

Betterment of the Mastiff Breed: Demystifying DNA
The Role DNA Plays in Pedigree Accuracy and the Mastiff Standard

DNA Committee Members

Mastiff Club of America

Abstract

The Mastiff breed is "a combination of grandeur and good nature as well as courage and docility" (American Kennel Club, 2014). Have you ever wondered where this grandeur and good nature comes from? DNA provides the blueprint behind the Mastiff standard. While the American Kennel Club (AKC) is "dedicated to upholding the integrity of its Registry, promoting the sport of purebred dogs and breeding for type and function" (American Kennel Club, 2014), the mission of the Mastiff Club of America (MCOA) is to "protect and promote the Mastiff" (Mastiff Club of America, 2014). The MCOA is focused on the betterment of the Mastiff breed. What makes the MCOA National Specialty different than other AKC sanctioned events? The word "Special"! The MCOA National Specialty provides the unique opportunity for Mastiff fanciers, breeders, exhibitors, and owners to participate in an event that only features the Mastiff breed. The National Specialty also provides an education venue to assist in understanding and competing against the Mastiff standard. Mastiff breeders are also able to gain valuable insight for their breeding program via pedigree and parentage analysis. "Knowledge is power" (Francis Bacon). Without the knowledge and confidence provided by the Mastiff presented to you, the integrity of the breed is in jeopardy.

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This paper is designed to provide an overview of why parentage analysis is important and should be required for entry into the MCOA National Specialty. Not only are the genes that make up a Mastiff responsible for its conformance to the standard, these genes are also responsible for temperament and health. The MCOA and its members are chartered to be responsible guardians of the Mastiff breed.

Demystifying DNA: DNA Profile Requirement for the National Specialty

In 1995, the Philadelphia Inquirer released an article that highlighted allegation of rampant fraud in the AKC registry (Stark, 1995). The AKC turned to DNA testing as a tool to ensure the integrity of the registry (American Kennel Club, 2014).

The AKC allows breed clubs to make AKC DNA Profiles mandatory for entries in their National Specialties and other AKC Events as long as all of the dogs in competition at the events have the same requirement. MCOA along with several other clubs have taken the opportunity to utilize AKC DNA Profile requirement as a way to validate and improve the integrity of the breeds' pedigrees at the National level. This is a pro-active approach towards ensuring accuracy and integrity of future pedigrees by having AKC DNA Profile numbers associated with both dogs and bitches (vertical and horizontal). The AKC DNA Profile number, with the exception of the Rescue Parade, is also listed in the MCOA National Specialty Catalog. A majority of the entries have at least one parent with an AKC DNA Profile number and many now have both sire and dam with an AKC DNA Profile number.

AKC DNA Profile numbers can be verified online by logging into the AKC Online Store, (<http://classic.akc.org/shop/index.cfm>) selecting “AKC Reports (Points & Awards)” and looking up the dog’s registration number or registered name. AKC shares DNA Profile numbers and other registry information with the Orthopedic Foundation for Animals. When owners and breeders add their Mastiffs’ health information to the Orthopedic Foundation for Animals, the public can view the dog’s AKC DNA Profile numbers along with their health testing information through the OFA public, online database (<http://www.offa.org>).

The Motion(s)

The MCOA DNA Sub-Committee submitted the following agenda item to the MCOA membership at the 2008 MCOA Member’s Annual Meeting in Crystal Lake, IL proposing that the MCOA require AKC DNA Profiles on all National Specialty entries. The request included (Mastiff Club of America Board of Directors, personal communication, 2008):

The MCOA DNA Sub-Committee is requesting that the Mastiff Club of America require AKC DNA Profiling on all National Specialty entries. Justification:

- Accuracy of Specialty Catalogs and Historical Documentation
- Un-refutable identification of our Mastiffs
- Integrity of our Mastiff's Pedigrees

A formal request for an agenda item was submitted to the MCOA Corresponding Secretary before the deadline, but the agenda item was not listed on the meeting agenda printed for distribution. This mishap was acknowledged in discussion during the 2008 MCOA Member’s Annual Meeting. The National Specialty DNA Requirement agenda item was presented as planned by a panel of MCOA members.

Following the presentation, a motion was made and approved by membership at the 2008 MCOA Member's Annual Meeting. Based on the motion that was passed, AKC DNA Profiles would be required on all entries in the MCOA National Specialty with the exception of the Rescue Parade.

Since the AKC DNA Profile Requirement was a concept that was new for many members and non-members, the Health Committee and DNA Sub-Committee felt it was important to educate members and non-members on the requirement. As a result, the Health Committee and DNA Sub-Committee submitted a new motion to the MCOA Board of Directors requesting that the MCOA move forward with implementing the new National Specialty DNA Profiling Requirement at the 2009 MCOA National Specialty, but not to enforce the DNA requirement until the 2010 MCOA National Specialty. This extension would allow time to bring awareness and educate members and non-members to the new requirement and to allow time for people to have their Mastiffs AKC DNA Profiled. The new motion was approved by the MCOA BOD.

