

# DNA: SOME THINGS TO KNOW BEFORE ASKING, "WHAT'S NEXT?"

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The AKC originated its DNA program in 1998. Today, with several years of operations under its belt, and new policies such as the Frequently Used Sires program and the provision for multiple sired litters, many fanciers are beginning to ask, "What's Next?" Topics such as mandatory DNA profiling for all breeding animals and DNA banking for health purposes are routinely being debated. As these discussions continue, it is important for all delegates to have a baseline understanding of DNA, what it is, how it is collected and stored, and what it can be used for.

DNA (deoxyribonucleic acid) is often described as the basic "building blocks of life". DNA is the genetic code which makes up the genes residing along the 39 pairs of canine chromosomes. The chromosomes reside in the nuclei of the cells, which by the trillions make up the canine body. It is estimated that the canine genome is made up of approximately 35,000 actual genes. These genes, working independently and in combination with other genes, are used to encode everything from color, to size, to disease susceptibility.

DNA is unique to each individual, and DNA within an individual is identical from cell to cell. At all locations (loci) along the chromosome pairs, the DNA sequence on one chromosome is inherited from the sire, and the DNA along the paired chromosome is inherited from the dam. These two principles make DNA analysis ideally suited for the AKC applications of identification and parentage verification. The AKC's DNA profiling involves analyzing DNA sequences (alleles) found at

14 specific variable sites (microsatellite markers) along the canine genome. The resulting profile will be unique to each dog and establishes identity with 100% certainty. Parentage verification works on a slightly different premise. Remembering that the sire and dam each contribute 50% of the offspring's DNA, comparing the DNA sequence found at these 14 markers to the DNA of possible sires or dams, it is possible to exclude certain candidates because there is no DNA match. Since parent-

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age verification works on the process of exclusion, it is difficult to establish with 100% certainty that a given dog is an offspring's sire or dam, however by finding DNA matches at all 14 markers, the probability of accurately determining, or accurately "including", the sire or dam is greater than 99%. AKC field inspectors routinely use DNA profiles in the inspection process to verify accurate record keeping. In addition, for all dogs born after 1/1/2000 that are registered with the AKC and have DNA profiles on record, AKC DNA Operations runs the parentage verification step if either parent has a DNA profile on record. When exclusions are found, the affected parties are notified and the AKC works

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with them to correct the registrations if possible. The combined result of these efforts is an increase in the data integrity of the AKC and its registry.

In the DNA profiling example above, the AKC uses buccal swabs to collect skin cells from the cheek lining. While the DNA yield is somewhat limited, several factors make this the

ideal solution for identity profiling and parentage verification. These tests do not require large amounts of DNA, the collection is noninvasive, sample shipping can be done via standard mail, and laboratories have well established processes to make DNA extraction and purification simple, reliable, and economical. Buccal swabs are obviously not the only means however of collecting DNA. Since DNA is found in all cells with nuclei, DNA

can be harvested from a number of sources. However, cell density and digestibility make certain sources far more practical and convenient in terms of both DNA yield and laboratory costs. The downstream applications such as parentage verification or genome wide scanning most often dictate the most practical and economical method of collecting DNA. While the buccal swabs are ideal for the AKC's DNA profiling activities, for research purposes such as the Canine Genome Project or projects searching for disease markers, the DNA yield from a single buccal swab is typically insufficient. Most researchers prefer blood

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samples. Blood is very cell rich and several cc's of whole blood will provide more than enough DNA to keep even the busiest researchers happy. Furthermore, most research laboratories are optimized to process blood. The downsides include invasive collection, special shipping and handling requirements, overall cost of the collection including a probable veterinary office call, and the need to extract the DNA soon after the sample collection since the sample has limited stability.

One method of DNA collection that is growing in interest is the use of FTA cards. FTA cards look like matchbooks. The inside of the "book" contains paper that is chemically treated and allows for rapid isolation of pure DNA. Samples can be obtained from a variety of sources including whole blood, buccal cells, plant material, tissue cells, microorganisms, and plasmids. When the samples are applied to the FTA-treated paper, cell lysis occurs and DNA is immobilized with

in the matrix of the card. The design also kills any pathogens to prevent future contamination by bacteria or fungi, and protects the DNA from microbial and environmental degradation over time. The samples can be shipped via standard mail, and can be stored at room temperature with the nucleic acids remaining stable for years. To prepare the DNA for use in assays, small punches of 1.2 to 2.0 mm are made in the card itself. The discs from the punches are washed several times in a purification reagent, and are then ready for most common methods of DNA analysis known as PCR amplification. Benefits of FTA include non invasive and economical sample collection from a variety of sources, the stability of the sample for many years, the ability to store the sample at room temperature, and the ability to avoid any further processing costs until such time that the sample is actually needed and used.

FTA paper is widely used today in many human forensic applications as

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well as by many livestock registries. Interestingly, the AKC uses FTA paper today when resubmissions are required after an unsuccessful DNA sample collection from a buccal swab. Protocols are currently in development that will allow elution of genomic DNA from FTA cards. In layman's terms this means that if successful, FTA cards may prove to be a viable alternative to blood in providing the quantity of genomic DNA needed for research activities along with the economic benefits of buccal swab collections. Organizations such as the AKC/CHF and the OFA are closely monitoring developments in this area.

With the rapid scientific advances being made in the area of DNA research and analysis, DNA will surely continue to be a hot topic of conversation, one that the delegates should be prepared to discuss further as we answer "What's Next!". 🐾