries shows that the majority of these dogs were suspected of being an Amstaff or a mixed-breed including this breed (Udvalget for Fødevarer Landbrug og Fiskeri, 2013).

1.2 Visual Breed Detection

According to the legislation a dog may be suspected of being illegal based on its phenotypic appearance and the police is not obligated to obtain a secondary opinion (Vejledning om hundelovens forbudsordning, 2016). This practice is problematic because studies show that visual identification of a dog's breeds in mixed-breed dogs is difficult: when people working with dogs, e.g. in shelters, were asked to decide which breeds a mixed-breed dog consisted of, there was great discrepancy between the visual identification and results from DNA testing, which was used as control (Voith *et al.*, 2009,

2013). In a study by Voith et. al (2013), for 14 of the 20 mixed-breed dogs investigated, fewer than 50% of the respondents identified the breeds of the dogs that were found by DNA identification. The study also revealed the level of inter-observer reliability as very poor, as for only seven of the 20 dogs more than 50% of the respondents agreed on the most predominant breed of a mixed-breed dog. A study by Olson et al. (2015) concerning Pit Bull-type dogs is consistent with the poor level of inter-observer reliability and illustrates that reliable inclusion or exclusion of dogs as Pit Bull-type dogs are not possible. It is also demonstrated in their study how one in five mixed-breed dogs containing Pit Bull-type breeds (American Staffordshire Terrier and Staffordshire Bull Terrier) were not labeled as such; and one in three mixed-breed dogs lacking any of these breeds were labeled as a Pit Bull-type by the participants. The overall mean sensitivity and mean specificity of visual identification of Pit Bull-type dogs were 50% and 83% respectively (Olson *et al.*, 2015).

1.3 History of Dog Breed Formation

The breed-specific legislation prohibits 13 individual dog breeds which is possible because of the fact that today's population of dogs is divided into well-defined subpopulations called breeds, which are possible to distinguish from each other.

The present population of domesticated dogs stems from a common ancestor, the grey wolf, and no other canid species have contributed to the genetic makeup. The domestication and co-living with humans began over 15,000 years ago, however, many details in the dog's geographical origin, evolution, history and domestication remain unclear (Ostrander *et al.*, 2017). It was not until nearly two centuries ago that the majority of dog breeds, as we know them today, were established. In the middle of the nineteenths century a new tendency was seen. People started to control dog breeding with the purpose to improve their animals. This new interest was combined with a sporting element in dog shows and field trials, where dog owners were rewarded for their work. In the beginning, dogs competed in both dog shows and field trials but later these activities became more specialized and most dogs were bred for the purpose of joining only one of the competitions. These specialized dogs became the first purebreds and they all had a documented pedigree stating their ancestors. Pedigrees or studbooks were established by including particularly good representative dogs of each breed, and after choosing these original animals, the studbooks were closed. After closure of the studbooks, only offspring from these chosen dogs were regarded as purebreds. To avoid conflicts regarding which dogs were accepted as purebreds and which were not, kennel clubs like American Kennel Club, The

Kennel Club and Danish Kennel Club were in the late 1800's established to manage this (Sandøe, Corr and Palmer, 2016; The Kennel Club, 2017b; American Kennel Club, 2018b; Dansk Kennel Klub, 2018). The controlled selection by man has led to the development of closed intraspecies groups, where each dog breed represents an isolated breeding population with relatively uniform physical characteristics defining each breed (Irion *et al.*, 2003; Parker, Shearin and Ostrander, 2010).

The first kennel club, The Kennel Club, recognized 40 breeds (Sandøe, Corr and Palmer, 2016). At present, 344 different dog breeds are recognized by the Fédération Cynologique Internationale (FCI), who organizes kennel clubs worldwide (Fédération Cynologique Internationale, 2018b). However, the overall number of breeds is in reality higher, as different breeds are accepted by different kennel clubs. Most modern dog breeds are a closed population and breed membership requires that both parents are registered members of the same breed before mating (Dansk Kennel Klub, 2017).

FCI classifies dog breeds in ten different groups[1] (Fédération Cynologique Internationale, 2018a). The Danish Kennel Club is a member of FCI and organizes dogs after FCI's international classification system. The American Kennel Club and The Kennel Club are not members of FCI, and upholds their own systems when it comes to classification[2] (The Kennel Club, 2017a; American Kennel Club, 2018a).

1.4 History of American Staffordshire Terrier

As earlier described, the Amstaff was the most popular of the 13 prohibited dog breeds in Denmark (Betænkning om farlige hunde, 2010). The history of the American Staffordshire Terrier, often called Amstaff, exists in slightly different versions, but there is great agreement on the fact that the Amstaff has a British ancestor in a dog type bred by crossing the Bulldog, which until the mid-19th century was primarily used for the purpose of acting in staged fights with bull or bear, with the White English Terrier or the Black-and-Tan Terrier, or any other game terrier. This new dog type was bred to

[1] 1: sheepdogs and cattledogs 2: pinscher and schnauzer – molossoids, swiss mountain and cattledog 3: terriers 4: dachshunds 5: spitz and primitive types 6: scent hounds and related breeds 7: pointing dogs 8: retrievers, flushing dogs and water dogs 9: companion and toy dogs 10: sighthounds.

[2] American Kennel Club: Sporting group, hound group, working group, terrier group, toy group, non-sporting group, herding group, miscellanous class, and foundation stock service.

The Kennel Club: Gundog, hound, pastoral, terrier, toy, utility and working.

combine the spirit and agility from the terrier and courage and tenacity from the bulldog for the purpose of dog fighting. These types of dogs were in the beginning called Half and Half, Bull-and-Terrier, Pit Dog or Pit Bullterrier. When brought to the United States of America around 1870 by immigrants from United Kingdom, they were known as Pit Dogs, Pit Bull Terriers, American Bull Terriers or Yankee Terriers. After being imported to the United States (U.S.) the breed developed into a heavier type of dog and was adjusted to general farm work, to hunt wild animals, to guard the farm and for general companionship. In 1936, the dog type was registered in American Kennel Club's studbook as its own breed, called Staffordshire Terrier. In 1972, the name was changed to American Staffordshire Terrier to distinguish these dogs from the British version of the Staffordshire Terrier now called Staffordshire Bull Terrier which had recently been recognized by the American Kennel Club (American Kennel Club, 2018c). Currently, the Amstaff is recognized by American Kennel Club but not by The Kennel Club in United Kingdom. In the late 1980's the Amstaff came to Scandinavia and in 1990 the first Swedish Amstaff litter was born (Svenska Amstaffklubben, 2018). The Amstaff became a popular dog in Denmark where the media drew special attention to the uptake of Amstaffs among a special segment of unexperienced owners, who used the dog as a part of their image; as a tool of power and as a "weapon" to frighten the public (Nyhedsavisen, Rene Fredensborg, 2007; Politikken, Morten Sørensen, 2009; Information, Lærke Cramon, 2017). It should be underlined that it is not known how many of the former Amstaff owners in Denmark fitted this stereotype view and how many were caring owners of Amstaffs as a family dog.

Today, the Amstaff is illegal in Denmark and Norway but not in Sweden where they are found in relatively great numbers, as 8,650 dogs were registered as Amstaff in 2017 (Forskrift om hunder, 2004; Bekendtgørelse af lov om hunde, 2017; Jordbruksverket, 2017).

1.5 Dog Breed Genetics

If dog breeds are to be distinguished on another basis than their phenotypical appearance, the underlying genetic structure must be studied. The present population of dogs express long range linkage disequilibrium (LD), long haplotype-blocks and great homozygosity within breeds in contrast to the great phenotypic diversity seen between breeds (Parker *et al.*, 2004; Sutter *et al.*, 2004; Lindblad-Toh *et al.*, 2005; Dreger *et al.*, 2016). This is the result of intense breeding, closing of subpopulations and several bottlenecks throughout the dog's history. The first bottleneck came with the divergence from the wolf. Another at the breed formation, which introduced breed-specific

bottlenecks due to closing the populations, popular sires and restricted breeding, which caused a decreased geneflow between breeds and an increase in the level of inbreeding. More recent events, such as the two World Wars, have left few founding animals in several breeds e.g. the Leonberger and the Cavalier King Charles and have contributed to new bottlenecks. This further limited the genetic pool (Ostrander and Kruglyak, 2000; Lindblad-Toh *et al.*, 2005; Dreger *et al.*, 2016). It has been suggested that only a 5% reduction in the genetic diversity was seen due to domestication, while a 35% loss of diversity occurred due to breed formation (Gray *et al.*, 2009).

1.5.1 Microsatellites

Because of the unique population structure and close interaction with humans, the dog has been used widely in genetic studies over the years (Ostrander and Kruglyak, 2000; Parker, 2012). Some of the previous analyses of the dog's genome were based on microsatellite markers that were used to differentiate breeds and to look at the canine genetic make-up. Microsatellites are repeated sequences of 1-6 base pairs (bp) and are also known as short tandem repeats or simple sequence repeats.

Several studies have examined the variation of microsatellites within and between dog breeds (Fredholm and Winterø, 1995; Koskinen and Bredbacka, 2000; Irion *et al.*, 2003). Microsatellite loci vary in a population because of different length of the repeated sequence in a given allele. The length depends on the number of repeats (Zajc *et al.*, 1994). Microsatellites are highly polymorphic, and this is demonstrated by the fact that different loci in different breeds have been analyzed finding wide variations in allele size. Breed specific alleles exist, but most of all, the difference in breeds is caused by different allele frequencies and allele distribution, and not in the allele length at a specific locus (Fredholm and Winterø, 1995; Koskinen and Bredbacka, 2000). There is a relative high level of allele heterozygosity between breeds, but the degree differs within the individual microsatellite. A lower degree of heterozygosity within breeds can be ascribed to a limited gene pool and non-random mating. Heterozygosity decreases concurrently with decrease in population size within a breed (Fredholm and Winterø, 1995; Irion *et al.*, 2003). In 2004, Parker et al. demonstrated that a genetic difference exists between dog breeds and that dogs can correctly be assigned to their individual breed based on their genotype using microsatellites. In this study, 414 dogs representing 85 different breeds were genotyped with 96 microsatellite loci revealing a genetic difference between breeds. More than one quarter (27%) of the genetic variation in a dog is the result of variation between breeds rather

than variation between individual dogs in contrast to a 5-10% of variation observed between human populations (Parker *et al.*, 2004).

In Denmark, one way to prove whether a dog is legal is through parentage testing. This is only possible if DNA from both parents is available (Vejledning om hundelovens forbudsordning, 2016). Paternity testing is based on microsatellite sequences, which are used because of their highly polymorphic nature and the fact that they show Mendelian codominant heritage. Microsatellites can be easily read with multiplexing Polymerase Chain Reaction (PCR) and the amplified PCR products pooled for electrophoresis. This makes microsatellites an efficient parentage testing assay (Zajc *et al.*, 1994; Koskinen and Bredbacka, 1999).

With the canine genome characterized partly in 2003 and fully in 2005, more genetic information has become available. Kirkness et al. (2003) established a partial reference genome by sequencing the canine genome to a sequence depth of 1.5X sequence coverage (Kirkness *et al.*, 2003). In 2005, Lindblad-Toh et al. (2005) succeeded with a full characterization of the canine genome by compiling data from the partially sequenced genome from 2003 with their own sequence information of a 7.5X sequence coverage of a female boxer's genome. As an increasing number of sequence information became available new genotyping tools based on single nucleotide polymorphism (SNP) have been established. Since a large number of SNPs can be genotyped together using SNP-chip this methodology has now to a large extend replaced microsatellites as a research and practical tool (Vaysse *et al.*, 2011).

1.5.2 Single Nucleotide Polymorphism (SNP)

A SNP-marker is a change in a single bp in a DNA sequence at a unique locus in the genome. SNP's are thereby responsible for some of the genetic variation existing among individuals. SNPs represent a unique genomic pattern for each dog breed and the SNP allele frequency differs between breeds (Mars Veterinary, 2007).

Several studies have worked with identification of canine SNPs and the establishment of a canine marker library to be used in canine mapping projects. When Lindblad-Toh et al. in 2005 sequenced the entire genome of a female boxer the study also reported an initial compilation of SNP markers covering the population of dogs. A comprehensive set of SNP markers (2.5 million in total) were

identified by comparing the boxer's genome to the previously sequenced poodle genome, to nine diverse dog breeds, to four grey wolves and to one coyote (Lindblad-Toh *et al.*, 2005). Since this original SNP map contained gaps, further development of the SNP markers has been done by targeted resequencing in order to ensure that the entire dog genome is covered. The combined efforts to identify SNP markers have let to the establishment of the CanineHD SNP array panel comprising a total of 170,000 SNPs (Vaysse *et al.*, 2011). The use of SNP chip in canine genetics is now widely known. In 2016 Dreger et al., used the CanineHD SNP array to evaluate genomic breed-specific homozygosity in 800 purebred dogs representing 80 different breeds. By comparing shared (between individuals of a breed) and individual homozygous regions in ten dogs from each breed, it was demonstrated that each dog breed has a unique profile of genome diversity caused by varying numbers and sizes of homozygous regions (Dreger *et al.*, 2016).

SNPs can be used in genetic studies because of their high density across the genome, their high polymorphism and the fact that they are evenly distributed across the genome. By comparing the genotypes of ten different breeds, a SNP-rate at one SNP pr. 900 bp were found to be reflecting of the polymorphism and variation among breeds. Reduced polymorphism was seen within breeds and was reflected by a SNP-rate at one SNP pr. 1600 bp. SNP genotyping has confirmed that dog genomes within breeds consist of large LD blocks and that homozygous regions extend over large regions (Lindblad-Toh *et al.*, 2005). This reflects the limited haplotype diversity seen in dog breeds. Long-range haplotypes are typical for most dog breeds, but the exact haplotypes vary between breeds and the location of homozygosity differs between individual dogs (Lindblad-Toh *et al.*, 2005; Dreger *et al.*, 2016). Haplotype frequencies differ between breeds and only 2-4 haplotypes accounts for a frequency of 80% of the chromosomes within each breed, thereby causing homozygosity (Sutter *et al.*, 2004; Lindblad-Toh *et al.*, 2005).

1.6 Wisdom Panel

By using the presence of breed specific genetic profiles and SNP markers, several breed-detector DNA tests have been developed. One of these products is Wisdom Panel developed by Wisdom Health, a business unit of Mars Petcare which is a part of Mars Incorporated (Wisdom Panel, 2017c). Wisdom Panel is a commercial, patented product and the first edition, Wisdom Panel MX, was presented in 2007. By analyzing DNA extracted from a blood sample, Wisdom Panel MX was able

to identify the different breeds combined in a specific dog's recent ancestry - mixed or purebred - to the great-grandparent levels. The first Wisdom Panel consisted of more than 300 SNP markers selected after analyzing 4,608 SNPs out of the total 2.5+ million existing SNP markers. The test made use of breed specific SNP allele frequencies to discriminate between different breeds. By analyzing the genome and comparing hundreds of SNPs across the chromosomes, the test was able to find various breed signatures and define the breed background in the dog being tested (Mars Veterinary, 2007).

Wisdom Panel has been improved over the last ten years and the latest version of the product is called Wisdom Panel 4.0. This is a cheek swab-based DNA test and the genotyping is now conducted on a canine Illumina® Infinium® chip consisting of 1,800 SNP markers created specifically for the test. Wisdom Panel 4.0. consists of a computer algorithm and a database containing more than 12,000 DNA samples covering over 250 different breeds, types and varieties (including all American Kennel Club recognized breeds). Based on the results received from examining DNA samples with the 1,800 SNP-markers, the algorithm finds over 18,000,000 different combinations of ancestry trees and gives each of them a score based on how well they match the specific dog's data. The pedigree tree with the best score is considered the best possible match and illustrates the dog's ancestry up to three generations (Wisdom Panel, 2017f, 2018a). The SNPs used in the genotyping are not chosen to cover the genes responsible for the breed specific traits, as many of the markers are found in the part of the genome that does not link to a phenotypic trait. Therefore, the pedigree tree result of a mixed-breed dog could show ancestors where only a very few evident traits are inherited (Wisdom Panel, 2018c).

