DNA testing:
What is it all about?

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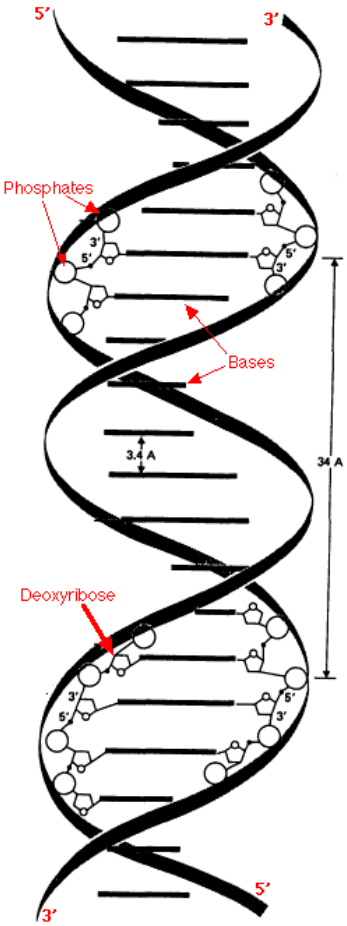
We hear about it in the news, on television, in the movies, and low and behold, at dog shows, dinners, veterinary hospitals, etc. What is the hoopla all about? DNA, otherwise known as deoxyribonucleic acid, the building block of genetics. Law enforcement uses DNA information to prosecute criminals, couples use DNA testing and genetic counseling for reproduction, Hollywood glamorizes DNA testing in movies like *GATACA* and on television shows like *Law and Order* and *CSI*. And what do breeders use DNA for? The AKC offers DNA testing to determine parentage in the case of uncertain sire syndrome (i.e. – oops, the bitch got out and oh no, I hope the unthinkable didn't happen…), and a DNA marker panel for dogs and bitches which is required if they produce above a certain number of litters. DNA testing is also offered by a variety of companies for many different diseases present in the canine population. For poodles, there are DNA tests for coat color genes, von Willebrand's disease, and progressive retinal atrophy. Currently, there are research institutes working on DNA markers for sebaceous adenitis, Addison's disease, epilepsy, and perhaps others. So, what is DNA? How does one go about finding markers? Why does it take so long for a test to become available? Why is it so expensive?

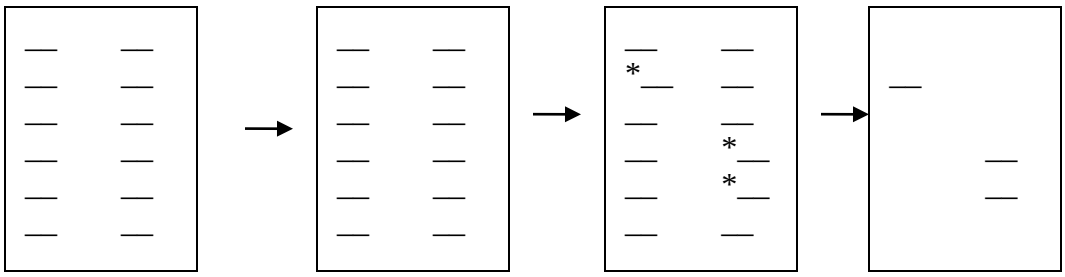


The discovery of DNA, the "building block of life" was announced in a paper published by James Watson and Francis Crick over 50 years ago in 1953. Described as a double helix which "unzips" itself to reproduce copies, DNA is made up of smaller components including nucleotide bases, sugar phosphate backbones, and hydrogen bonds. Just four chemical bases – adenine (A), guanine (G), thymine (T) and cytosine (C), are combined in infinite combinations that result in unique individuals. Certain DNA sequences make up genes, which then determine the expression of a trait. Many diseases are causes by a mutation, or alteration, of the DNA sequence of a gene, resulting in an abnormal physiological or physical trait, such as PRA or epilepsy. So, how does one determine the DNA sequence of an undesirable trait in order to create a test for it? First of all, the researcher has to at least narrow down the possibilities of the gene's location to a segment of DNA. Then the researcher must begin comparing the segments isolated from healthy individuals to those known to express the undesirable trait.