EM (Email Motion) #06/08-01: It was moved by Cindy Furr and seconded by David Henson to accept the request from the MCOA Health Committee and the DNA Sub-Committee, and change the effective date of the motion to require DNA Profiling of all entries in the MCOA National Specialty from the year 2009 to the year 2010. Aye: Collings, Henson, Furr, LaBombard, Ropes, Plezbert, Hudspeth; NR: Murphy, Speer; Motion Carried.

MCOA also allows temporary numbers for National Specialty Entry Forms. The AKC DNA Operations Department will provide priority service to Parent Clubs with AKC DNA Profile National Specialty Requirements. Instructions on how to expedite a DNA sample and obtain a temporary AKC DNA Profile number can be found in the MCOA National Specialty

Premium and on the MCOA National Specialty (<http://www.mcoanationalspecialty.com/>) web site.

The Requirement

The MCOA National Specialty AKC DNA Profile requirement takes the importance of the integrity of Mastiff pedigrees to a higher level by requiring AKC DNA Profiles on all entries, dogs, bitches, and their progeny, with the exception of the Rescue Parade.

Although AKC DNA Profiles are not National Specialty requirements for most AKC Parent Clubs, MCOA is setting an example and standing in the forefront of ensuring integrity of some of the most important breed pedigrees (conformation and working ability), along with the American Brittany Spaniel, Bernese Mountain Dog, German Shorthaired Pointer, Gordon Setter, and Vizsla AKC Parent Clubs. Additional AKC Parent Clubs and Canine Event Clubs are also requiring DNA Profiles for entry in National Field Trials.

The Survey

The price of an AKC DNA Profile is less than the price of many premium dog foods, less than one night at most hotels, and less than the price of a tank of gas for most vehicles. There are AKC DNA Live Discount Events (American Kennel Club, 2014) at many popular dog shows and discounted AKC DNA Profiles are offered at the MCOA National Specialty.

In 2013, MCOA membership received a survey question that asked whether the MCOA should continue to require DNA testing. Based on the 239 responses, 60.25% indicated YES. In July 2013, the 2014 National Show Chair requested a potential waiver for requiring DNA at the 2014 MCOA National Specialty to determine if the DNA requirement was one of the reasons for lower entries. The waiver for the 2014 MCOA National Specialty was not granted (Mastiff Club

of America, 2013). It is also important to note that 2014 MCOA National Specialty entries went up from previous years with the current DNA requirement in place.

Don't Blame Low Entries on the Genes

National Specialty entries go up and down for various reasons: location, date, graduations, reunions, vacations, employment, weddings, illness, death, puppies, judges, cost of hotel, cost of showing, number of dogs allowed in hotel room, number of dogs that will fit in vehicles, etc.

The economy has also played an important factor in entries and the United States saw a tremendous dive around the same time the requirement was enforced and the unemployment rate went up (as shown in Figure 1).

The genetic make up that includes conformation, temperament, working ability, and genetic health is inherited from both parents. Not only is an accurate pedigree applicable to Mastiffs competing at a National level and viable options for one's breeding program, it is equally important for Mastiffs placed in non-show homes.

Demystifying DNA: The Science

In order to make an educated decision on the role you feel DNA plays in the betterment of the Mastiff breed, it is important that you understand what DNA is, what parentage analysis is and how it works, what are DNA tests for traits and diseases, and how parentage analysis is different than the DNA tests for traits and disease.

Basic Terminology

DNA can be a very broad and sometimes confusing subject. Therefore, to help set the stage for the discussion to follow, some basic genetic terminology will be summarized:

- Genetics: According to Merriam Webster dictionary (2014), genetics is “the scientific study of how genes control the characteristics of plants and animals”.

- **DNA:** DNA stands for deoxyribonucleic acid and contains instructions for how plants and animals will look, how they will survive, and how they will interact with their surrounding environments. DNA is made of building blocks known as nucleotides. Nucleotides consist of: a phosphate group, a sugar group and one of four types of nitrogen bases (adenine - A, thymine - T, guanine - G, and cytosine – C). To form a strand of DNA, nucleotides are linked into chains, with the phosphate and sugar groups alternating. These bases form units called base pairs (A pairs with T and C pairs with G). Figure 2 shows how nucleotides form a double helix. The DNA double helix is often compared to a ladder. The base pairs are like the steps of a ladder and the sugar and phosphate molecules make up the vertical sides of the ladder. The sequence of these bases determines the instructions for that strand of DNA. A comprehensive overview of DNA can be found in the National Human Genome Research Institute DNA fact sheet (National Human Genome Research Institute, 2014).
- **Genes:** A gene is a unit of DNA. That DNA contains the instructions required to produce a specific protein or set of proteins. Organs and tissues in the human (and canine) body are made up of these proteins. For example, these proteins are responsible for characteristics of the Mastiff breed (e.g. height, weight, coat color, fur length, topline). Proteins are also responsible for controlling chemical reactions and for carrying signals between cells. If an abnormal protein is produced as a result of a cell's DNA being mutated, it can lead to diseases like cancer. "The canine genome has approximately 2.5 billion bases (letters), divided