The database is based on DNA samples mostly from American dogs but dogs from United Kingdom, Canada, Australia and Germany have also been included during the recent years and therefore, the test is useable in these countries. According to Wisdom Health, differences in the genetic breed signature across geographical areas have been found during the development of Wisdom Panel, and the use of the test on Scandinavian dogs can therefore be questionable (Wisdom Panel, 2017a).

In 2007, when the first panel (Wisdom Panel MX) was developed, the company promised an accuracy of 84% (Mars Veterinary, 2007). At the present moment, it is not possible to find exact data indicating how accurate the DNA test is, as this depends on the quality of the DNA samples. However, to maintain a high quality, Wisdom Health ensures that tests are run in an USDA-accredited laboratory

(quality controlled), that repeated tests of a dog's data are run and that independent third parties review the test (Wisdom Panel, 2017d).

1.7 Genetic Differences Between American and European Dog Populations

The differences in the genetic breed signature between American and European dog breed populations found by Wisdom Health have also been demonstrated in a study by Quignon et al. (2007) who showed how the Golden Retriever shared 70.1% of its haplotypes between the breed populations in U.S. and Europe. This displays a higher diversity in the Golden Retriever compared to the other breeds included in the study, such as the Bernese Mountain Dog who shared 76.2 % of its haplotypes between the populations in U.S. and Europe. At the same time, the Golden Retriever shows a higher number of total haplotype blocks compared to the Bernese Mountain Dog, Rottweiler and Flat-coated Retriever. This correlates well with the popularity and size of the Golden Retriever population (Quignon *et al.*, 2007).

A recent study has shown that when importing a dog breed to a new country, genetic differences between the breed in its original country and in the new country, can occur (Parker *et al.*, 2017). This was seen in the Cane Corso, a breed of Italian origin. When analyzing haplotypes in the Cane Corso the U.S. population significantly shared haplotypes with the Rottweiler and the Mastiff. This was not seen in the Italian population. The study also implies that when a breed is introduced to a new country the genetic pool is decreased compared to the origin population. This contributes to a possible genetic difference between breeds in different geographical regions (Parker *et al.*, 2017).

1.8 Study Purpose

This project was established in light of the ban of the so called dangerous dog breeds which is a legislation that presently relies on the reversed burden of proof using visual judgment of phenotypes. The aim of the present study was to establish if a DNA test can be used in Denmark for identification of American Staffordshire Terrier (Amstaff) and mixed-breed dogs containing Amstaff.

2. Materials and Methods

2.1 Study Design

The study was designed to establish if a DNA test, in this study Wisdom Panel 4.0, could be used to identify the genetic profile of Amstaffs sampled in Sweden. To validate the use of the test in Denmark, samples from other dog breeds were analyzed as well.

2.1.1 Animal Material

The DNA material used for this study consists of purified DNA from 192 Danish dogs distributed on 174 dogs representing 58 different specific breeds and 18 samples from Danish mixed-breed dogs. Furthermore, purified DNA material from six American Staffordshire Terriers born and raised in the United States of America and DNA samples from 20 American Staffordshire Terriers born and raised in Sweden, were included. See appendix 1 for a complete list of samples.

2.1.2 Sample Collection

DNA samples from the 192 Danish dogs were selected from a Biobank established at Section of Animal Genetics, Bioinformatics and Breeding, Department of Veterinary and Animal Sciences at the University of Copenhagen. Selection of the specific DNA samples was a result of dog breeds available in the Biobank and an estimation of dog breeds that would be informative for the sake of establishing info on Amstaffs and mixed-breeds. The final list included samples from dog breeds with phenotypic similarities to the Amstaff (e.g. Mastiff, Rottweiler, Staffordshire Bullterrier), worldwide popular dog breeds (e.g. Labrador Retriever, Golden Retriever, German Shepherd), dog breeds with origin in Denmark (e.g. Broholmer, Danish-Swedish Farmdog, Old Danish Pointing Dog), randomly selected breeds from the Biobank (e.g. Saluki, Xoloitzcuintli, Wippet) and some samples registered as mixed-breeds. Two of the Biobank samples represents DNA from Danish Amstaffs. Eight of the mixed-breed samples derived from the same litter of puppies, which had been confiscated by the Danish police under the suspicion of being illegal regarding the breed-specific legislation.

DNA material from the six American Amstaffs was kindly provided by Professor Kerstin Lindblad-Toh, Broad Institute, Harvard, United States of America.

DNA material from the 20 Swedish purebred Amstaffs was collected in November 2017. Since Sweden does not have a breed-specific legislation and the Amstaff is a common breed in Swedish households, it was ideal to collect samples in our neighboring country. Because of the small

geographic distance between Denmark and Sweden, the population of Swedish Amstaffs is a good representative for the Danish population of Amstaffs. The animals were recruited via the Swedish Kennel Club. DNA from the Swedish dogs was obtained by buccal swabs. Two swabs per dog were collected with Wisdom Panel 4.0 Canine DNA Test Kit and one swab per dog was collected with a gyno brush to use in the laboratory at Section of Animal Genetics, Bioinformatics and Breeding, Department of Veterinary and Animal Sciences at the University of Copenhagen. The dogs did not eat food or shared toys or water bowls with any other dog an hour before sample collection as this could disturb the test results.

2.1.3 DNA Extraction

DNA from the 20 Swedish Amstaffs was extracted and purified from gyno brushes using the Promega Kit with a protocol for DNA extraction from the Section of Animal Genetics, Bioinformatics and Breeding, Department of Veterinary and Animal Sciences at the University of Copenhagen.
See appendix 2.

2.2 DNA Analysis

2.2.1 Microsatellite Genotyping

The raw data from the Wisdom Panel analyses were inaccessible for this study. Therefore, a microsatellite genotyping of the DNA samples from the Swedish and American Amstaffs were conducted to compare the genetic profile of the two populations. PCR-analysis was run with the extracted DNA from the 20 Swedish and six American Amstaffs and a microsatellite assay (Canine Genotypes^{TM} Panel 1.1). The microsatellite assay is used at present for parentage testing at the Department of Veterinary and Animal Sciences at the University of Copenhagen and is approved and standardized by International Society for Animal Genetics (ISAG). The assay encompasses 19 different loci. The PCR product was visualized with electrophoresis using ABI PRISM 3130 XL Genetic Analyzer and the results were read with Genemapper version 3.7.

2.2.2 DNA Test

DNA material from the 192 Danish dogs, the six American dogs and the 20 Swedish dogs were sent to Wisdom Health. Here the DNA genotyping was conducted on a canine Illumina® Infinium® chip and analyzed by the patented method, Wisdom Panel 4.0. For further description of the test, see section "Wisdom Panel" in the background section.

3. Results

3.1 Wisdom Panel Reports

The results from Wisdom Panel were received as individual sample reports on email. For each dog the report contains sections named: ancestry, ancestry tree, breed tests (for purebreds), breed description and adult weight. For an example of a purebred and a mixed-breed report see appendix 3 and 4 respectively.

In the ancestry section, a calculated percentage of the most likely breeds in the specific dog is shown. Figure 1 shows three illustrative examples of different ancestries.

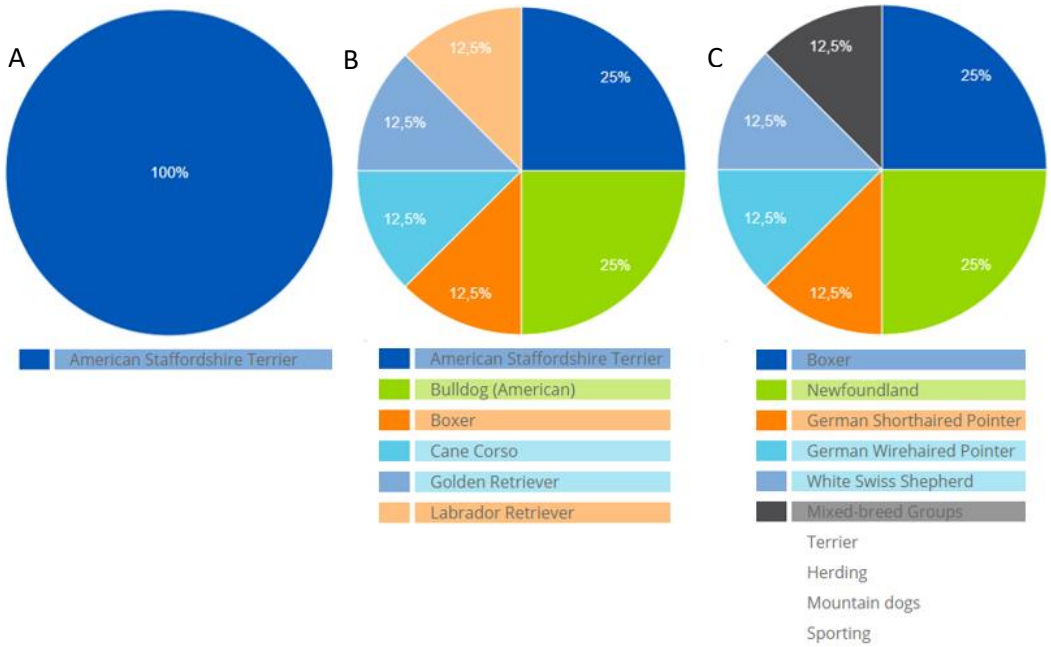

Figure 1: Examples from the Wisdom Panel reports, showing the calculated percentage of breeds involved in a dogs ancestry. A) illustrates the reults from a purebred Amstaff. B) illustrates the results from a mixed-breed containing Amstaff. C) illstrates a mixed-breed dog containing DNA from breeds not reprensented above 12.5% of the total DNA. These breeds are gatherd in a ”mixed-breed group”. *Note: reprinted from Wisdom Panel reports.*

The lowest possible breed percentage to detect for a single specific breed is 12.5%. If the dog's DNA consists of breeds not represented above 12.5% in the total DNA the test cannot detect those specific breeds. Instead these percentages are gathered in a "mixed-breed group" with information on which groups (Asian, companion, guard, herding, hound, Middle East and African, mountain dogs, sighthound, sporting, terrier or wild canids) the DNA is most similar to. An example of this is seen in figure 1, C.

Besides the calculated percentages of breeds in the specific dog, Wisdom Panel illustrates the dog's most likely ancestry tree. For an example see appendix 3 or 4, page 49 and 57. Currently, the test does not identify who the maternal and paternal ancestors are.

If the result of a dog turns out to be a purebred dog or an F1 mix of two breeds, the panel performs several breed tests to see how consistent the sample is to the suggested breed. In the Single Breed PCA Test and the All Breed PCA Test, Wisdom Panel uses Principal Component Analysis (PCA) to illustrate how well the dog's DNA sample is consistent with other samples from the same breed and to a single representative sample from every other breed in the Wisdom Panel database. A PCA is a statistical method that reduces the numbers of variables in a dataset. It is an analysis that emphasizes variations and illustrates strong patterns of relatedness in a dataset. Samples from the same breed or the same subpopulation of a breed are expected to be closer together compared to other breeds and this tends to create a cluster. If a sample falls within such a cluster, the dog is most likely a purebred of the specific breed. PCA can be used to visualize genetic variation and relatedness in a population analysis.

Another way to compare the sample to the breed profile in the database is with a homozygosity profile. This profile shows to what percent a sample's genetic markers are identical and compares this to the breed specific range of homozygosity score found in the Wisdom Panel database. All of these breed tests are carried out to secure the highest accuracy when deciding a dog's breed and each analysis is performed individually.

3.2 Wisdom Panel Results

3.2.1 DNA Sample Results

The breed results from Wisdom Panel for each DNA sample appeared in the individual reports and a complete list of the results are registered in appendix 1.

Out of a total number of 218 samples sent to Wisdom Panel only the three samples KP70, KP151, KP153 were unable to be successfully processed (for abbreviations see appendix 1). Regarding KP70, failure was due to not enough high-quality DNA to meet the minimum standards for analysis. The reason for failure for KP151 and KP153 is unknown. Two samples, KP47 and KP74, were lost during transportation and sample reports were never received.

The results of the ancestry reports from eleven dogs (KP99, KP106, KP107, KP115, KP118, KP121, KP130, KP132, KP134, KP166, KP167) did not match the breed the samples were registered as in the Biobank. Most of these dogs were reported to be mixed breeds and going back to the information that was registered on these dogs in the Biobank, it turned out that none of them are pedigreed dogs, and that insufficient information had been provided on the ancestry. Thus, they should in fact have been registered as unknown. Because KP106, KP107, KP121, KP166 and KP167 are unknown breeds, Cavapoo, Large Münsterlander and Xoloitzcuintli are no longer represented in the samples and the total number of breeds sent to Wisdom Panel are 55.

The results from three of the six American Amstaffs (KP87, KP88, KP89) were reported as mixed-breeds after being analyzed by Wisdom Panel. Discussing this with Professor Kerstin Lindberg-Toh, who provided the DNA, it could not be ruled out that they were actually mixed-breeds.

In some breeds, different standards of the breed exist e.g. the Poodle, which is represented in a miniature, a toy and a standard version. In the samples sent to Wisdom Panel it is not distinguished which standard of the breed the sample represented, and the results were therefore reported as the overall right breed but sometimes with more specific information on the standard. This is seen in the samples representing the Poodle (KP113, KP114), the Bull Terrier (KP103, KP104) and the Dachshund (KP116, KP117). Also, the Belgian Sheepdog covers a breed variation of four different standards. Three samples are listed as Belgian Tervuren and one of these samples was reported back as purebred Belgian Sheepdog (KP159) and the two other samples were reported back as a mix of Belgian Tervuren and Belgian Sheepdog (KP160, KP161). The sample representing the Jack Russel Terrier (KP133) was reported as a mix of Jack Russel Terrier and Parson Russel Terrier.

In a total, Wisdom Panel was able to detect 46 of the 55 represented breeds sent to analysis. These breeds are listed in table 1.

Table 1: Breeds detected correctly by Wisdom Panel in DNA samples sent from the Biobank

Airedale Terrier	English Cocker Spaniel	Leonberger
American Staffordshire Terrier	French Bulldog	Maltese
Beagle	German Shepherd Dog	Mastiff
Belgian Sheepdog	German Shorthaired Pointer	Neapolitan Mastiff
Belgian Tervuren	Golden Retriever	Newfoundland
Bernese Mountain Dog	Great Dane	Poodle
Boxer	Great Pyrenees	Pug
Bulldog (English)	Greater Swiss Mountain Dog	Rottweiler
Bullmastiff	Greyhound	Saluki
Bull Terrier	Havanese	Samoyed
Cavalier King Charles Spaniel	Hovawart	Siberian Husky
Coton de Tulear	Irish Glen of Imaal	Staffordshire Bull Terrier
Dandie Dinmont Terrier	Irish Wolfhound	West Highland White Terrie
Dachshund	Jack Russel Terrier	Whippet
Dobermann Pinscher	Labrador Retriever	
Dogue de Bordeaux	Lagotto Romagnolo	

In nine out of the 55 represented breeds, Wisdom Panel was not able to detect the correct breed. These nine breeds are listed in table 2.

Tabel 2: Breeds Wisdom Panel was unable to detect in DNA samples sent from the Biobank

Broholmer
Danish/Swedish Farmdog
Eurasier
Greenland Dog
Gross Spitz
Landseer
Old Danish Pointing Dog
Polski Owczarek Podhalanski
Scharpendos

The eight mixed-breed samples (KP58, KP59, KP60, KP101, KP102, KP171, KP185, KP186) were reported as mixed-breeds. In all samples, multiple breeds were detected. Se appendix 1 for a complete list of the dogs' breed composition.

3.2.2 Purebred Amstaff Results

All 20 Swedish Amstaff samples were correctly assigned to the American Staffordshire Terrier breed by Wisdom Panel. The same was the case for the three American Amstaffs (KP85, KP86, KP90) and the two Danish Amstaffs samples (KP91, KP92). See appendix 1.