In order to begin comparing sequences of DNA, the researcher must first separate the DNA from the cells which carry it. This involves using the knowledge of chemical reactions to break down cellular components and "sift" out the DNA from the nucleus of the cell. The DNA is then "harvested" or purified from the cellular soup until the resulting solution is a concentrated DNA sample containing many thousands of copies of each gene. In fact, for large scale DNA analysis,

such as the human genome project, robotic machines are used for DNA isolation. DNA can also be cloned using bacterial vectors which can create many exact copies of the genetic sequence to be studied. This is not to be confused with cloning of entire organisms, which is a much more complicated process than cloning segments of DNA itself.

Once the DNA strands have been isolated, it is necessary to break them down into smaller segments to eventually isolate a single gene or strand which contains the gene desired. The DNA molecules are run through a gel material in a buffer solution with an electric current flowing through the tank. As the current is running, the DNA strands will migrate across the gel at different rates, depending on the size of the strand. Once the electrical current is stopped, the DNA is transferred onto a blotter membrane, and then "probed" with radioactive, fluorescent, or other "tags" which will attach to the desired sequence being studied. If one then desires to sequence the individual strands, further chemical reactions can be used to discover the actual order of individual base pairs making up the code for the gene in question.



DNA strands separated on a gel *DNA transferred to a blot membrane* *DNA strands tagged with probe* *DNA strands seen with attached tag*

Currently, there are two commonly used types of DNA tests. The first, called the linkage based test, relies on identifying a sequence of DNA (called a "marker") which lies physically close to the gene sequence of the disease of interest. The closer the marker is located to the gene of interest, the more accurate the DNA test will be, even in cases where multiple variations can produce the disease. Linkage based tests are available in both human and canine research, for several reasons. First, it is not necessary to identify the actual gene sequence of the mutation producing the disease. This can be advantageous if there may be more than one mutation which can produce or combine to produce the disease in question. Secondly, researchers can often identify a segment of DNA in which the mutation is suspected to reside, without having to spend the extra hours and money necessary to identify the actual genetic sequence and all of the mutations involved. The disadvantage is that the accuracy of the test depends upon the marker being close to the gene in question: the greater the separation between the two, the less accurate the test. This can sometimes be overcome if several markers are available, particularly if markers are located both upstream and downstream from the sequence of the gene in question.

The second type of DNA test is the mutation based test. This involves identifying the actual genetic sequence and the mutation(s) involved in producing the disease in

question. Keep in mind that the mutation which produces the disease in one breed may not be the same mutation which produces the same disease in another breed. Therefore, there must be a high degree of specificity in marketing mutation based tests because the mutation(s) must be identified in each individual breed. Often, identifying the sequence of specific mutations within each breed can be very costly. Companies may have difficulty justifying the expense of undergoing such research, if the return will not result in a sufficient profit margin. It is more common for companies to invest resources in identifying genetic mutations for which there is a human counterpart, such as Addison's disease or epilepsy, in which the company may have two markets available to promote the product. Of course, there is also much more grant money available from a variety of sources if the outcome may result in an advance in human genetics as well.

Once either a linkage based DNA test or a mutation based DNA test is available, breeders can use it to help make decisions concerning traits which they wish to either strengthen or reduce within their line. While cost can be a factor in a breeder's decision over whether or not to utilize such a test, keep in mind the amount of time, effort and cost involved in a company even being able to produce such a DNA test for the myriad of diseases in canine genetics. This article only very briefly summarized a skeleton of events which take place in DNA research. The actual amount of hours, specialized equipment, specialized personnel, supplies, marketing, etc. involved in the production of a DNA test for a genetic disease, is astronomical.

Whether a researcher is developing a linkage or a mutation based DNA test, he/she needs plenty of volunteers from the canine population. This requires working closely with breeders and breed clubs. In order to identify carriers versus affected and clear dogs, the researcher must have people who are willing to provide DNA samples as well as pedigrees and follow-up information on dogs which are affected with the disease as well as those which appear normal. The dogs which appear normal must be further sub-divided into either carriers or clear individuals in the case of a genetic mutation which is recessive. It is accepted practice for researchers to guarantee anonymity of those volunteering their dog's DNA in order to encourage as many people as possible to participate in a study without fear of recrimination. It can be particularly difficult for a researcher to obtain samples and pedigrees from affected dogs if breeders fear repercussions for their breeding program. In an ideal world, breeders would be open and honest about all of the problems that may crop up in their breeding experience, encouraging an exchange of knowledge which can only better the health of their chosen breed. Regardless, the more DNA tests which are available for different diseases which plague our canine companions, the more breeders can strive for producing healthier, long-lived puppies.