among almost 20,000 genes (books), across 39 chromosomes (bookshelves)” (Star, 2011).

- **Allele:** An allele is a specific variation of a gene. For example, there is a gene for eye color. There is an allele for blue eyes, an allele for brown eyes, etc.
- **Chromosome:** DNA is packaged into units called chromosomes. A chromosome is made up of DNA that is coiled around proteins that are known as histones. In humans, each cell normally contains 46 chromosomes (National Human Genome Research Institute, 2011). As indicated above, the canine genome has 39 chromosomes. Therefore, each normal canine cell would contain 78 chromosomes (39 chromosomes from the sire and 39 chromosomes from the dam). The only cells that do not contain a pair of chromosomes are reproductive cells (sperm and egg). Reproductive cells contain just one copy of each chromosome. When a sperm and egg unite, they become a single cell that contains two copies of each chromosome. This cell will divide and eventually result in an offspring.
- **Heredity:** Heredity is the passing of traits from parents to offspring. Traits are defined by the instructions contained in genes. Environmental influences can also have an effect on traits. For example, genes play a critical role in the bone structure of a canine (Chase et al., 2002). Nutrition can also have an effect on that bone structure. An offspring receives one set of chromosomes from each parent. This takes place during conception. Each offspring inherits a unique set of chromosomes since each parent contributes chromosomes randomly. Common terms that one may hear include: dominant and recessive traits. Several inheritance patterns have been identified. These inheritance patterns can include:

Autosomal dominant, Autosomal recessive, X-linked dominant, X-linked recessive, Codominant, and Mitochondrial. An overview of inheritance patterns can be found on the National Coalition for Health Profession Education in Genetics (2012) web site.

- **DNA Sequencing:** DNA sequencing is the mapping of the sequence of nucleotides that make up a strand of DNA. DNA sequencing of the canine genome first began in 2003 with a female boxer named Tasha with the complete sequence completed in 2005 (Lindbald-Toh et al., 2005). This sequencing effort was conducted as part of the National Human Genome Research Institute Large Scale Sequencing Research Network. The cost associated with this effort was \$30 million (National Human Genome Research Institute, 2005). According to Starr (2005), “Having the sequence – the specific order of the 2.5 billion As, Ts, Cs, and Gs – allows researchers to examine coding and non-coding regions of DNA, determine the number of genes (approximately 20,000), and compare the canine genome to that of humans and other species. Knowing the similarities among species has propelled advances in understanding which genes are involved in canine phenotypes and disease.”
- **Parentage Analysis:** DNA typing is a procedure where DNA is extracted from a biological sample (e.g. blood, cheek swab) from the subject being analyzed. The set of genetic markers that are identified make up the DNA or genetic profile. Based on this profile, parentage can be analyzed since the sample being analyzed contains two DNA copies (maternal and paternal). Short tandem repeat (STR) sequences are used to determine the DNA profile. These repetitive sequences are

called microsatellites. For the AKC, parentage analysis is based on the AKC DNA Profile that is based on the SuperPlex plus Gender Marker (SuperPlex-G) universal panel (American Kennel Club, 2003).

- **DNA Tests for Traits and Diseases:** DNA tests can be valuable tools to help improve the genetic health of the Mastiff breed while still maintaining genetic diversity. There are a number of DNA tests available that are associated with inherited genetic traits or diseases in canines. Some examples of these tests include: Progressive Renal Atrophy (PRA), Canine Multifocal Retinopathy (CMR), Degenerative Myelopathy (DM), Cystinuria (Non Type I), Von Willebrand's Disease (vWD), coat length, and coat color.
- **DNA Bank:** A DNA bank is a repository of DNA and medical information. Submission to a DNA bank is typically done via submitting a blood or cheek swab sample. One of the common DNA banks is the Canine Health Information Center (CHIC) DNA Repository that is co-sponsored by the Orthopedic Foundation for Animals (OFA) and the AKC/CHF (Canine Health Foundation). More detail can be found on the Canine Health Information Center (2014) web site.