The Single Breed PCA Test and the All Breeds PCA Test placed the samples from the Amstaffs into three subpopulation clusters. An example from a Single Breed PCA Test is seen in figure 2. The 20 Swedish Amstaffs can be found in the light blue cluster. In this cluster the two Danish Amstaffs (KP91, KP92) are also found. Two out of three American Amstaffs (KP85, KP86) are found in the light purple cluster and the last one (KP90) is found in the dark purple cluster.

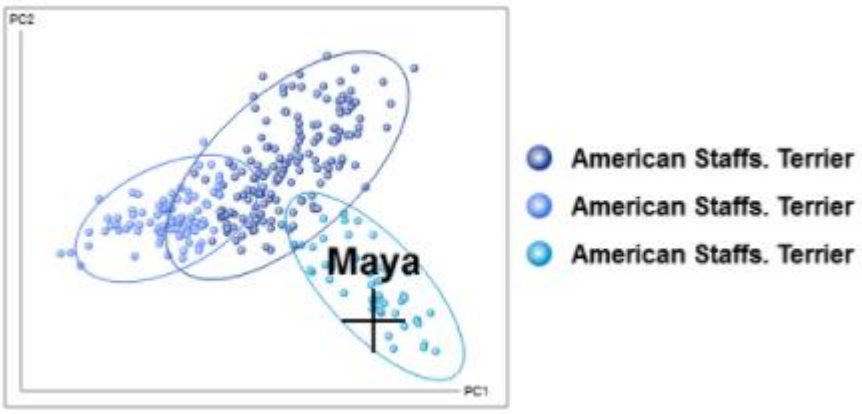

Figure 2: Wisdom Panel results from the Single Breed PCA test for the Swedish Amstaff Maya, showing three different clusters in the Amstaff population. Maya is placed in the light blue cluster. .
Note: reprinted from Maya's Wisdom Panel report.

3.2.3 Amstaff Mixed-Breeds Results

In the mixed-breed litter, confiscated by the Danish police, the ancestry results differed between the puppies - both in ancestry percentage and in presence of breeds in the ancestry. However, all eight samples included Amstaff. The results are listed in table 3.

Table 3: Wisdom Panel results from the mixed-breed litter confiscated by the Danish police.

Sample name of the puppy	Breeds detected by Wisdom Panel in the samples
KP172	25% American Staffordshire Terrier, 25% American Bulldog, 12,5% Boxer, 12,5% Cane Corso, 12,5% Golden Retriever, 12,5% Labrador Retriever
KP173	25% American Staffordshire Terrier, 25% Cane Corso, 12,5% Boxer, 12,5% American Bulldog, 12,5% Bullmastiff, 12,5% Labrador Retriever
KP174	37,5% American Staffordshire Terrier, 25% Cane Corso, 12,5% Boxer, 12,5% American Bulldog, 12,5% Labrador Retriever
KP175	37,5% American Staffordshire Terrier, 25% Cane Corso, 12,5% American Bulldog, 12,5% Bullmastiff, 12,5% Labrador Retriever
KP176	25% American Staffordshire Terrier, 25% American Bulldog, 25% Cane Corso, 12,5% Labrador Retriever, 12,5% Mixed-breed Groups (sporting, guard, terrier)
KP177	37,5% American Staffordshire Terrier, 25% American Bulldog,12,5% Labrador Retriever, 25% Mixed-breed Groups (guard, herding)
KP178	25% American Staffordshire Terrier, 12,5% Boxer, 12,5% American Bulldog, 12,5% Golden Retriever, 12,5% Labrador Retriever, 25% Mixed-breed Groups (guard, herding, sporting)
KP179	25% American Staffordshire Terrier, 25% American Bulldog, 25% Cane Corso, 12,5% Golden Retriever, 12,5% Labrador Retriever

3.3 Microsatellite Genotyping

To compare the genetic profile of the American and Swedish populations of Amstaffs, a microsatellite genotyping assay was conducted. The results consist of alleles found in each dog in the 19 loci. For a complete list of the alleles see appendix 5.

When looking at the results from the microsatellite panel, some differences in the alleles present in the American Amstaff population and the Swedish Amstaff populations are seen, as some alleles are found in only one of the populations. We analyzed how the American samples differentiate from the Swedish samples and not the reverse because three American dogs are not sufficient to represent the American population of Amstaffs. The results of the genotyping show that of a total of 54 different

alleles found in the American dogs in the 19 loci of the panel, 19 alleles were not represented in the Swedish population. The size and locus of the alleles are listed in table 4.

Two of the Swedish dogs, Santos and Bruno, did not run properly in test and therefore, the results are not included. Also, the three American Amstaffs (KP187, KP188, KP189) which were determined as mixed-breeds by Wisdom Panel were excluded.

The results from the loci on the sex chromosomes, Amelogenin, are not included in our analysis as it is not relevant for this study.

Table 4: alleles found with the microsatellite analysis only in the American Amstaff population compared to the Swedish population.
*The results from the loci on the sex chromosomes, Amelogenin, are not included in our analysis.

Locus name	**Allele size of alleles found only in the American dogs**
AHT121	102
AHT137	143, 149, 151
AHTh171	239
AHTh260	250, 252
AHTk211	91, 95
AHTk253	
Amelogenin*	
CXX279	116, 120
FH254	176
FH2848	242, 244
INRA21	101
INU005	130
INU030	
INU055	
REN162C04	
REN169D01	
REN169O18	164
REN247M23	
REN54P11	222, 234

4. Discussion

4.1 Results from the DNA Test

This study reveals that a DNA test, in this study Wisdom Panel 4.0, can be used to detect purebred and mixed-bred Amstaff in the Danish dog population.

Because Wisdom Panel is mainly based on DNA material from U.S. and dog breed populations from different geographical areas might have different genetic profiles, it was expected that the test could have difficulties detecting all the breeds in the Danish and Swedish samples correctly. Also, during development of the test, Wisdom Health observed that certain breeds sometimes have different genetic breed signatures in different countries (Wisdom Panel, 2017a). Nevertheless, all samples from Amstaffs in this study were correctly assigned to their breed, however the samples from different geographical origins were found in different clusters in the PCA tests (see figure 2). The PCA results and the results from the microsatellite panel assay, where some alleles in the American population are not found in the Swedish population (see table 4), might indicate a minor difference in the genetic profile across geographical origin. There seem to be a 'continent' specific DNA profile, however this can be overcome by Wisdom Panel 4.0.

Also, when looking at the results from the samples registered as Golden Retriever in the Biobank, Wisdom Panel seems to overcome the genetic difference in the European and U.S. population previously described by Quignon et al. (2007). The fact that Wisdom Panel is able to determine the Golden Retriever correctly could indicate that including DNA material from United Kingdom and Germany in the database has increased the reliability of the breed test in the European samples, and therefore also in the Danish samples.

To evaluate the use of Wisdom Panel to identify Amstaff in the Danish dog population, this study also demonstrated Wisdom Panel's accuracy in Danish samples from other dog breeds. In the current study Wisdom Panel was able to detect 46 out of 55 different breeds in the samples from the Biobank. These 46 breeds are all listed on www.wisdompanel.com as detectable breeds included in the test (Wisdom Panel, 2018b). The nine undetectable breeds are not mentioned on this list and were therefore not expected to be detected correctly. Based on the results of this study, it is suggested that the genetic difference between geographical distinct breed populations does not determine whether Wisdom Panel is able to detect the correct breeds in Denmark or not. More likely, this is determined by the fact that some breeds are not yet included in the Wisdom Panel database.

Included in the nine undetectable breeds are four dog breeds with origin in Denmark – Broholmer, Danish/Swedish Farm Dog, Old Danish Pointing Dog and Greenland Dog. If these breeds and other breeds of relevance in Denmark, such as the 13 banned dog breeds (except American Staffordshire Terrier, Boerboel and American Bulldog, which are already recognized by Wisdom Panel), should be included in the Wisdom Panel database, Wisdom Panel needs to be provided with DNA from a large number of dogs of these breeds.

The results for the eight puppies from the Amstaff mixed-breed litter, revealed a difference in ancestry between the puppies. This difference is to be expected as a result of crossover events where maternal and paternal homologues chromosomes exchange random pieces of DNA during meiosis and every puppy inherits a unique combination of DNA with a new assortment of genes (Hartwell *et al.*, 2011). The final genetic make-up depends on which part of the chromosomes the puppy inherits. If some of the great-grandparents were mixed-breeds, it is possible that some of the puppies inherited DNA from one breed involved in the ancestors and not from another breed. Since an offspring inherits different breed-specific alleles from its ancestors the ancestor tree can be different for each puppy even though the same ancestors are shared in real life, as seen for the Amstaff mixed-breed puppies in the current study (Wisdom Panel, 2018c). Another reason that may result in different ancestry in a litter is the fact that a litter of puppies can have more than one father (Wisdom Panel, 2017e). Both reasons to different ancestry in litter mates demonstrate the importance of testing an entire litter, as one puppy's genetic profile does not necessarily represent the rest.

Since doubts about ancestry often arise regarding mixed-breed dogs, like the litter with the eight puppies, it would be optimal to establish Wisdom Panel's accuracy in breed determination in such mixed-breed samples. In order to estimate this accuracy, it would have been ideal to include samples from several mixed-breeds with known descent. We tried to recruit such dogs, but this turned out to be very difficult within the time frame of this study since owners of mixed-breeds rarely know the entire ancestry of their dogs. We are therefore not able to document the accuracy of Wisdom Panel in mixed-breeds, but it seems reasonable to think that the received results regarding mixed-breeds are reliable. This is due to the high accuracy reported in purebred dogs, demonstrated with the fact that Wisdom Panel was able to detect 46 out of the 55 represented breeds in Danish dogs.

In dog breeds where different standards (size, color, coat etc.) are detected by Wisdom Panel, the results might be considered incorrect when reported back as mixes of these standards. This applies

for the samples representing the Poodle (KP113, KP114), the Dachshund (KP116, KP117), the Bull Terrier (KP103, KP104), the Belgian Tervuren (KP160, KP161) and the Jack Russel Terrier (KP133), see appendix 1. It is not registered in the Biobank what specific standards are involved in these samples and since some mating between the different standards is allowed it is possible that a dog's genetic profile contains DNA from more than one standard and therefore is reported as a mix of standards. For example the Belgian Sheepdog consists of four different types and in Denmark mating between specific types are allowed (Belgiske Hyrdehunde, 2015). The breed registered as Belgian Sheepdog in Wisdom Panel is known as the Groenendael in Denmark and since mating between Tervuren and Groenendael is allowed the results reported for KP160 and KP161 might illustrate such mating. Regarding the Jack Russel Terrier, sample KP133 was reported as 50% Jack Russel Terrier and 50% Parson Russel Terrier. These two breeds rise from the same ancestor and according to Wisdom Panel, the Parson Russel Terrier is a show variant of the Jack Russel Terrier. Therefore, it is possible that Wisdom Panel will detect some ancestral contribution from both dog types in a single dog depending on the dog's ancestry and report it as a mix of the two breeds, even though it is registered as only one of the breeds (Wisdom Panel, 2018d). Despite this, the sample is still correctly assigned to the "Russel Terrier" type breed and no other breeds are involved.

It is not possible to evaluate the accuracy of the Wisdom Panel test regarding the standard determinations, since the standards and types of the dogs in the samples are not registered in the Biobank and the pedigrees are not available. However, the main focus of this study is Wisdom Panel's ability to assign a dog to the correct breed and in this case more specific information regarding the breed is not necessary. In all the samples reported as mixed standard breeds, the correct overall breed has been determined.

Since Wisdom Panel is a commercial product, the raw data from the analyses was not accessible to us, and it was therefore not possible to perform a more thorough analysis of the method used by Wisdom Panel. Therefore, the only way to estimate the accuracy and value of Wisdom Panel in Danish dogs was to send anonymized DNA samples from purebred dogs to Wisdom Panel, where the specific breeds were known by us, and then compare the results from Wisdom Panel to the samples' registered breed. It would have been interesting to review which breed specific SNPs that were used, how these were selected and how they are used to calculate the pedigrees. Wisdom Health has been contacted several times regarding this, but it is not possible to receive the data from the analysis. This

makes it difficult to evaluate the method and the results on a scientific basis. Nonetheless, our results based on microsatellite genotyping support the PCA results on Amstaff reported by Wisdom Panel.

4.2 Use of Wisdom Panel in Denmark

Based on our results, Wisdom Panel can be expected to detect the breed of a dog as long as the breed is included on the list of detectable breeds at www.wisdompanel.com. The Amstaff is included on this list and as it is seen in this study Wisdom Panel had no problems assigning the Swedish population of Amstaffs as purebred Amstaffs. Only two other dog breeds (American Bulldog and Boerboel) of the 13 prohibited dogs in Denmark, are found on the list. Based on our results, it is reasonable to believe that American Bulldog and Boerboel (both purebred and mixed-breeds) will be detected in Danish dogs. The remaining ten prohibited dogs are not represented in Wisdom Panel's DNA database and these breeds will not be detected by Wisdom Panel. This is a point of critique of the use of Wisdom Panel as a tool regarding the breed-specific legislation. However, none of these ten breeds were represented in great numbers in Denmark before 2010 and most of them do not share phenotypic characteristics with the Amstaff (Betænkning om farlige hunde, 2010). As a result of this, a mixed-breed containing these breeds will probably not be suspected as being illegal very frequently. If considered necessary that the entire list of banned dogs must be represented in the Wisdom Panel database, they can be included, as earlier mentioned, by submitting a sufficient amount of DNA samples to Wisdom Health. However, this does not apply for the Pit Bull Terrier as the term "Pit Bull", according to Wisdom Health, does not refer to a single recognized breed but rather to a genetically diverse group of breeds, and it is therefore not possible to establish a breed-specific DNA profile of the Pit Bull Terrier (Wisdom Panel, 2017b).

Today, there is no lower limit for illegal admixture allowed in a dog specified in the Danish breed-specific legislation, which means that the legislation is currently being practiced with a limit of zero per cent. If DNA testing should be implemented in the legislation, it would be reasonable to accept the lowest limit possible with the available technology. Wisdom Panel's lower limit of detection of a breed involved in a specific dog is 12.5% and therefore this limit needs to be accepted as the lower limit of admixture of illegal breeds in a dog if DNA testing with Wisdom Panel should be implemented in the Danish breed-specific legislation. Acceptance of this limit would make Wisdom Panel a useful tool to document if a dog is illegal or not. If this limit is not accepted, Wisdom Panel

could still be used as documentation to rule out the presence of any illegal breeds in a dog if the result shows a 100% admixture of legal breeds, e.g. 50% Rottweiler and 50% Boxer. It is important to mention that Wisdom Panel does not distinguish between the maternal or paternal contribution of genes. The ancestry tree of a dog is only illustrating the best possible match made by the algorithm and not necessarily the true distribution of ancestors. Therefore, the results cannot be used to proceed against the mother or father of a tested dog, as they will need to be tested individually.

Wisdom Panel could be a helpful tool in determination of breeds in a dog that is suspected to be illegal regarding the breed-specific legislation in Denmark. This applies particularly to mixed-breed dogs, which are present in relatively great numbers in Denmark. Since the accusation of a dog being illegal is based on the phenotypic appearance, and since owners of mixed-breed dogs are often unable to present a reliable pedigree, the cases are often based on the owners' word against the words of the police, which may result in prolonged cases. By accepting Wisdom Panel as a tool to prove the descent of a dog, the matter of dispute would rarely exist, which would improve legal rights of dog owners and lower both economic and emotional costs. Even though the accuracy in mixed-breed samples could not be established based on the samples in this study, the use of Wisdom Panel still contributes to a higher accuracy in breed identification than the visual identification, which have been proved insufficient in several studies (Voith *et al.*, 2009, 2013; Olson *et al.*, 2015).

DNA testing as a tool to prove that a dog is of a legal breed or mix, must be implemented in the Danish breed-specific legislation, if Wisdom Panel is to be used in cases regarding this legislation in Denmark. Accepting DNA testing would improve legal rights for dog owners in Denmark and by demonstrating in the this study how Wisdom Panel can be used for breed identification in the Danish dog population, it is now possible to use DNA testing and therefore, it seems reasonable to suggest an implementation.

5. Conclusion

The aim of the present study was to establish if a DNA test can be used in Denmark for identification of American Staffordshire Terrier (Amstaff) and mixed-breed dogs containing Amstaff. Our study demonstrates that a DNA test can be used in Denmark to breed identification of purebred and mixed-bred Amstaff in this case to a limit of 12.5%. In light of the ban of the so called dangerous dogs this can improve legal rights for dog owners compared to visual judgement of phenotypes.

6. Limitations and Future Research

The evaluation of Wisdom Panels' accuracy in breed identification in mixed-breed dogs were limited by the fact that it was very difficult to recruit and collect samples from mixed-breed dogs with known ancestry. To encourage the use of Wisdom Panel in mixed-breed dogs we recommend that future research focus on stating the accuracy of Wisdom Panel in such samples. Another focus of future research and improvement of the use of Wisdom Panel in Denmark is to extent the database of Wisdom Panel to include DNA from dog breeds with Danish origin. As earlier mentioned, this can be done by providing Wisdom Panel with DNA from these breeds. A collaboration between Section of Animal Genetics, Bioinformatics and Breeding, Department of Veterinary and Animal Sciences at the University of Copenhagen and Wisdom Health could provide such DNA material to establish these breeds' genetic profiles in the Wisdom Panel database. The more genetic profiles of dog breeds existing in Wisdom Panels' database, the better the test works, both in Denmark and worldwide.

This study investigated the use of Wisdom Panel as a breed-detector DNA test in Denmark. However, several other breed-detector tests exist on the market and it would be interesting to evaluate the accuracy and usability of these in Denmark.

Three of the six American Amstaffs turned out to be mixed-breeds and this limited the use of the microsatellite genotyping in this study. To further investigate the geographical significance in relation to genetic variation between two dog breed populations and to make a more adequate comparison of the American and Swedish population of Amstaffs, more American samples would be required.

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8. Appendixes

Appendix 1

A complete list of the Biobank samples and the Swedish Amstaff samples sent to Wisdom Health and analyzed by Wisdom Panel 4.0.

* represent samples from the Biobank where information of the registered breed turned out to be insufficient.

** represents samples from American Amstaffs that turned out be mixed-breeds.
*** represents samples of breeds Wisdom Panel was not able to detect.

ID	Breed	Results from Wisdom Panel
KP1	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP2	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP3	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP4	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP5	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP6	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP7	Staffordshire Bull Terrier	100% Staffordshire Bull Terrier
KP8	Bullmastiff	100% Bullmastiff
KP9	Bullmastiff	100% Bullmastiff
KP10	Bullmastiff	100% Bullmastiff
KP11	Bullmastiff	100% Bullmastiff
KP12	Pug	100% Pug
KP13	Mastiff	100% Mastiff
KP14	Mastiff	100% Mastiff
KP15	Pug	100% Pug
KP16	Mastiff	100% Mastiff
KP17	German Shepherd Dog	100% German Shepherd Dog
KP18	German Shepherd Dog	100% German Shepherd Dog
KP19	German Shepherd Dog	100%German Shepherd Dog
KP20	German Shepherd Dog	100% German Shepherd Dog
KP21	German Shepherd Dog	100% German Shepherd Dog
KP22	German Shepherd Dog	100% German Shepherd Dog
KP23	German Shepherd Dog	100% German Shepherd Dog
KP24	German Shepherd Dog	100% German Shepherd Dog
KP25	Labrador Retriever	100% Labrador Retriever

KP26	Labrador Retriever	100% Labrador Retriever
KP27	Labrador Retriever	100% Labrador Retriever
KP28	Labrador Retriever	100% Labrador Retriever
KP29	Labrador Retriever	100% Labrador Retriever
KP30	Labrador Retriever	100% Labrador Retriever
KP31	Labrador Retriever	100% Labrador Retriever
KP32	Labrador Retriever	100% Labrador Retriever
KP33	Golden Retriever	100% Golden Retriever
KP34	Golden Retriever	100% Golden Retriever
KP35	Golden Retriever	100% Golden Retriever
KP36	Golden Retriever	100% Golden Retriever
KP37	Golden Retriever	100% Golden Retriever
KP38	Golden Retriever	100% Golden Retriever
KP39	Golden Retriever	100% Golden Retriever
KP40	Golden Retriever	100% Golden Retriever
KP41	French Bulldog	100% French Bulldog
KP42	French Bulldog	100% French Bulldog
KP43	French Bulldog	100% French Bulldog
KP44	French Bulldog	100% French Bulldog
KP45	French Bulldog	100% French Bulldog
KP46	French Bulldog	100% French Bulldog
KP47	French Bulldog	Sample gone during transportation, no report received
KP48	French Bulldog	100% French Bulldog
KP49	Great Dane	100% Great Dane
KP50	Great Dane	100% Great Dane
KP51	Great Dane	100% Great Dane
KP52	Great Dane	100% Great Dane
KP53	Great Dane	100% Great Dane
KP54	Great Dane	100% Great Dane
KP55	Great Dane	100% Great Dane
KP56	Great Dane	100% Great Dane
KP57	Belgian Sheepdog	100% Belgian Sheepdog
KP58	Mixed	50% Munsterlander (large), 12,5% Golden Retriever, 12,5% Chow Chow, 12,5% Collie, 12,5% German Shepherd Dog
KP59	Mixed	50% Havanese, 50% Shih Tzu
KP60	Mixed	37,5% American Staffordshire Terrier, 12,5% Dobermann Pinscher, 12,5% Golden Retriever, 12,5% Bull Terrier (standard), 12,5% German Shepherd Dog, 12,5% Mixed-breed Groups (guard, sighthound, terrier)

KP61	Old Danish Pointing Dog***	25% German Shorthaired Pointer, 25% Chihuahua, 12,5% Pointing Griffon (Wire), 37,5% Mixed-breed Groups (sporting, hound, terrier, herding)
KP62	Old Danish Pointing Dog***	12,5% Pointer, 25% German Shorthaired Pointer, 62,5% Mixed-breed Groups (sporting, terrier)
KP63	Old Danish Pointing Dog***	12,5% Pointer, 12,5% German Shorthaired Pointer, 12,5% Chihuahua, 12,5% Weimaraner, 50% Mixed-breed Groups (sporting, herding)
KP64	Old Danish Pointing Dog***	25% German Shorthaired Pointer, 12,5% Keeshond, 12,5% Pointer, 12,5% Poodle (miniature), 37,5% Mixed-breed Groups (terrier, sporting)
KP65	Boxer	100% Boxer
KP66	Old Danish Pointing Dog***	25% English Setter, 25% Chihuahua, 25% German Shorthaired Pointer, 12,5% Weimaraner, 12,5% Mixed-breed Groups (sporting, terrier)
KP67	Cocker Spaniel	100% English Cocker Spaniel
KP68	Boxer	100% Boxer
KP69	Boxer	100% Boxer
KP70	Boxer	Test failed due to low DNA quality
KP71	English Bulldog	100% Bulldog (English)
KP72	Danish/Swedish Farmdog***	12,5% Cocker Spaniel, 12,5% Fox Terrier (smooth), 12,5 % Parson Russel Terrier, 62,5% Mixed-breed Groups (terrier, sporting)
KP73	Danish/Swedish Farmdog***	50% Russel Terrier, 12,5% Parson Russel Terrier, 37,5% Mixed-breed Groups (terrier, hound, sporting)
KP74	Engelsk Bulldog	Sample gone during transportation, no report received
KP75	Dobermann	100% Dobermann Pinscher
KP76	Dobermann	100% Dobermann Pinscher
KP77	Dobermann	100% Dobermann Pinscher
KP78	Dobermann	100% Dobermann Pinscher
KP79	Dobermann	100% Dobermann Pinscher
KP80	Rottweiler	100% Rottweiler
KP81	Rottweiler	100% Rottweiler
KP82	Rottweiler	100% Rottweiler
KP83	Rottweiler	100% Rottweiler
KP84	Rottweiler	100% Rottweiler
KP85	American Staffordshire Terrier (U.S)	100% American Staffordshire Terrier
KP86	American Staffordshire Terrier (U.S)	100% American Staffordshire Terrier
KP87	American Staffordshire Terrier (U.S)**	62,5% American Staffordshire Terrier, 12,5% Bullmastiff, 12,5% Chow Chow, 12,5% Mixed-breed Groups (guard, sporting, Asian)

KP88	American Staffordshire Terrier (U.S)**	25% American Staffordshire Terrier, 25% Rottweiler, 12,5% Bull Terrier (miniature), 12,5% Bulldog (standard), 25% Mixed- breed Groups (hound, sighthound, sporting, herding)
KP89	American Staffordshire Terrier (U.S)**	50% American Staffordshire Terrier, 12,5% Golden Retriever, 12,5% Rottweiler, 25% Mixed-breed Groups (guard, Asian, companion)
KP90	American Staffordshire Terrier (U.S)	100% American Staffordshire Terrier
KP91	American Staffordshire Terrier	100% American Staffordshire Terrier
KP92	American Staffordshire Terrier	100% American Staffordshire Terrier
KP93	Beagle	100% Beagle
KP94	Beagle	100% Beagle
KP95	Bernese Mountain Dog	100% Bernese Mountain Dog
KP96	Bernese Mountain Dog	100% Bernese Mountain Dog
KP97	Bernese Mountain Dog	100% Bernese Mountain Dog
KP98	Broholmer	37,5% Newfoundland, 12,5% American Bulldog, 12,5% German Wirehaired Pointer, 12,5% Vizsla, 12,5% White Swiss Shepherd, 12,5% Mixed-breed Groups (terrier, sporting, guard)
KP99	Havanese*	87,5% Havanese, 12,5% Maltese
KP100	Havenese	100% Havanese
KP101	Mixed	50% Labrador Retriever, 50% Bouvier des Flandres
KP102	Mixed	50% Labrador Retriever, 50% Bouvier des Flandres
KP103	Bull Terrier	50% Bull Terrier (Standard), 50% Bull Terrier (Miniature)
KP104	Bull Terrier	75% Bull Terrier (Standard), 25% Bull Terrier (Miniature)
KP105	Bull Terrier	100% Bull Terrier (Standard)
KP106	Cavapoo*	100% Cavalier King Charles Spaniel
KP107	Cavapoo*	50% Poodle (Miniature), 25% Poodle (Toy), 25% Mixed-breed Groups (Companion, Terrier)
KP108	Cavalier King Charles Spaniel	100% Cavalier King Charles Spaniel
KP109	Cavalier King Charles Spaniel	100% Cavalier King Charles Spaniel
KP110	Cavalier King Charles Spaniel	100% Cavalier King Charles Spaniel
KP111	Dogue de Bordeaux	100% Dogue de Bordeaux
KP112	Dogue de Bordeaux	100% Dogue de Bordeaux
KP113	Poodle (toy)	100% Poodle (Toy)
KP114	Poodle (toy)	62,5% Poodle (Miniature), 37,5% Poodle (Toy)
KP115	Daschhund*	25% Dachshund (Miniature Shorthaired), 25% Dachshund (Miniature Wirehaired), 25% Dachshund (Wirehaired), 12,5%

		Dachshund (Miniature Longhaired), 12,5% Mixed-breed Groups (sporting, companion, terrier)
KP116	Daschhund	37,5% Dachshund(Longhaired), 37,5% Dachshund (Miniature Longhaired), 25% Dachshund (Miniature Shorthaired)
KP117	Daschhund	50% Dachshund (Longhaired), 37,5% Dachshund (Miniature Longhaired), 12,5% Dachshund (Miniature Shorthaired)
KP118	Greyhound*	100% Munsterlander (Large)
KP119	Greyhound	100% Greyhound
KP120	Greyhound	100% Greyhound
KP121	Large Münsterlander*	25% German Shepherd Dog, 25% Golden Retriever, 12,5% Chow Chow, 12,5% Collie, 12,5% German Shorthaired Pointer, 12,5% Mixed-breed Groups (herding, mountain dogs, sporting, Asian)
KP122	Gross Spitz***	12,5% German Spitz, 12,5% Keeshond, 12,5% Rhodesian Ridgeback, 62,5% Mixed-breed (herding, terrier, mountain dogs, hound)
KP123	Greenland Dog***	75% Canadian Eskimo Dog, 12,5% Alaskan Malamute, 12,5% Siberian Husky
KP124	Greenland Dog***	75% Canadian Eskimo Dog, 12,5% Alaskan Malamute, 12,5% Siberian Husky
KP125	Samoyed	100% Samoyed
KP126	Greater Swiss Mountain Dog	100% Greater Swiss Mountain Dog
KP127	Hovawart	100% Hovawart
KP128	Irish Glen of Imaal Terrier	100% Glen of Imaal Terrier
KP129	Irish Glen of Imaal Terrier	100% Glen of Imaal Terrier
KP130	Irish Wolfhound*	50% German Shepherd Dog, 50% Irish Wolfhound
KP131	Irish Wolfhound	100% Irish Wolfhound
KP132	Jack Russel Terrier*	37,5% Parson Russel Terrier, 25% Russel Terrier, 12,5% Fox Terrier (smooth), 25% Mixed-breed Groups (terrier, sporting, companion)
KP133	Jack Russel Terrier	50% Parson Russel Terrier, 50% Russel Terrier
KP134	Jack Russel Terrier*	62,5% Russel Terrier, 12,5% Parson Russel Terrier, 25% Mixed-breed Groups (terrier, sporting, herding)
KP135	German Shorthaired Pointer	100% German Shorthaired Pointer
KP136	German Shorthaired Pointer	100% German Shorthaired Pointer
KP137	German Shorthaired Pointer	100% German Shorthaired Pointer
KP138	Lagotto Romagnolo	100% Lagotto Romagnolo
KP139	Lagotto Romagnolo	100% Lagotto Romagnolo
KP140	Landseer***	50% Newfoundland, 12,5% Poodle (standard), 12,5% Saint Bernard, 25%, Mixed-breed Groups (sporting, guard, sighthound)
KP141	Leonberger	100% Leonberger

KP142	Maltese	100% Maltese
KP143	Maltese	100% Maltese
KP144	Neapolitan Mastiff	100% Neapolitan Mastiff
KP145	Neapolitan Mastiff	100% Neapolitan Mastiff
KP146	Newfoundland	100% Newfoundland
KP147	Newfoundland	100% Newfoundland
KP148	Newfoundland	100% Newfoundland
KP149	Polski Owczarek Podhalanski***	25% Kritikos Lagonikos, 12,5% English Setter, 62,5% Mixed-breed Groups (Mountain dogs, Middle East and African, Sporting, Asian, Herding)
KP150	Polski Owczarek Podhalanski***	12,5% Kuvasz, 12,5% Schipperke, 12,5% White Swiss Shepherd, 62,5% Mixed-breed Goups (companion, herding)
KP151	Great Pyrenees	Sample failed
KP152	Great Pyrenees	100% Great Pyrenees
KP153	Great Pyrenees	Sample failed
KP154	Samoyed	100% Samoyed
KP155	Samoyed	100% Samoyed
KP156	Scharpendos***	12,5% English Cocker Spaniel, 12,5% Irish Water Spaniel, 75% Mixed-breed Groups (herding, Middle East and African, sporting, terrier)
KP157	Scharpendos***	25% Dutch Shepherd Dog, 12,5% Puli, 62,5% Mixed-breed Groups (terrier, sporting, herding)
KP158	Scharpendos***	12,5% English Cocker Spaniel, 12,5% Parson Russel Terrier, 75% Mixed-breed Groups (sporting, herding)
KP159	Belgian Tervueren	100% Belgian Sheepdog
KP160	Belgian Tervueren	50% Belgian Sheepdog, 50% Belgian Tervuren
KP161	Belgian Tervueren	75% Belgian Sheepdog, 25% Belgian Tervuren
KP162	West Highland White Terrier	100% West Highland White Terrier
KP163	West Highland White Terrier	100% West Highland White Terrier
KP164	Whippet	100% Whippet
KP165	Whippet	100% Whippet
KP166	Xoloitzquintli*	25% Chihuahua, 12,5% Boykin Spaniel, 12,5% Manchester Terrier (Toy), 50% Mixed-breed Groups (companion, terrier, hound)
KP167	Xoloitzquintli*	25% Chihuahua, 25% Yorkshire Terrier, 12,5% Manchester Terrier (Toy), 37,5% Mixed-breed Groups (herding, sporting, companion, Middle East and African)
KP168	Eurasier***	37,5% Chow Chow, 25% Samoyed, 12,5% Keeshond, 25% Mixed-breed (Middle East and African, guard, sporting)
KP169	Eurasier***	62,5% Chow Chow, 12,5% Keeshond, 12,5% Korean Jindo, 12,5% Samoyed