Parentage Analysis

A high level description of parentage analysis was presented above. Let's take a closer look at the AKC DNA Profile Program (G. Lycan, personal communication, February 2014). The AKC leverages the DNA Profile Program to ensure the integrity of the AKC Registry. To participate in the DNA Profile Program:

1. Obtain an AKC DNA Kit from the American Kennel Club (2014) web site.

2. Use the cheek swab brush to collect a sample. When collecting the sample, it is important to minimize the risk of sample contamination (canine should be isolated from possible containments such as other canines, toy, food, and water) and make sure enough DNA has been obtained (the source of DNA is the cheek cells, drool/saliva may prevent a good contact between the brush and the cheek cells).
3. Send the DNA sample, required paperwork, and payment (if required) to the AKC.
4. When the DNA Test Kit is received by AKC DNA Operations in Raleigh, North Carolina, it is logged in the AKC DNA Database where a temporary DNA Profile number is assigned and the samples are bar-coded for reference.
5. The DNA sample is sent to the AKC's DNA service provider, Neogen. Note: Processing time can vary, but the average is six to eight weeks.
6. The DNA Profile and parentage verification results are returned to the AKC. The processing results are entered into the AKC DNA Database. The information obtained is also used to verify parentage.
7. The canine owner will receive a letter from the AKC that includes the DNA Profile number and resulting genotype. The DNA Profile number will also be added to the canine's AKC registration record. In cases where DNA proves the litter is registered to an incorrect sire or dam, the litter owner will be notified and "every effort will be made to cooperate with the breeders in establishing correct parentage based on additional information and submitted DNA samples...Litter corrections will be made based on DNA profiles...Litters that cannot be corrected will, unfortunately, be cancelled" (American Kennel Club, 2002).

What type of processing is Neogen doing? Parentage Analysis is based on Neogen Genomic's standardized DNA markers. Also known as the SuperPlex plus Gender Marker (SuperPlex-G) universal panel, seventeen microsatellite markers have been identified. The criteria for identifying these markers were: reproducibility, information content, ease of scoring, multiplex assay robustness, and low mutation rates (DeNise et al., 2004). Figure 3 depicts an example of a DNA Profile. The Neogen Genomic DNA Parentage Analysis is able to distinguish between related sires or dams, even if it is a close relation (e.g. litter mates, siblings). In instances where the results are inconclusive, additional DNA markers can be used. Based on the study conducted by S. DeNise et al. (2004):

The primary panel of 10 markers exceeded 99% power of exclusion for canine parentage verification of 61% of the breeds. In combination with the secondary panel of seven markers, 100% of the tested breeds exceeded 99% power of exclusion. The minimum probability match rate of the first panel was 3.6×10^{-5} averaged across breeds, and with the addition of the second panel, the probability match rate was 3.2×10^{-8} ; thus the probability of another random, unrelated canine with the same genotype is very low. The results of this analysis indicated that, on average, the primary panel meets the AKC's needs for routine parentage testing, but that a combination of 10 - 15 genetic markers from the two panels could yield a universal canine panel with enhanced processing efficiency, reliability and informativeness (p. 1).

Figure 4 shows the analysis where there were two potential sires. Based on the parentage analysis, Solomon is the sire of Viking.

DNA Tests for Traits and Diseases

A high level description of what DNA tests for traits and diseases are was presented above. As indicated, for the Mastiff breed, there are DNA tests available for various traits and diseases. In this particular section, the genetics of one of the DNA tests available for diseases will be examined.

Per the Mastiff Club of America (2014), “Progressive Retinal Atrophy (PRA) is a family of inherited progressive degenerative eye disease affecting the retina which ultimately result in blindness.” To date, Mastiffs and Bullmastiffs are the only breeds where the mode of inheritance for PRA has been confirmed as dominant. As a result, only a single copy of the mutant gene will cause PRA. Genetic analysis identified two synonymous mutations and one non-synonymous mutation within the canine rhodopsin gene. PRA in Mastiffs is caused by the non-synonymous mutation (T4R) (Kijas, Miller, Pearce-Kelling, Aguirre, & Acland, 2003). Per Optigen (2014), the DNA test for Dominant PRA will result in:

- Homozygous Normal – this dog is not and will not become affected with PRA. It has two normal PRA gene copies and will pass along only the normal gene to its offspring.
- Heterozygous Affected – this dog is or will be affected with PRA. It has one dominant PRA mutant gene copy and one normal gene copy. On average, half of its offspring will receive the mutant gene copy and half of its offspring will receive the normal gene copy.
- Homozygous Affected – this dog is or will be affected with PRA. It has two dominant PRA mutant gene copies and no normal gene copies. All of its offspring will receive the mutant gene causing PRA. (p. 1)

What role does genetics play in PRA? An offspring receives one set of chromosomes from each parent. In the study conducted by Kijas, Miller, Pearce-Kelling, Aguirre, & Acland (2003),

the integrity of the pedigree of the canines used in the study played a crucial role in identifying the rhodopsin T4R mutation. Figure 5 shows three pedigrees for a canine form of PRA. Figure 5A depicts the six generation Mastiff subset. In Figure 5B, an affected Mastiff female was bred to a normal Beagle male (laboratory derived strain) which resulted in two out of six offspring being affected. In Figure 5C, an affected Mastiff female was bred to a normal Irish Wolfhound which resulted in two affected offspring.