KP170	Eurasier***	50% Chow Chow, 25% Samoyed, 12,5% Keeshond, 12,5% Mixed-breed Groups (Middle East and African, companion, sporting, guard)
KP171	Mixed	37,5% Labrador Retriever, 25% German Shepherd, 12,5% Golden Retriever, 12,5% Rottweiler, 12,5% Samoyed
KP172	Mixed puppy from police	25% American Staffordshire Terrier, 25% American Bulldog, 12,5% Boxer, 12,5% Cane Corso, 12,5% Golden Retriever, 12,5% Labrador Retriever
KP173	Mixed puppy from police	25% American Staffordshire Terrier, 25% Cane Corso, 12,5% Boxer, 12,5% American Bulldog, 12,5% Bullmastiff, 12,5% Labrador Retriever
KP174	Mixed puppy from police	37,5% American Staffordshire Terrier, 25% Cane Corso, 12,5% Boxer, 12,5% American Bulldog, 12,5% Labrador Retriever
KP175	Mixed puppy from police	37,5% American Staffordshire Terrier, 25% Cane Corso, 12,5% American Bulldog, 12,5% Bullmastiff, 12,5% Labrador Retriever
KP176	Mixed puppy from police	25% American Staffordshire Terrier, 25% American Bulldog, 25% Cane Corso, 12,5% Labrador Retriever, 12,5% Mixed-breed Groups (sporting, guard, terrier)
KP177	Mixed puppy from police	37,5% American Staffordshire Terrier, 25% American Bulldog, 12,5%Labrador Retriever,25% Mixed-breed Groups (guard,herding)
KP178	Mixed puppy from police	25% American Staffordshire Terrier, 12,5% Boxer, 12,5% American Bulldog, 12,5% Golden Retriever, 12,5% Labrador Retriever, 25% Mixed-breed Groups (guard, herding, sporting)
KP179	Mixed puppy from police	25% American Staffordshire Terrier, 25% American Bulldog, 25% Cane Corso, 12,5% Golden Retriever, 12,5% Labrador Retriever
KP180	Dandie Dinmont Terrier	100% Dandie Dinmont Terrier
KP181	Dandie Dinmont Terrier	100% Dandie Dinmont Terrier
KP182	Siberian Husky	100% Siberian Husky
KP183	Siberian Husky	100% Siberian Husky
KP184	Siberian Husky	100% Siberian Husky
KP185	Mixed	25% Dachshund (Miniature Wirehaired), 25% Shih Tzu, 12,5% Dachshund (Wirehaired), 12,5% Parson Russel Terrier, 12,5% Pekingese, 12,5% Tibetan Spaniel
KP186	Mixed	37,5% Dachshund (Wirehaired), 25% Shih Tzu, 25% Tibetan Spaniel, 12,5% Lhaso Apso
KP187	Saluki	100% Saluki
KP188	Airedal Terrier	100% Airedal Terrier
KP189	Airedal Terrier	100% Airedal Terrier
KP190	Coton de Tulear	100% Coton de Tuelar
KP191	Coton de Tulear	100% Coton de Tuelar
KP192	Coton de Tulear	100% Coton de Tuelar
KP193	Saluki	100% Saluki
KP194	Saluki	100% Saluki

KP195	Broholmer***	25% Newfoundlander, 12,5% Boxer, 12,5% German Wirehaired Pointer, 12,5% Mastiff, 37,5% Mixed-breed Groups (sporting, herding, companion, mountain dogs)
KP196	Broholmer***	25% Boxer, 25% Newfoundlander, 12,5% German Shorthaired Pointer, 12,5% German Wirehaired Pointer, 12,5% White Swiss Shepherd, 12,5% Mixed-breed Groups (terrier, herding, mountain dogs, sporting)
KP197	Broholmer***	37,5% Newfoundlander, 62,5% Mixed-breed Groups (herding, sporting,guard)
KP198	Havanese	100% Havanese

Samples from the Swedish Amstaffs sent to Wisdom Health to be analyzed by Wisdom Panel 4.0:

ID	**Breed**	**Results from Wisdom Panel**
Andy	American Staffordshire Terrier	100% American Staffordshire Terrier
Asko	American Staffordshire Terrier	100% American Staffordshire Terrier
Boss	American Staffordshire Terrier	100% American Staffordshire Terrier
Bruno	American Staffordshire Terrier	100% American Staffordshire Terrier
Ciara	American Staffordshire Terrier	100% American Staffordshire Terrier
Daisy	American Staffordshire Terrier	100% American Staffordshire Terrier
Doris	American Staffordshire Terrier	100% American Staffordshire Terrier
Ella	American Staffordshire Terrier	100% American Staffordshire Terrier
Franko	American Staffordshire Terrier	100% American Staffordshire Terrier
Harry	American Staffordshire Terrier	100% American Staffordshire Terrier
Inka	American Staffordshire Terrier	100% American Staffordshire Terrier
Kenny	American Staffordshire Terrier	100% American Staffordshire Terrier
Loke	American Staffordshire Terrier	100% American Staffordshire Terrier
Maya	American Staffordshire Terrier	100% American Staffordshire Terrier
Nea	American Staffordshire Terrier	100% American Staffordshire Terrier
Oscar	American Staffordshire Terrier	100% American Staffordshire Terrier
Zafira	American Staffordshire Terrier	100% American Staffordshire Terrier
Santos	American Staffordshire Terrier	100% American Staffordshire Terrier
Shanti	American Staffordshire Terrier	100% American Staffordshire Terrier
Stella	American Staffordshire Terrier	100% American Staffordshire Terrier

Appendix 2

Protocol from Section of Animal Genetics, Bioinformatics and breeding, Department of Veterinary and Animal Sciences at University of Copenhagen for DNA extraction with Promega Kit.

Oprensning af DNA fra Swab eller Gyno brush (PROMEGA KIT)

Noter: Anvend filterspidser når der tages fra kittet. Skyl saks samt pincet med ethanol mellem hver prøve.

1. Tænd for ryste-varmeblok på 55 °C
2. Overfør swab'en til et 2,0 mL rør (klip forsigtigt skaftet af swab'en over, så røret kan lukkes) Brug rene og klorbehandlede picetter og sakse, husk at gøre dem rene til næste gang.
3. Tilsæt 900 µL Cell lysis solution og inkuber i 20 min. Vortex prøverne 2-3 gange undervejs.
4. Tag forsigtigt swab'en op og overfør til nyt rent 2,0 mL rør **(NB! Den skal tilbage igen)**
5. Spin v. max speed i 5 min, fjern supernantanten og kom swab'en og evt. rester af væsken tilbage.
6. Tilsæt 600 µL nuclei lysis solution, 15 µL EDTA og 20 µL Proteinase K. (Tilsæt proteinase K i stinkskabet)
7. Inkubér 3 timer ved 55°c v. 700 rpm.
8. Spind kort ned i mini spind og tilsæt 3 µL RNase, vortex. Inkuber v. 37°c i 15 min (Hvis der er kommet blodprøver af forældredyrene, kan de startes her og køres parallelt)
9. Tag swab'en op og smid væk. **NB! Kør den op af siden så al væsken kommen ned i røret.**
10. (Stinkskab!!!) Tilsæt 200 µL Protein Precipitation solution, vortex 20 sek., stå på is i 5 min.
11. Spin v. max speed i 10 min
12. Overfør supernantanten til nyt 2,0 mL rør.
13. Fæld med 650 µL isopropanol. Vend og ryst prøven godt (Stinkskab!!!)
14. Spin 10 min. v. max speed.
15. Fjern supernantanten til affaldsbøtte C2
16. Vask m. 500 µL 70% EtOH, spin 5 min ved max.
17. Fjern supernantanten og lufttørre i 5 min.
18. Genopløs i 16 µl 1xTE buffer.
19. Vortex godt, spin ned og lad stå O.N. v.16 °c på rystebordet mål derefter OD.

Appendix 3

Wisdom Panel report from a purebred dog.

Wisdom Panel® Results

Print Report

Sample ID# EN203794

Maya's Results Are In!

Maya's Unique Genetic Signature

GTTCCCCGGGGNNCCTTCCAAAAACAGGGTTAGAGAAGGCCG
GCCAAAAAAAAAGGGAACCCCAGTCACCCCCAGGGAGTCGGT
CAGGGTTGGAGTCTCAAGGGGAACCTCTAAAGGTCCCAGCCA
GAACGGGAACCAAAATTTTTTAGTTTCAGAAGGAATTGGTTTC
AAAATTAAGGAATTCCNNCCCCCCTCCCCGGGTTAAAATCCCN
NTCCCGGCCCCTTGGTTAAAAGGAATTTTTTTCTCTTAATTAGT
CAGATGGCCAAGGCCGGGGGGGGCCAGTTNNAGAGTCGGG
GAAGGACAAAACCTTTTCCTAAGAGAGTTAAGGAGTTACTTAG
CCGGGGTAAATTAAGGAGTTCCCCAGCCTTTCGGTCGGTCCCA
AGGCCAGGGAGAGTCAGTCGCTTGGGGAGTTTGGGTTAAACA
GTCGGGGCCAGGGGGTCGGTTAGTCAGCCTCTCCCAGTTAGT
TCCCCTTTTTTAAGGTCTCTCAATCCCGGGGGCAACCTCAGAA
GGTTCCAAGGGGCCGGCCGGCCAAGGAACCAGTGAAAACCG
GAGGGGGCCAGGGAATTTTTGGGAATCGGAGGGCCCCAAAG
AGTTGGAAAATTCCCCTGTCAAGGTCCCTGTCCCTCAGAAGGC
CCCNNAATTTTCCAACCAGCCTCGGTTAACCGGAACCAAAGAA
GGTGAGGGAGGGACAGAGGGAGTCAAGGGGAAGGCCAAAC
GGAAAGTCTTAGTGAGCCNNGGACTTGGCCGG

This is a portion of the code—or "fingerprint"—from your dog's DNA that we use as a unique identifier.

Notifications :

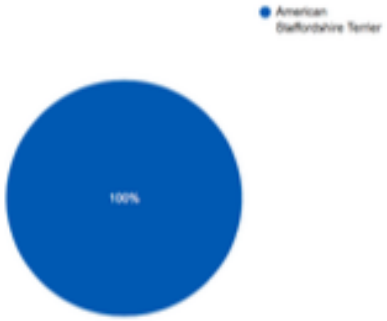
As part of the ***Wisdom Panel*** ® Canine DNA Test analysis, we have considered whether Maya's results are consistent with the purebred American Staffordshire Terrier samples in our database, using a variety of analyses. The results are in! Our findings show that Maya's results are consistent with the purebred American Staffordshire Terrier samples in our database!

Each analysis performed is independent of the others. Analyses vary in specificity and therefore may not always agree with each other. As a result, the overall outcome is based on a weighted average of all individual findings. A more detailed explanation of the specific tests performed on Maya's DNA data can be found on the Breed Tests page of your report.

If you have any questions about this information enclosed, please contact our customer care team at **customercare@marsveterinary.co.uk**.

Best wishes,
Mars Veterinary

Ancestry



How did we get these percentages?

Once your sample is received at our lab it is scanned into our database and assigned to a batch for testing. It then undergoes processing to extract the DNA from your dog's cells, which is examined for the 1800 markers that are used in the tests. The results of these markers are sent to a computer that evaluates them using an algorithm designed to consider all of the pedigree trees that are possible in the last three generations for your dog. Our computer algorithm uses information from our extensive breed database of more than 12,000 samples to analyze these potential pedigrees and determine which one is the best fit.

The computer algorithm gives each of the 18,000,000+ combinations of ancestry trees built and considered, a score representing how well that selected combination of breeds matches your dog's data. The pedigree with the overall best score is selected and provided to you in your dog's individualized report. It normally takes 2-3 weeks from the time a sample is received for the genetic testing and analysis to be completed.

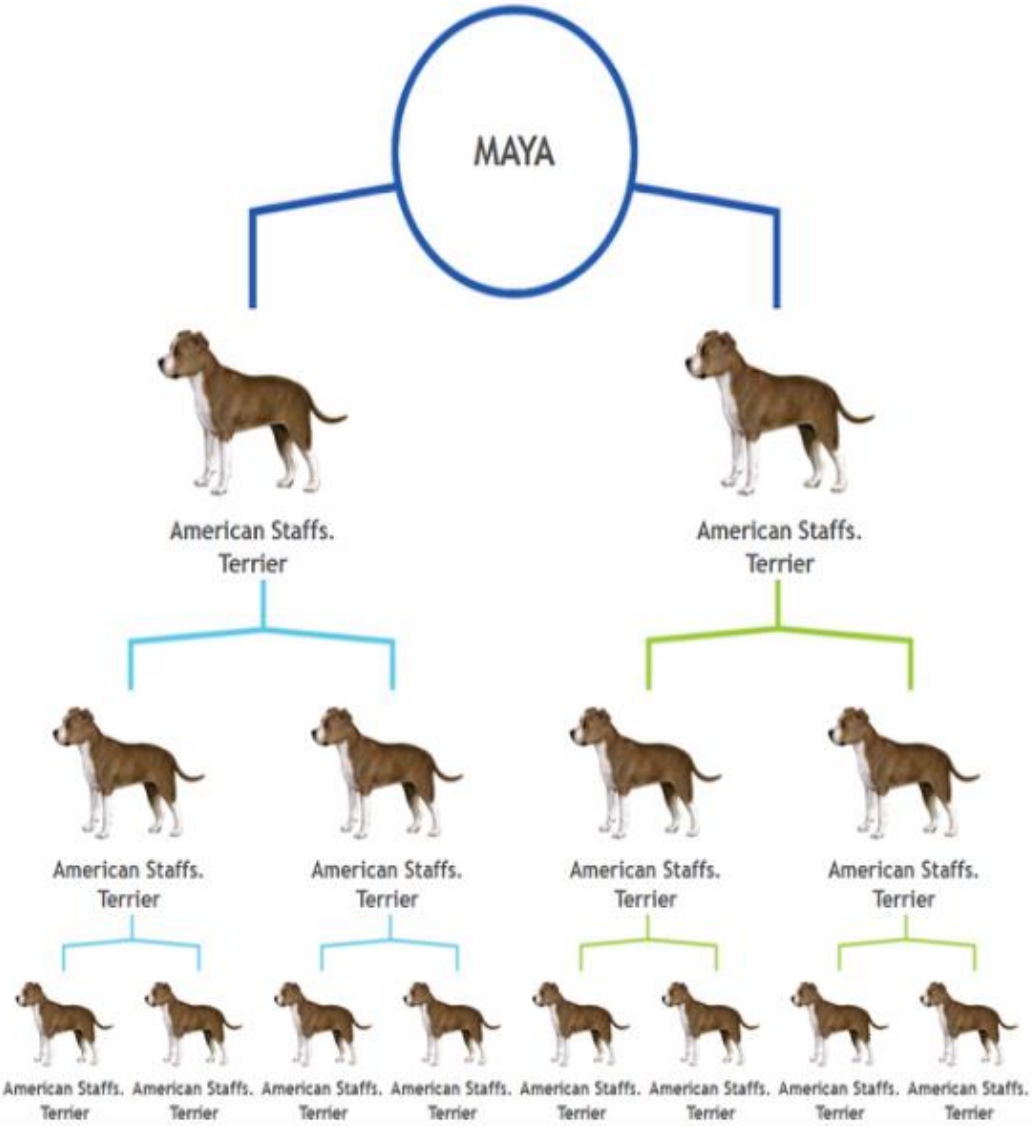
In addition, the **Wisdom Panel** 2.5, 3.0 and 4.0 versions screen for the MDR1 genetic mutation that affects drug sensitivity. This mutation in the MDR1 or Multi-Drug Resistance 1 gene is found in many of the herding breeds. The MDR1 gene is responsible for production of a protein called P-glycoprotein. The P-glycoprotein molecule is a drug transport pump that plays an important role in limiting drug absorption and distribution (particularly to the brain) and enhancing the excretion of many drugs used in dogs. As a result, dogs with this mutation may have adverse reactions to some common drugs, so it is important to test your dog and share your results with your veterinarian so they can provide you with the best possible care.

Wisdom Panel 4.0 also screens for Exercise-induced Collapse (EIC) and specific phenotypic traits (visual characteristics of the dog). EIC is most commonly found in some retrieving breeds or mixed-breed dogs with retrieving ancestry and is an inherited disorder of nerve and muscle that was first identified in Labrador Retrievers. It is caused by a mutation in the DNM1 gene, and is characterized by exercise intolerance in otherwise normal dogs. You will want to make sure to share these results with your veterinarian so they can update your dog's records. They may be critical to the care and everyday health of your dog.

The results are in! Our findings show that Maya's results are consistent with the purebred American Staffordshire Terrier samples in our database.

The **Wisdom Panel** computer algorithm performed over 18 million calculations using 11 different models (from a single breed to complex combinations of breeds) to predict the most likely combination of pure and mixed breed dogs in the last three ancestral generations that best fit the DNA marker pattern observed in Maya. Here you will find the results of these calculations. To view more about the breed(s) found in your dog, click on the individual breed name(s) next to the pie chart. You can also continue onto the next page to see this same information in ancestry tree format.

Ancestry Tree



American Staffordshire Terrier



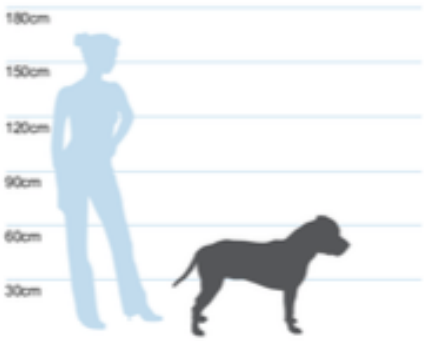
Description

The American Staffordshire Terrier can trace its roots all the way back to the nineteenth century in England. The English Staffordshire Terrier was created when various terriers were crossed with the Bulldog. The crossbreeding resulted in the active and powerful breed that came to the attention of the United States public in 1870. American breeders then focused their attention and efforts to increasing the size and weight of the American Staffordshire Terrier. These breeding efforts resulted in a Staffordshire Terrier that was recognised as a separate breed by the American Kennel Club in 1936. In 1972, the current name of "American Staffordshire Terrier" was adopted. The American Staffordshire Terrier was once used primarily for fighting. That practice, however, was banned in the early 1900's and two separate variations of the breed were gradually developed.

- Intelligent, hard-working, and stoic dogs.
- Very loyal to family and usually good with children. May not get along well with other dogs.
- American Staffordshire Terriers seem to enjoy dog sports such as agility, flyball, rally and competitive obedience.
- The American Staffordshire Terrier was originally bred for various purposes including the guarding of people and/or property. Individual members of this breed will benefit from firm and dedicated training to temper this guarding tendency, and thus help to maintain safety as a household companion.

All dogs should be considered individual animals. Because each is a product of their unique environment and handling, they may exhibit different traits and behaviors than those listed here.


Appearance



Height: 43 - 48 cm
Weight (Show): 18 - 27 kg
Weight (Pet): 17 - 31 kg

Ear

Muzzle

Tail


Certificate

Wisdom Panel®

MARS veterinary

STATEMENT OF AUTHENTICATION

Owner's name: **Laura Pedersen**

Dog's name: **Maya**

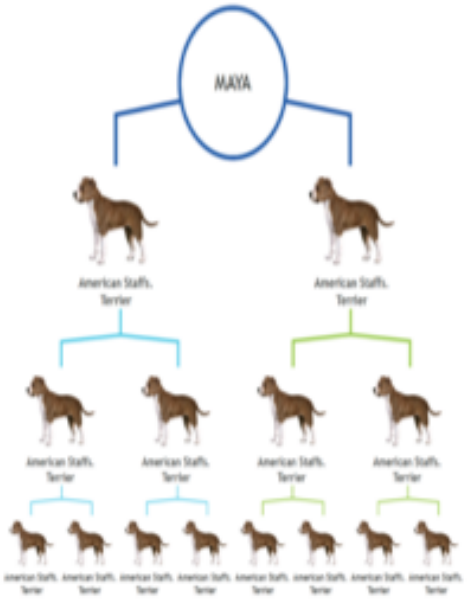
Date: **December 6, 2017**

This certifies the authenticity of Maya's canine genetic background as determined following careful analysis of more than 1800 genetic markers using **Wisdom Panel**. The purebred dog breed signature matches included in this analysis are those that were detected in the last three generations of Maya's ancestry using the proprietary breed detection algorithm at Mars Veterinary.

Cynthia Cole

Dr. Cynthia Cole DVM, PhD, DACVCP

Research & Development Director



Adult Weight

One of the main questions people ask when adopting a mixed-breed puppy is "What size will my puppy grow up to be?" To help answer this question we've developed a proprietary algorithm that uses the breeds identified on each chromosome in a mixed-breed dog to predict the likely size range a puppy will develop into when fully mature. For adult dogs this weight prediction along with recommendations from your veterinarian can be used to maintain a healthy weight for you dog.

Maya's predicted adult weight is:

- **Between 17 - 27 kg**

How is Weight Calculated?

Our weight-predictive algorithm has been developed and calibrated using a combination of important elements:

- The published weight ranges of more than 200 purebred dog breeds.
- The observed weights of more than 100,000 client-owned purebred dogs, each with an ideal Body Condition Score.
- Dog breeds identified by Wisdom Panel that reflects a dog's true heritage and genetic complexity.
- A genetic algorithm developed and optimized using observed mixed-breed dog data that serves to weight the real contribution of each set of chromosomal genetic markers to the final predicted weight of an adult dog.
- The observed weights of over 800 mixed-breed dogs of varied backgrounds.

Environmental Effects on Weight

A dog's early life is extremely important in determining its overall growth potential and final adult weight. Some key factors that can decrease a dog's weight from the expected include:

- Nutrition of dam (mom) during pregnancy and nursing
- Nutrition of puppy in critical first year during period of rapid growth and development
- Illness/disease
- Heavy parasite load either internal (eg.roundworms) OR external (eg. fleas, ticks)

Differences in Body Weight

We've factored everything we know about Maya in predicting a healthy adult weight. However there are several other factors that can play an important role. Through the use of your report information and by working with your veterinarian you can help your dog reach and/or maintain a healthy adult body weight.

Breed Tests

Single Breed PCA Test: PASS

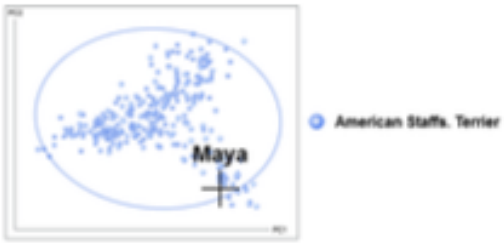
Principle Component Analysis (PCA) allows us to see how similar samples are. Closely related samples, like dogs from the same breed, are expected to be closer together than samples from other breeds. This tends to create a cluster of points for each breed or sub-population within a breed. If a sample is within the cluster for the breed, this is a very good indication that it is likely a pure member of this breed. The PCA below shows Maya's data compared to American Staffordshire Terrier samples from the **Wisdom Panel** database. The sample falls within the American Staffordshire Terrier cluster indicating that its genetic profile is consistent with other dogs from this breed.

All Breeds PCA Test: PASS

The PCA below shows Maya's data compared to samples of American Staffordshire Terrier, as well as an "All Breeds" outgroup comprised of a single representative sample from the other breeds in the **Wisdom Panel** database. This is another way of verifying that Maya's data is more consistent with the American Staffordshire Terrier than with any other breed and here the sample is clustering closely with the purebred American Staffordshire Terrier cluster.

Next Closest Breed(s) PCA Test: PASS

The PCA below shows Maya's data compared to American Staffordshire Terrier samples and, when applicable, the samples of the next two best matched breeds from the ancestry analysis. In some cases, a dog's genetic signature matches the signature of the primary breed so closely that while the other breeds are considered, statistically they have no bearing on the overall results and are therefore not illustrated. In this case only the primary breed and sometimes one other will be shown. Maya's DNA sample clusters tightly with the purebred American Staffordshire Terrier cluster. This is what we would expect from a purebred American Staffordshire Terrier.

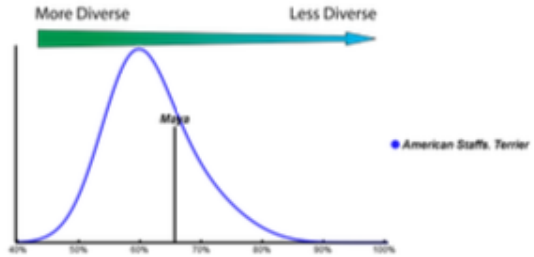


Pairwise Overall Breed Test: PASS

A pairwise comparison test was performed between Maya's DNA data and that of each of more than 12,000 dogs in the **Wisdom Panel** purebred dog database. In this analysis the top matching breed detected is the American Staffordshire Terrier.

Homozygosity Profile: PASS

Homozygosity is a measure of how many of Maya's genetic markers are identical because both the sire and dam passed down the same marker variant. Purebred dogs tend to have a higher homozygosity than most mixed breed dogs. Each breed within the **Wisdom Panel** database has a specific range of homozygosity scores. Maya's homozygosity score falls within the range that is seen for purebred American Staffordshire Terrier samples.



Appendix 4

Wisdom Panel report from a mixed-breed dog.

Wisdom Panel® Results

Print Report

Sample ID# UCO0175

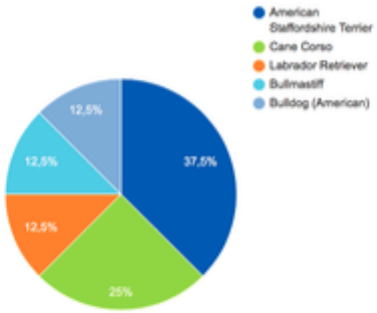
0175's Results Are In!

0175's Unique Genetic Signature

GTTCCCCGGGGNNACTGCGAGAAACAAAATCGGAGAAGGCC
GGCCAAAGAAAGAGAGGGTCCCAGTTCCCCCCAGAGAGTCTT
TCAGGGTCGGAGCCTTAAAGGGAACCTCTAAGAGTCCCAGTC
GGAGCGTGAAACAAAATTTGTCAGTCCCAGACGGAGGGCCTT
TCCCAGTCAGGGACGGTTNNTCTCCCTTTCCGAGTCAAAGCCC
CNNTCCCGGACACTTGGTTAAAGAGAAATTTTGTCTCATAGTT
AACCAAATAGAAGGAATCAGAGAGAGCCAATTNNAAAGTCGG
GGAGGGAAACAGCCTCTTCCTAAAAGAGTTCCTGAACCACTTA
GCCAGAGAAAGTTAAGGGGTCCCCCAGTCTCTTGGTCGGCCC
CGGAGCCAAGGAGAGTCGGCCGCTTGGAGAGTGTTGGTCAG
ACGGTCTGAGTTGGAGAGCCGGTTGGTCAGGCTTTCTTAATCA
ATCCCTCTCTTTTAGGGCCTCTCACTCAAAGGGGCGGCCTCAA
AGGGTTCCCCTGGGCCAGCCAGTCAATGACGCGGGGAGAGA
CGGAGAGAGCCGGGGAGTCTCGGGGGGGTTAAGGGGGGCCA
AAAAGCCAAAGAGTTTCCCGGTCAAGGTTCCTTCCCCTCAAAA
TGACTCNNAACCTGTCGGCCAGTCTTGGGGGGCCGGAAGGA
AAGAAAGTGAGAGAGAGCCAGGGAGAGTTAAGGCGAAAGCC
AAAATGAGAATCTCAGTTAGCCNNAGAATCAGCCGG

This is a portion of the code—or "fingerprint"—from your dog's DNA that we use as a unique identifier.

Ancestry



How did we get these percentages?

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The computer algorithm gives each of the 18,000,000+ combinations of ancestry trees built and considered, a score representing how well that selected combination of breeds matches your dog's data. The pedigree with the overall best score is selected and provided to you in your dog's individualized report. It normally takes 2-3 weeks from the time a sample is received for the genetic testing and analysis to be completed.

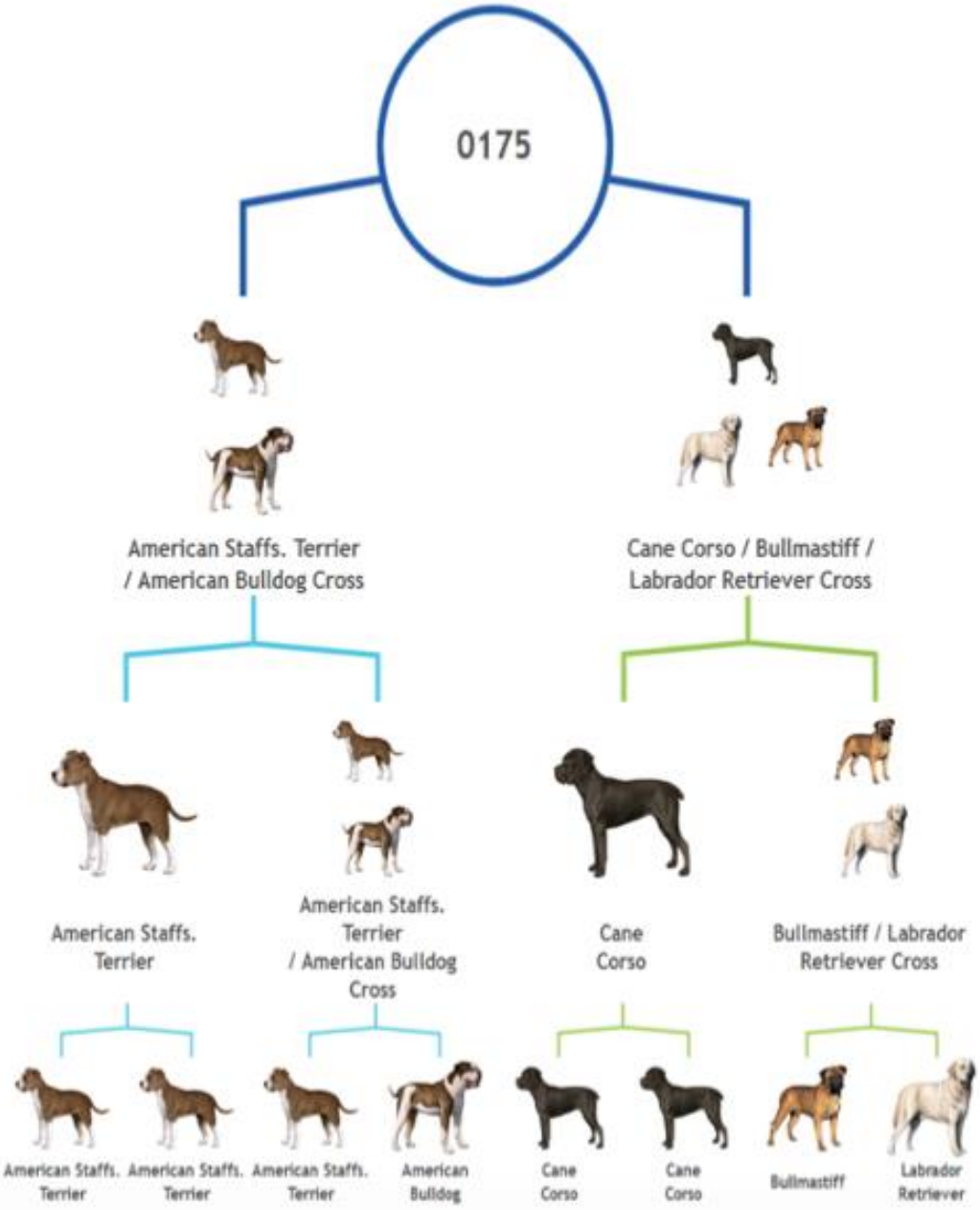
In addition, the **Wisdom Panel** 2.5, 3.0 and 4.0 versions screen for the MDR1 genetic mutation that affects drug sensitivity. This mutation in the MDR1 or Multi-Drug Resistance 1 gene is found in many of the herding breeds. The MDR1 gene is responsible for production of a protein called P-glycoprotein. The P-glycoprotein molecule is a drug transport pump that plays an important role in limiting drug absorption and distribution (particularly to the brain) and enhancing the excretion of many drugs used in dogs. As a result, dogs with this mutation may have adverse reactions to some common drugs, so it is important to test your dog and share your results with your veterinarian so they can provide you with the best possible care.