Per the Mastiff Club of America (2014), a Mastiff is proven clear of the PRA gene if:

- both parents are DNA tested clear
- or, both of the sire's parents are DNA tested clear and the dam is DNA tested clear
or, both of the dam's parents are DNA tested clear and the sire is DNA tested clear
- or, all four grandparents are DNA tested clear (p. 1)

For scenarios where the offspring are “cleared by parentage”, it is important that the parentage is confirmed via DNA analysis.

Parentage Analysis versus DNA Tests for Traits and Diseases

While parentage analysis and DNA tests for traits and diseases are very complimentary in nature, they are independent tests. Sequencing a canine’s genome is not only costly, but time consuming as well (National Human Genome Research Institute, 2005). As a result, researchers have identified alternative mechanisms to leverage DNA in parentage analysis and in the identification of traits and diseases. In both cases, the technology does not use actual genes, but other DNA sequences that have been identified through research. Therefore, the AKC DNA Profile (American Kennel Club, 2014) used for parentage analysis does not provide information about the confirmation of the canine or the presence or absence of genetic diseases. The AKC

DNA Profile is also not able to determine the breed of the canine. Likewise, DNA tests for traits and diseases do not verify parentage.

Demystifying DNA: MCOA Constitution, By-Laws and Code of Ethics

As the delegated AKC Parent Club for the Mastiff breed and as MCOA members, we must do all that we can “above and beyond the AKC” to assure that Mastiff are included in the American Kennel Club's Mission Statement. It is the responsibility of MCOA to protect the Mastiff breed and do all that we can for the betterment of the breed and its future. To achieve this, the MCOA’s goals must include protecting the integrity of Mastiff pedigrees while promoting the sport of purebred Mastiffs and encourage breeding for type and function.

Per the MCOA Constitution and By-Laws: Section 2, Article 1, (a), (d), (e)

SECTION 2 OBJECTIVES

(1) The objectives of the club shall be:

- (a) to encourage and promote the selective breeding of quality purebred Mastiffs and to do all possible to bring their natural qualities to fit the standard;
- (d) to do all in its power to protect and advance the interests of the breed and to encourage sportsmanlike competition at dog shows and obedience trials;
- (e) to provide for the welfare of the breed through a program of Mastiff Rescue and continuing education;

Objectives (a), (d), and (e) acknowledge that the Mastiff Club of America encourages and urges its members to promote selective breeding of quality purebred Mastiffs, to do all in its power to protect and advance the interests of the breed and to provide for its welfare (Mastiff Club of America, 2013).

The Mastiff Club of America's Code of Ethics require all MCOA members to consider only the betterment of the breed when breeding and they must provide the buyer with a five (5) generation pedigree on the litter when they sell a puppy or adult Mastiff .

1. I will consider only the betterment of the breed when breeding a bitch or allowing a breeding with my stud dog, being conscientious of controlling and eliminating inherited problems. A breeder and stud dog owner shall plan each breeding with the paramount intention of protecting the breed, and only when the parties involved agree the breeder is in a position and has the knowledge to give proper care to both the bitch and offspring.

4. An MCOA member will sell each Mastiff puppy/adult on a written contract signed by all parties. Said contract shall contain, but is not limited to the following provisions:

C. Provide the buyer with a five (5) generation pedigree on the litter (Mastiff Club of America, 2013).

To consider the betterment of the breed with breeding decisions and to conscientiously control and eliminate inherited problems the pedigrees must be correct for accurate pedigree analysis. Historically reputable breeders have planned each breeding with in depth pedigree analysis focusing on the various dogs' positive attributes and weaknesses to improve conformation, temperaments, health and working ability within their lines. The most convenient way to validate the accuracy of current & future pedigrees (after the death of the owners, breeders and dogs), is to have AKC DNA Profile numbers listed next to all the names (male and female) on pedigrees.

Demystifying DNA: Your Call to Action

Vote YES to KEEP the MCOA National Specialty AKC DNA Profile Requirement

Please vote yes to keep the AKC DNA Profile requirement for entries in the MCOA National Specialty events, excluding the Rescue Parade. MCOA made a significant, historical accomplishment when it made AKC DNA Profiles a requirement for National Specialty entries. The science behind DNA Profiles has been proven to be valid and the same science is used by law enforcement worldwide for forensics, identification and paternity of humans. DNA Profiles (genetic fingerprints) are used to verify parentage and pedigrees, to put criminals in prison, to release innocent people from prison, for child support and to identify bodies. Please consider the betterment of the Mastiff breed and make your vote count.

Acknowledgement: In memory, honor and recognition of Dr. William Newman for making the “Betterment of the Mastiff Breed” a priority and looking out for its future. May your legacy of making AKC DNA Profiles a requirement for entry in Mastiff Club of America National Specialty Events remain mandatory and may your vision of seeing future Mastiff Pedigrees with AKC DNA Profile numbers next to all the names (both vertical and horizontal) become a reality (Mastiff Club of America, 2014).

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Figure Captions

Figure 1. Unemployment rates from labor force statistics from the current population survey – January 2004 through August 2014 (Bureau of Statistics, 2014).

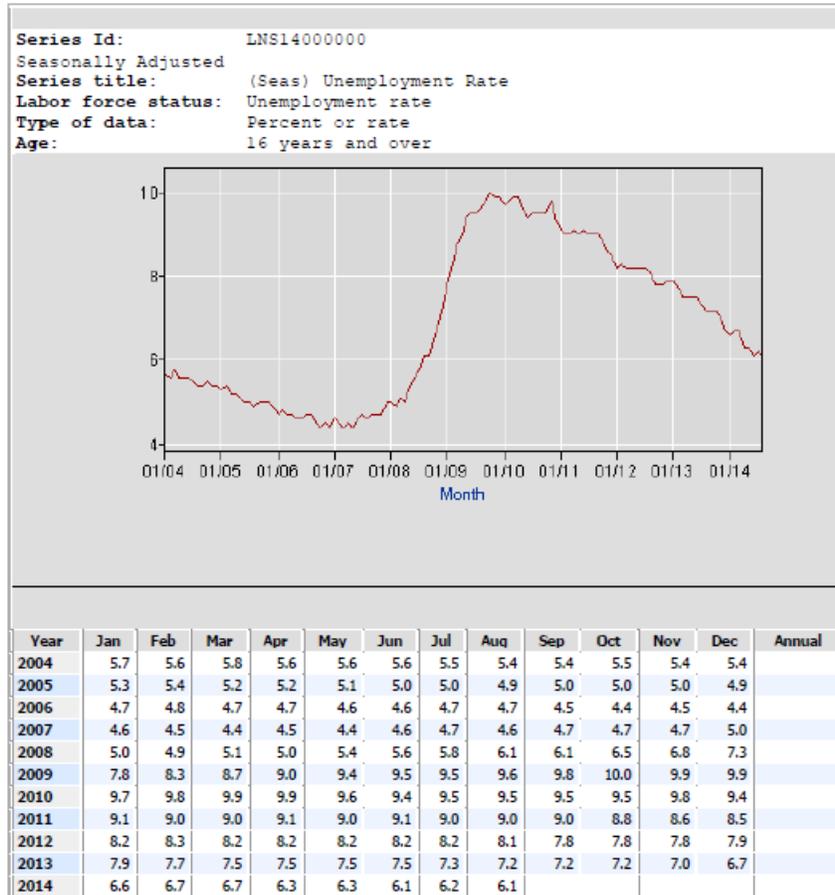


Figure 2. Deoxyribonucleic Acid (DNA) Double Helix (U.S. National Library of Medicine, 2014). This figure shows how nucleotides form a double helix. The DNA double helix is often compared to a ladder. The base pairs are like the steps of a ladder and the sugar and phosphate molecules make up the vertical sides of the ladder.