Wisdom Panel 4.0 also screens for Exercise-induced Collapse (EIC) and specific phenotypic traits (visual characteristics of the dog). EIC is most commonly found in some retrieving breeds or mixed-breed dogs with retrieving ancestry and is an inherited disorder of nerve and muscle that was first identified in Labrador Retrievers. It is caused by a mutation in the DNM1 gene, and is characterized by exercise intolerance in otherwise normal dogs. You will want to make sure to share these results with your veterinarian so they can update your dog's records. They may be critical to the care and everyday health of your dog.

Breed Percentages

The **Wisdom Panel** computer algorithm performed over 18 million calculations using 11 different models (from a single breed to complex combinations of breeds) to predict the most likely combination of pure and mixed-breed dogs in the last three ancestral generations that best fit the DNA marker pattern observed in 0175. Here you will find the results of these calculations. To view more about the breed(s) found in your dog, click on the individual breed name(s) next to the pie chart. You can also continue onto the next page to see this same information in ancestry tree format.

Ancestry Tree



American Staffordshire Terrier



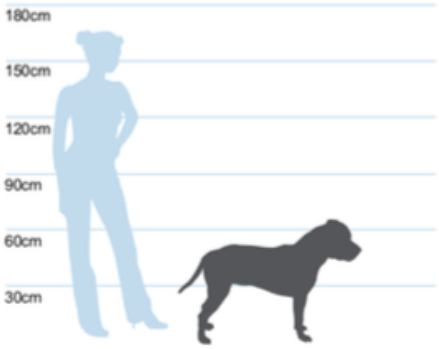
Description

The American Staffordshire Terrier can trace its roots all the way back to the nineteenth century in England. The English Staffordshire Terrier was created when various terriers were crossed with the Bulldog. The crossbreeding resulted in the active and powerful breed that came to the attention of the United States public in 1870. American breeders then focused their attention and efforts to increasing the size and weight of the American Staffordshire Terrier. These breeding efforts resulted in a Staffordshire Terrier that was recognised as a separate breed by the American Kennel Club in 1936. In 1972, the current name of "American Staffordshire Terrier" was adopted. The American Staffordshire Terrier was once used primarily for fighting. That practice, however, was banned in the early 1900's and two separate variations of the breed were gradually developed.

- Intelligent, hard-working, and stoic dogs.
- Very loyal to family and usually good with children. May not get along well with other dogs.
- American Staffordshire Terriers seem to enjoy dog sports such as agility, flyball, rally and competitive obedience.
- The American Staffordshire Terrier was originally bred for various purposes including the guarding of people and/or property. Individual members of this breed will benefit from firm and dedicated training to temper this guarding tendency, and thus help to maintain safety as a household companion.

All dogs should be considered individual animals. Because each is a product of their unique environment and handling, they may exhibit different traits and behaviors than those listed here.


Appearance



Height: 43 - 48 cm

Weight (Show): 18 - 27 kg

Weight (Pet): 17 - 31 kg

Ear

Muzzle

Tail

Cane Corso





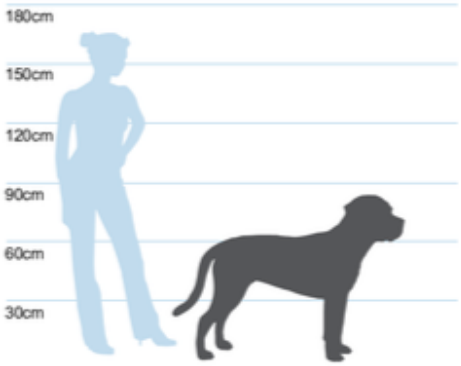
Description

The Cane Corso comes from Italy and is a likely ancestor of the Roman Molosser dogs. The breed was primarily used as a catch dog, a task where the dog is used to subdue a boar or another wild animal until the farmer arrived. They may also have been used as guard dogs. The name is thought to come from the Latin "Cohors", which could either refer to a farmyard, enclosure or corral, or a bodyguard. The breed was fully recognised by the American Kennel Club in 2010 and is a member of the working group.

- Require good socialization when young and benefit from firm handling and an experienced owner.
- Usually very Intelligent and highly trainable dogs.
- The Cane Corso was originally bred for various purposes including the guarding of people and/or property. Individual members of this breed will benefit from firm and dedicated training to temper this guarding tendency, and thus help to maintain safety as a household companion.
- Very loyal to family and usually good with children though need careful supervision. May not get along well with other dogs.

All dogs should be considered individual animals. Because each is a product of their unique environment and handling, they may exhibit different traits and behaviors than those listed here.

Appearance



Height: 58 - 71 cm
Weight (Show): 40 - 50 kg
Weight (Pet): 36 - 54 kg

Ear

Muzzle

Tail


Labrador Retriever



Description

The Labrador Retriever can trace its roots to the coast of Newfoundland, Canada. The breed dates back to at least the seventeenth century when they were known as the "Lesser Newfoundland." The breed is believed to have descended from the extinct "St. John's Water Dog" which was a cross between native water dogs and the Newfoundland. Labrador Retrievers were initially trained to retrieve fishing nets from the cold waters of the North Atlantic. Fisherman brought them to England in the nineteenth century where they were lauded for their swimming, retrieving and hunting skills. The Earl of Malmesbury is believed to have coined the name Labrador in order to differentiate them from their Newfoundland ancestors. During the 1800's, a heavy dog tax in Canada and quarantine laws in Britain drastically cut the number of Labradors in the U.K., but a good breeding program replenished the stock.

- Usually happy-go-lucky, calm, or easygoing dogs, though some may be energetic.
- Usually friendly and are generally good family dogs.
- Labrador Retrievers enjoy dog sports such as agility, hunting, tracking, rally and competitive obedience; retrieving and swimming.
- Quite food motivated, which may make it easier to teach the Labrador Retriever to drop retrieved items not intended for play.

All dogs should be considered individual animals. Because each is a product of their unique environment and handling, they may exhibit different traits and behaviors than those listed here.


Appearance

Height: 53 - 61 cm

Weight (Show): 25 - 30 kg

Weight (Pet): 22 - 35 kg

Ear

Muzzle

Tail

Bullmastiff



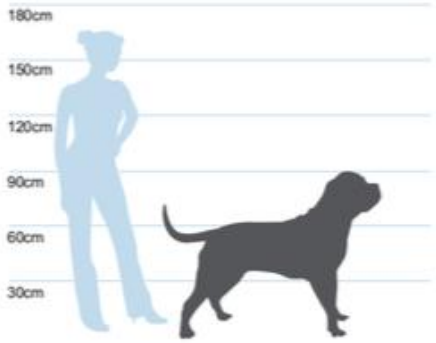
Description

The history of the Bullmastiff dates back to the mid-nineteenth century when English estate owners found themselves in need of a guard dog that would protect their properties from poachers. Breeders began crossing English Bulldogs with Mastiffs and the Bullmastiff is a result of those efforts. The goal of the breeders was to develop a powerful breed that would be capable of knocking down an intruder and holding that intruder to the ground until its master arrived. The efforts were successful and the Bullmastiff was so good at its job, it was often referred to as the gamekeeper's night dog.

- Intelligent, alert, and powerful dogs.
- Enjoy dog sports such as agility, tracking, carting, rally obedience, and competitive obedience to provide physical and mental stimulation.
- Respond well to a reward-based approach to training to reduce any stubbornness.
- Because of their background as guard dogs, may be aloof around strangers or other dogs.

All dogs should be considered individual animals. Because each is a product of their unique environment and handling, they may exhibit different traits and behaviors than those listed here.


Appearance



Height: 61 - 69 cm

Weight (Show): 41 - 59 kg

Weight (Pet): 35 - 65 kg

Ear

Muzzle

Tail

Bulldog (American)



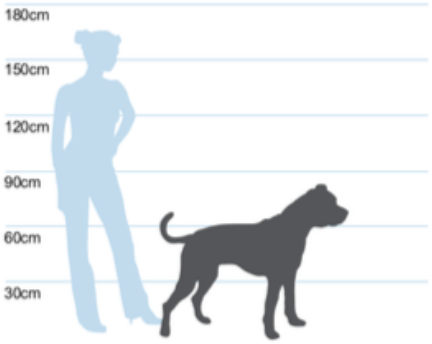
Description

This breed is the closest surviving relative of the Old English Bulldog, which were used as working and guard dogs. The dogs moved with their owners across to South America, where the American Bulldog was developed. In the late 1940's the Bulldogs were near extinction, but due to the breeding programs set up by John Johnson and Alan Scott, the American Bulldog breed was preserved. There are two types named after the breeders, the Johnson, known as the classic or bully type, and the Scott type, which is also referred to as the standard or performance type. The Johnson type is a large dog with a shorter muzzle and the Scott type is smaller with a longer muzzle. Today's dog is a mixture of the two types. The American Bulldog is used as a guard, hunting and working dog. The breed was first officially recognised and registered by The National Kennel Club in 1970. The American Bulldog Association was established in 1989, and a year later the United Kennel Club recognised the breed in the working class.

- Strong, alert, self-confident, physically active dogs that require daily mental and physical activity to prevent hyperactivity and difficulty in handling.
- Forms strong family bonds and possesses strong protective instincts. There have been reported incidents of American Bulldogs being aggressive with other pets or people.
- Requires firm, consistent obedience training and socialization to prevent reservation with strangers and to ensure the dog is controllable in all situations.
- Can be wary of cats and other small pets and may react to strange dogs, especially those of the same sex. This can be reduced through early socialization.

All dogs should be considered individual animals. Because each is a product of their unique environment and handling, they may exhibit different traits and behaviors than those listed here.


Appearance



Height: 51 - 71 cm

Weight (Show): 27 - 54 kg

Weight (Pet): 28 - 54 kg

Ear

Muzzle

Tail

Certificate

Wisdom Panel®

BREED ANCESTRY CERTIFICATE

MARS veterinary

STATEMENT OF AUTHENTICATION

Owner's name: **Laura Pedersen**

Dog's name: **0175**

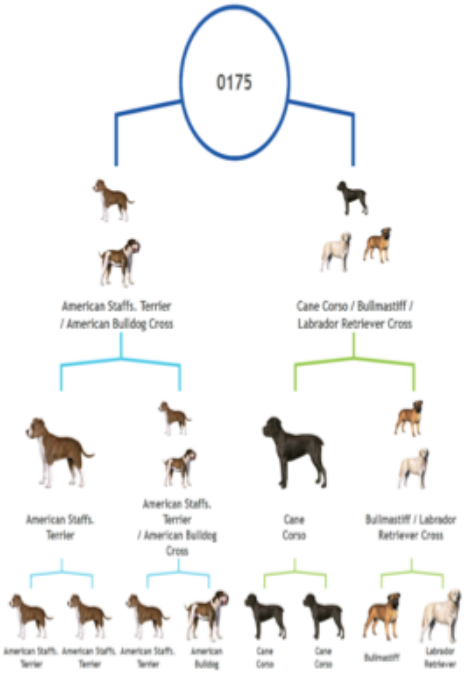
Date: **December 27, 2017**

This certifies the authenticity of 0175's canine genetic background as determined following careful analysis of more than 1800 genetic markers using **Wisdom Panel**. The purebred dog breed signature matches included in this analysis are those that were detected in the last three generations of 0175's ancestry using the proprietary breed detection algorithm at Mars Veterinary.

Cynthia Cole

Dr. Cynthia Cole DVM, PhD, DACVCP

Research & Development Director



Adult Weight

One of the main questions people ask when adopting a mixed-breed puppy is "What size will my puppy grow up to be?" To help answer this question we've developed a proprietary algorithm that uses the breeds identified on each chromosome in a mixed-breed dog to predict the likely size range a puppy will develop into when fully mature. For adult dogs this weight prediction along with recommendations from your veterinarian can be used to maintain a healthy weight for you dog.

0175's predicted adult weight is:

- **Between 28 - 45 kg**

How is Weight Calculated?

Our weight-predictive algorithm has been developed and calibrated using a combination of important elements:

- The published weight ranges of more than 200 purebred dog breeds.
- The observed weights of more than 100,000 client-owned purebred dogs, each with an ideal Body Condition Score.
- Dog breeds identified by Wisdom Panel that reflects a dog's true heritage and genetic complexity.
- A genetic algorithm developed and optimized using observed mixed-breed dog data that serves to weight the real contribution of each set of chromosomal genetic markers to the final predicted weight of an adult dog.
- The observed weights of over 800 mixed-breed dogs of varied backgrounds.

Environmental Effects on Weight

A dog's early life is extremely important in determining its overall growth potential and final adult weight. Some key factors that can decrease a dog's weight from the expected include:

- Nutrition of dam (mom) during pregnancy and nursing
- Nutrition of puppy in critical first year during period of rapid growth and development
- Illness/disease
- Heavy parasite load either internal (eg.roundworms) OR external (eg. fleas, ticks)

Differences in Body Weight

We've factored everything we know about 0175 in predicting a healthy adult weight. However there are several other factors that can play an important role. Through the use of your report information and by working with your veterinarian you can help your dog reach and/or maintain a healthy adult body weight.

Appendix 5

Complete list of alleles found by microsatellite genotyping in the Swedish and American Amstaff samples.

Samples marked with red were not included in the analysis.

Sample Name	Marker	Allele 1	Allele 2
Andy	AHT121	94	96
Asko	AHT121	98	104
Boss	AHT121	98	104
Bruno	AHT121	100	
Ciara	AHT121	94	104
Daisy	AHT121	96	
Doris	AHT121	94	
Ella	AHT121	98	104
Franko	AHT121	94	104
Harry	AHT121	94	96
Inka	AHT121	96	98
KP85	AHT121	96	98
KP86	AHT121	96	104
KP87	AHT121	94	100
KP88	AHT121	98	
KP89	AHT121		
KP90	AHT121	96	102
Kenny	AHT121	100	108
Loke	AHT121	98	108
Maya	AHT121	98	
Nea	AHT121	96	98
Oscar	AHT121	98	104
Santos	AHT121	104	
Shanti	AHT121	98	104
Stella	AHT121	98	104
Vand	AHT121		
Zafira	AHT121	98	104

Sample Name	Marker	Allele 1	Allele 2
Andy	INRA21	95	
Asko	INRA21	95	
Boss	INRA21	95	
Bruno	INRA21	95	
Ciara	INRA21	95	
Daisy	INRA21	95	
Doris	INRA21	95	113
Ella	INRA21	95	
Franko	INRA21	95	97
Harry	INRA21	95	113
Inka	INRA21	95	
KP85	INRA21	101	
KP86	INRA21	95	97
KP87	INRA21	95	101
KP88	INRA21	95	99
KP89	INRA21		
KP90	INRA21	95	
Kenny	INRA21	95	
Loke	INRA21	95	
Maya	INRA21	95	
Nea	INRA21	95	
Oscar	INRA21	95	
Santos	INRA21	95	
Shanti	INRA21	95	
Stella	INRA21	95	
Vand	INRA21		
Zafira	INRA21	95	

Sample Name	Marker	Allele 1	Allele 2
Andy	AHT137	137	147
Asko	AHT137	137	147
Boss	AHT137	137	147
Bruno	AHT137	147	?
Ciara	AHT137	137	147
Daisy	AHT137	137	147
Doris	AHT137	147	
Ella	AHT137	137	147
Franko	AHT137	137	147
Harry	AHT137	137	
Inka	AHT137	137	145
KP85	AHT137	143	
KP86	AHT137	143	149
KP87	AHT137	143	147
KP88	AHT137	131	137
KP89	AHT137		
KP90	AHT137	151	
Kenny	AHT137	147	
Loke	AHT137	137	147
Maya	AHT137	137	
Nea	AHT137	137	147
Oscar	AHT137	137	
Santos	AHT137	149	
Shanti	AHT137	147	
Stella	AHT137	145	147
Vand	AHT137		
Zafira	AHT137	137	147

Sample Name	Marker	Allele 1	Allele 2
Andy	INU005	132	
Asko	INU005	124	
Boss	INU005	110	124
Bruno	INU005	110	124
Ciara	INU005	126	132
Daisy	INU005	124	
Doris	INU005	110	
Ella	INU005	110	
Franko	INU005	118	132
Harry	INU005	110	
Inka	INU005	110	
KP85	INU005	124	
KP86	INU005	124	130
KP87	INU005	124	
KP88	INU005	124	132
KP89	INU005		
KP90	INU005	124	126
Kenny	INU005	110	124
Loke	INU005	110	124
Maya	INU005	124	132
Nea	INU005	110	124
Oscar	INU005	110	132
Santos	INU005	124	126
Shanti	INU005	124	132
Stella	INU005	126	132
Vand	INU005		
Zafira	INU005	124	132

Sample Name	Marker	Allele 1	Allele 2
Andy	AHTh171	231	233
Asko	AHTh171	225	
Boss	AHTh171	219	229
Bruno	AHTh171	215	
Ciara	AHTh171	219	233
Daisy	AHTh171	233	
Doris	AHTh171	225	
Ella	AHTh171	221	229
Franko	AHTh171	229	233
Harry	AHTh171	231	233
Inka	AHTh171	225	235
KP85	AHTh171	219	
KP86	AHTh171	219	225
KP87	AHTh171	219	225
KP88	AHTh171	229	
KP89	AHTh171		
KP90	AHTh171	225	239
Kenny	AHTh171	225	229
Loke	AHTh171	231	233
Maya	AHTh171	225	233
Nea	AHTh171	221	233
Oscar	AHTh171	221	229
Santos	AHTh171	233	
Shanti	AHTh171	221	225
Stella	AHTh171	221	233
Vand	AHTh171		
Zafira	AHTh171	225	

Sample Name	Marker	Allele 1	Allele 2
Andy	INU030	150	
Asko	INU030	150	
Boss	INU030	150	
Bruno	INU030	150	
Ciara	INU030	150	
Daisy	INU030	124	
Doris	INU030	150	
Ella	INU030	150	
Franko	INU030	150	
Harry	INU030	150	
Inka	INU030	150	
KP85	INU030	150	
KP86	INU030	144	150
KP87	INU030	144	152
KP88	INU030	150	
KP89	INU030		
KP90	INU030	150	
Kenny	INU030	144	150
Loke	INU030	150	
Maya	INU030	150	
Nea	INU030	150	
Oscar	INU030	150	
Santos	INU030	150	
Shanti	INU030	150	
Stella	INU030	150	
Vand	INU030		
Zafira	INU030	150	

Sample Name	Marker	Allele 1	Allele 2
Andy	AHTh260	238	240
Asko	AHTh260	246	
Boss	AHTh260	238	246
Bruno	AHTh260	246	
Ciara	AHTh260	240	246
Daisy	AHTh260	238	240
Doris	AHTh260	238	
Ella	AHTh260	238	
Franko	AHTh260	240	246
Harry	AHTh260	246	248
Inka	AHTh260	238	246
KP85	AHTh260	250	252
KP86	AHTh260	246	
KP87	AHTh260	240	250
KP88	AHTh260	238	246
KP89	AHTh260		
KP90	AHTh260	246	
Kenny	AHTh260	238	246
Loke	AHTh260	238	246
Maya	AHTh260	238	246
Nea	AHTh260	238	246
Oscar	AHTh260	238	
Santos	AHTh260	238	250
Shanti	AHTh260	238	246
Stella	AHTh260	240	246
Vand	AHTh260		
Zafira	AHTh260	240	246

Sample Name	Marker	Allele 1	Allele 2
Andy	INU055	210	218
Asko	INU055	218	
Boss	INU055	210	
Bruno	INU055	218	
Ciara	INU055	218	
Daisy	INU055	218	
Doris	INU055	218	
Ella	INU055	210	218
Franko	INU055	218	
Harry	INU055	210	214
Inka	INU055	210	214
KP85	INU055	218	
KP86	INU055	218	
KP87	INU055	210	214
KP88	INU055	210	212
KP89	INU055		
KP90	INU055	214	218
Kenny	INU055	210	218
Loke	INU055	210	218
Maya	INU055	214	218
Nea	INU055	218	
Oscar	INU055	210	218
Santos	INU055	218	220
Shanti	INU055	218	
Stella	INU055	218	
Vand	INU055		
Zafira	INU055	214	

Sample Name	Marker	Allele 1	Allele 2
Andy	AHTk211	87	
Asko	AHTk211	87	
Boss	AHTk211	87	
Bruno	AHTk211	87	
Ciara	AHTk211	87	89
Daisy	AHTk211	87	89
Doris	AHTk211	87	
Ella	AHTk211	87	
Franko	AHTk211	87	
Harry	AHTk211	87	
Inka	AHTk211	87	89
KP85	AHTk211	87	91
KP86	AHTk211	87	95
KP87	AHTk211	87	91
KP88	AHTk211	89	97
KP89	AHTk211		
KP90	AHTk211	87	
Kenny	AHTk211	87	
Loke	AHTk211	87	
Maya	AHTk211	87	
Nea	AHTk211	87	
Oscar	AHTk211	87	
Santos	AHTk211	87	
Shanti	AHTk211	87	
Stella	AHTk211	87	
Vand	AHTk211		
Zafira	AHTk211	87	

Sample Name	Marker	Allele 1	Allele 2
Andy	REN162C04	202	206
Asko	REN162C04	202	
Boss	REN162C04	202	208
Bruno	REN162C04		
Ciara	REN162C04	200	208
Daisy	REN162C04	202	
Doris	REN162C04	202	208
Ella	REN162C04	202	208
Franko	REN162C04	200	206
Harry	REN162C04	202	
Inka	REN162C04	202	
KP85	REN162C04	202	208
KP86	REN162C04	202	206
KP87	REN162C04	200	202
KP88	REN162C04	202	206
KP89	REN162C04		
KP90	REN162C04	202	208
Kenny	REN162C04	202	206
Loke	REN162C04	202	208
Maya	REN162C04	202	206
Nea	REN162C04	202	
Oscar	REN162C04	202	208
Santos	REN162C04	206	
Shanti	REN162C04	202	208
Stella	REN162C04	202	
Vand	REN162C04		
Zafira	REN162C04	202	

Sample Name	Marker	Allele 1	Allele 2
Asko	AHTk253	286	288
Boss	AHTk253	286	
Bruno	AHTk253		
Ciara	AHTk253	288	292
Daisy	AHTk253	292	
Doris	AHTk253	286	
Ella	AHTk253	292	
Franko	AHTk253	286	292
Harry	AHTk253	288	292
Inka	AHTk253	292	
KP85	AHTk253	288	
KP86	AHTk253	288	292
KP87	AHTk253	288	
KP88	AHTk253	288	
KP89	AHTk253		
KP90	AHTk253	288	292
Kenny	AHTk253	288	292
Loke	AHTk253	286	292
Maya	AHTk253	286	
Nea	AHTk253	286	
Oscar	AHTk253	286	
Santos	AHTk253		
Shanti	AHTk253	286	288
Stella	AHTk253	286	292
Vand	AHTk253		
Zafira	AHTk253	286	

Sample Name	Marker	Allele 1	Allele 2
Asko	REN169D01	212	216
Boss	REN169D01	210	218
Bruno	REN169D01		212
Ciara	REN169D01	210	212
Daisy	REN169D01	210	216
Doris	REN169D01	210	212
Ella	REN169D01	212	216
Franko	REN169D01	210	212
Harry	REN169D01	210	216
Inka	REN169D01	210	212
KP85	REN169D01	210	216
KP86	REN169D01	212	216
KP87	REN169D01	212	216
KP88	REN169D01	214	218
KP89	REN169D01		
KP90	REN169D01	210	216
Kenny	REN169D01	212	218
Loke	REN169D01	212	216
Maya	REN169D01	218	
Nea	REN169D01	212	218
Oscar	REN169D01	212	
Santos	REN169D01	212	216
Shanti	REN169D01	212	
Stella	REN169D01	210	216
Vand	REN169D01		
Zafira	REN169D01	216	

Sample Name	Marker	Allele 1	Allele 2
Andy	Amelogenin	Y	X
Asko	Amelogenin		
Boss	Amelogenin	Y	X
Bruno	Amelogenin	?	X
Ciara	Amelogenin	X	
Daisy	Amelogenin		
Doris	Amelogenin	X	
Ella	Amelogenin		
Franko	Amelogenin	Y	X
Harry	Amelogenin		
Inka	Amelogenin		
KP85	Amelogenin	Y	X
KP86	Amelogenin	X	
KP87	Amelogenin	Y	X
KP88	Amelogenin	X	
KP89	Amelogenin		
KP90	Amelogenin	X	
Kenny	Amelogenin	Y	X
Loke	Amelogenin	Y	X
Maya	Amelogenin	X	
Nea	Amelogenin	X	
Oscar	Amelogenin	Y	X
Santos	Amelogenin	?	X
Shanti	Amelogenin	X	
Stella	Amelogenin	X	
Vand	Amelogenin		
Zafira	Amelogenin	X	

Sample Name	Marker	Allele 1	Allele 2
Andy	REN169O18	156	168
Asko	REN169O18	168	170
Boss	REN169O1	170	
Bruno	REN169O18		
Ciara	REN169O18	166	168
Daisy	REN169O18	162	168
Doris	REN169O18	162	170
Ella	REN169O18	156	170
Franko	REN169O18	166	170
Harry	REN169O18	162	170
Inka	REN169O18	170	
KP85	REN169O18	156	170
KP86	REN169O18	164	
KP87	REN169O18	164	168
KP88	REN169O18	168	170
KP89	REN169O18		
KP90	REN169O18	156	170
Kenny	REN169O18	168	170
Loke	REN169O18	170	
Maya	REN169O18	168	170
Nea	REN169O18	168	170
Oscar	REN169O18	156	170
Santos	REN169O18	168	170
Shanti	REN169O18	162	170
Stella	REN169O18	168	170
Vand	REN169O18		
Zafira	REN169O81	170	

Sample Name	Marker	Allele 1	Allele 2
Andy	CXX279	124	
Asko	CXX279	124	126
Boss	CXX279	118	126
Bruno	CXX279	118	
Ciara	CXX279	124	
Daisy	CXX279	124	
Doris	CXX279	118	126
Ella	CXX279	124	126
Franko	CXX279	124	
Harry	CXX279	124	
Inka	CXX279	118	124
KP85	CXX279	120	130
KP86	CXX279	118	120
KP87	CXX279	118	120
KP88	CXX279	116	118
KP89	CXX279		
KP90	CXX279	116	124
Kenny	CXX279	124	
Loke	CXX279	124	
Maya	CXX279	118	
Nea	CXX279	124	
Oscar	CXX279	124	126
Santos	CXX279	124	
Shanti	CXX279	124	126
Stella	CXX279	124	130
Vand	CXX279		
Zafira	CXX279	118	

Sample Name	Marker	Allele 1	Allele 2
Andy	REN247M23	268	270
Asko	REN247M23	271	
Boss	REN247M23	268	272
Bruno	REN247M23	268	
Ciara	REN247M23	272	
Daisy	REN247M23	268	272
Doris	REN247M23	268	
Ella	REN247M23	268	272
Franko	REN247M23	268	272
Harry	REN247M23	270	
Inka	REN247M23	268	270
KP85	REN247M23	268	272
KP86	REN247M23	268	272
KP87	REN247M23	272	
KP88	REN247M23	270	272
KP89	REN247M23		
KP90	REN247M23	268	
Kenny	REN247M23	270	272
Loke	REN247M23	272	
Maya	REN247M23	270	272
Nea	REN247M23	272	
Ocar	REN247M23	272	
Santos	REN247M23	272	
Shanti	REN247M23	272	
Stella	REN247M23	266	272
Vand	REN247M23		
Zafira	REN247M23	272	

Sample Name	Marker	Allele 1	Allele 2
Andy	FH2054	160	164
Asko	FH2054	152	160
Boss	FH2054	160	164
Bruno	FH2054		
Ciara	FH2054	160	164
Daisy	FH2054	152	160
Doris	FH2054	160	
Ella	FH2054	152	160
Franko	FH2054	156	160
Harry	FH2054	152	160
Inka	FH2054	160	164
KP85	FH2054	152	160
KP86	FH2054	152	176
KP87	FH2054	152	176
KP88	FH2054	156	160
KP89	FH2054		
KP90	FH2054	160	
Kenny	FH2054	152	160
Loke	FH2054	152	164
Maya	FH2054	152	160
Nea	FH2054	152	156
Oscar	FH2054	160	
Santos	FH2054	156	160
Shanti	FH2054	152	160
Stella	FH2054	156	164
Vand	FH2054		
Zafira	FH2054	160	164

Sample Name	Marker	Allele 1	Allele 2
Andy	REN54P11	236	
Asko	REN54P11	236	
Boss	REN54P11	236	
Bruno	REN54P11	236	
Ciara	REN54P11	236	
Daisy	REN54P11	236	
Doris	REN54P11	236	
Ella	REN54P11	236	
Franko	REN54P11	236	
Harry	REN54P11	236	
Inka	REN54P11	236	
KP85	REN54P11	236	
KP86	REN54P11	222	236
KP87	REN54P11	226	236
KP88	REN54P11	226	236
KP89	REN54P11		
KP90	REN54P11	234	236
Kenny	REN54P11	228	236
Loke	REN54P11	236	
Maya	REN54P11	236	
Nea	REN54P11	236	
Oscar	REN54P11	236	
Santos	REN54P11	236	
Shanti	REN54P11	236	
Stella	REN54P11	236	
Vand	REN54P11		
Zafira	REN54P11	236	

Sample Name	Marker	Allele 1	Allele 2
Andy	FH2848	238	240
Asko	FH2848	238	240
Boss	FH2848	240	
Bruno	FH2848	238	
Ciara	FH2848	240	
Daisy	FH2848	238	240
Doris	FH2848	238	240
Ella	FH2848	238	240
Franko	FH2848	238	240
Harry	FH2848	240	
Inka	FH2848	238	240
KP85	FH2848	240	242
KP86	FH2848	238	240
KP87	FH2848	228	244
KP88	FH2848	238	240
KP89	FH2848		
KP90	FH2848	240	244
Kenny	FH2848	240	
Loke	FH2848	238	240
Maya	FH2848	238	240
Nea	FH2848	240	
Oscar	FH2848	238	
Santos	FH2848	238	
Shanti	FH2848	240	
Stella	FH2848	240	
Vand	FH2848		
Zafira	FH2848	240	