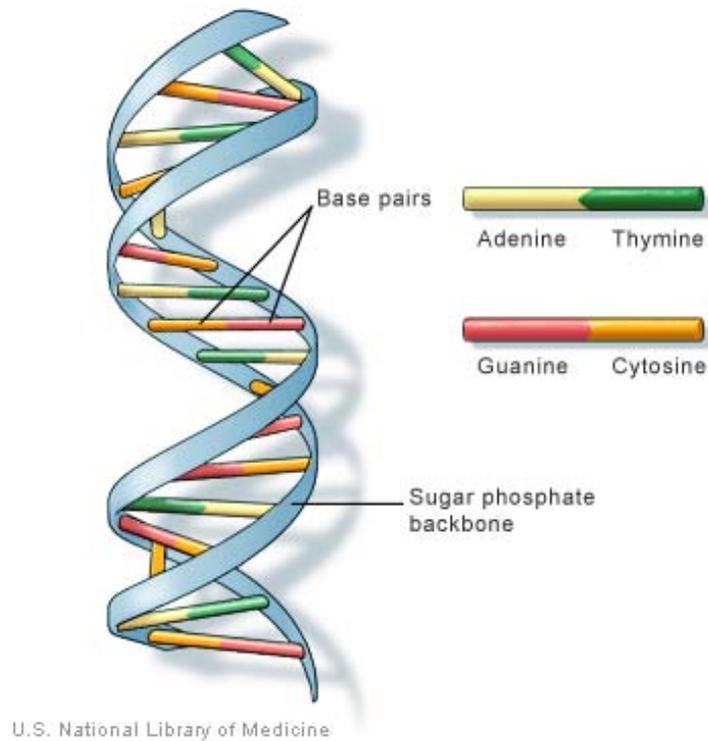


Figure 3. Example of a DNA Profile (Neogen, 2014). The DNA Profile is based on Neogen Genomic's standardized DNA markers. Also known as the SuperPlex plus Gender Marker (SuperPlex-G) universal panel, seventeen microsatellite markers have been identified.

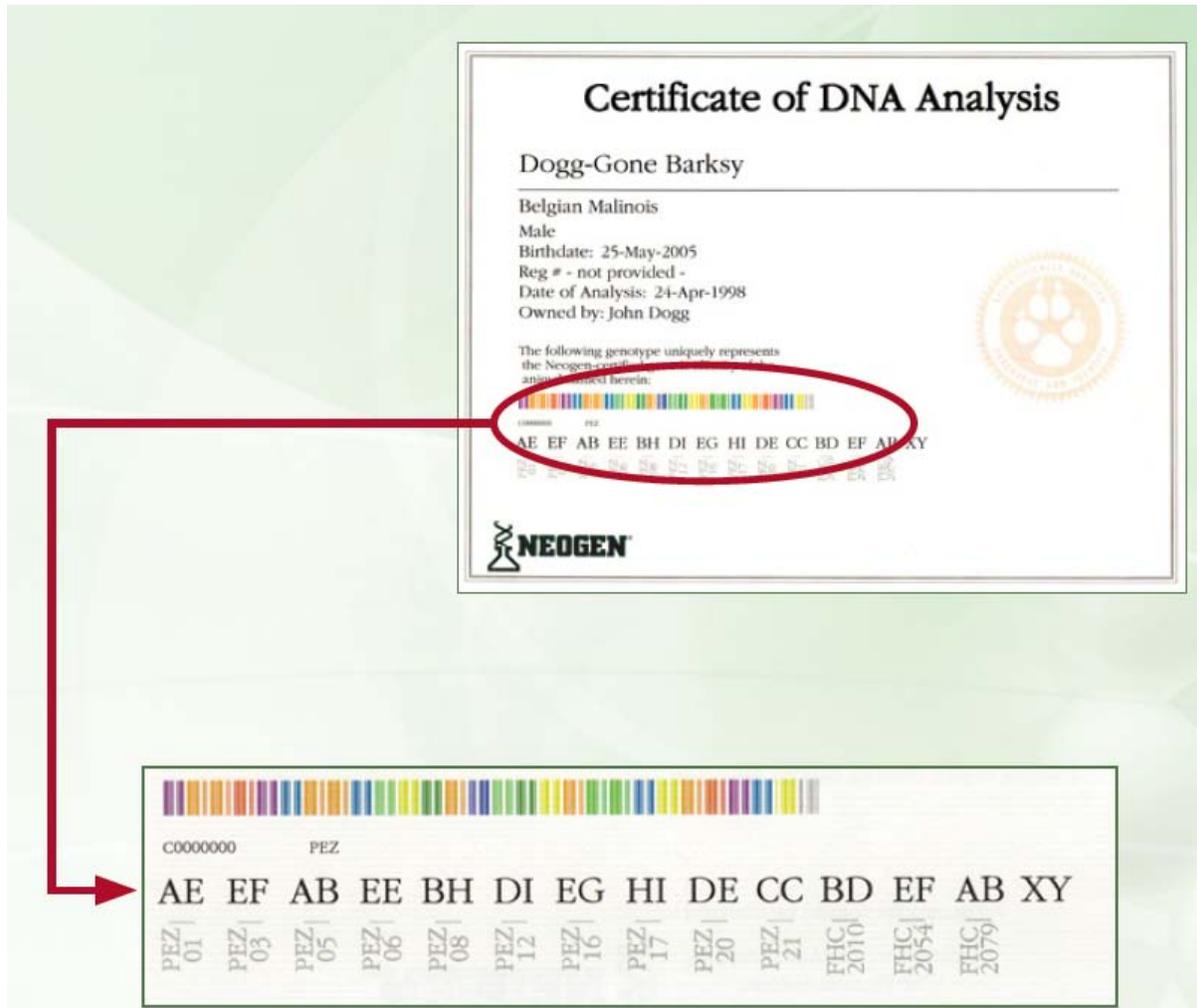


Figure 4. Example of parentage analysis (Neogen, 2014). The analysis is between two potential sires. Based on the parentage analysis, Solomon is the sire of Viking.

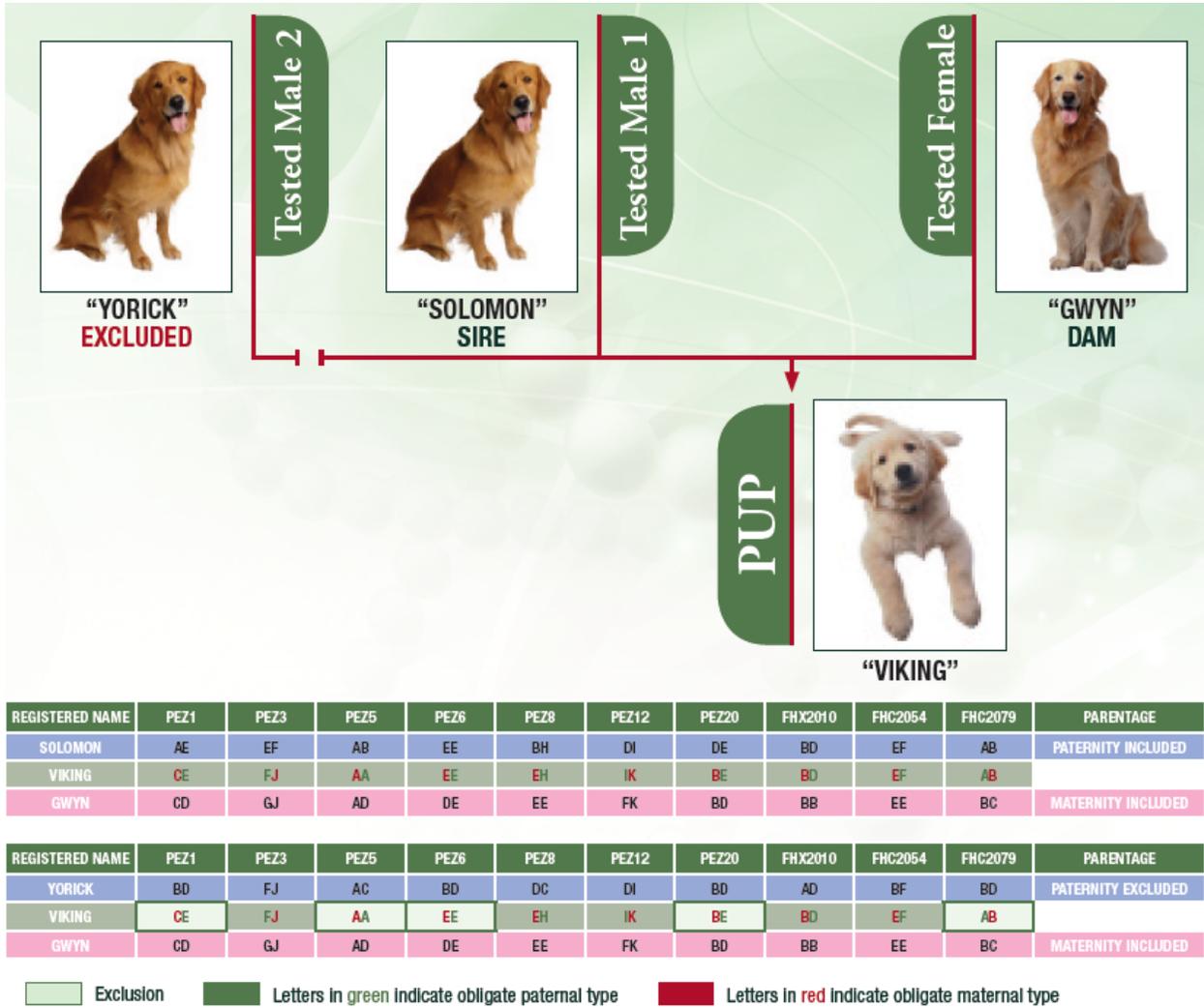


Figure 5. Figure 4 shows three pedigrees for a canine form of PRA (Kijas, Miller, Pearce-Kelling, Aguirre, & Acland, 2003). Figure 4A depicts the six generation Mastiff subset. In Figure 4B, an affected Mastiff female was bred to a normal Beagle male (laboratory derived strain) which resulted in two out of six offspring being affected. In Figure 4C, an affected Mastiff female was bred to a normal Irish Wolfhound which resulted in two affected offspring.

