

## Chapter 10: DNA tests for Hereditary Eye Disease

### Introduction

Advances in molecular biology have facilitated the identification of the causal DNA mutations underlying many different hereditary eye diseases. New mutations are regularly being identified and the resulting list of DNA-based tests developed from these discoveries is expanding. A list of DNA-based tests is available on the ECVO web page as a service for members, dog breeders, kennel clubs and dog owners ([www.ecvo.org](http://www.ecvo.org)).

The availability of DNA-based tests confers many advantages to our efforts to eliminate hereditary eye disease:

- **Accuracy.** Mutation detection DNA-based tests are specific for the gene mutation that they are designed to detect and are very accurate.
- **Early diagnosis.** DNA-based tests can be used to genotype an animal for an hereditary disease prior to the onset of clinical signs. This is particularly valuable for conditions that may not be apparent on eye screening performed prior to the animal being used for breeding.
- **Detection of carriers of recessive conditions.** Detecting whether an animal is a carrier of a recessive trait is one of the major advantages of DNA-based testing. Many of the hereditary eye diseases in companion animals are recessively inherited; meaning that animals heterozygous for the DNA mutation do not develop disease themselves but can pass on the gene mutation to their offspring. Those offspring receiving a mutated allele from both parents will develop the condition. Prior to DNA testing, test mating had been used in some instances to identify carriers. But this is expensive, may result in more affected animals being born and is only really practical for early-onset conditions. In some instances obligate carrier status can be inferred if one of the parents is affected. The presence of carriers in a population is a major barrier to eradication of recessive hereditary eye disease.
- **Specificity of tests.** DNA-based mutation detection tests are specific for the DNA variation being tested for.

Limitations of DNA-based tests:

- The specificity of each DNA-test could also be considered a limitation because it does have the potential of causing some confusion, in particular in breeds where there is genetic heterogeneity (where the same clinical disease can be caused by more than one different gene mutation). Progressive retinal atrophy is an example of such a condition where in several breeds of dog there is more than one form of the condition. Each individual test will be specific for only one form of the condition (this is discussed further below under “Considerations for DNA Testing”).
- Some testing companies offer tests without providing information about the gene mutation being tested for. This is often done to protect the exclusivity of the test and allow the company to make a return on the investment in identifying the putative causal mutation. However, this does not allow the scientific community the opportunity to review the evidence that the DNA variation detected is truly causal for the condition. Peer-reviewed publications that provide convincing evidence that the putative mutation does cause the condition are preferable.

## **What Tests are Available?**

A list of DNA-based tests for hereditary eye diseases is posted on the ECVO web site ([www.ecvo.org](http://www.ecvo.org)). Because this is a rapidly changing field we recommend that the web sites of the individual laboratories offering DNA tests be consulted for up-to-date information from each laboratory.

Most tests are designed to directly identify the presence, or absence, of a DNA mutation that is reported to cause the condition being tested for. These are known as *mutation detection* tests. In certain circumstances in the search for the DNA variation that causes particular hereditary condition researchers may identify a DNA marker that is very closely linked to the disease causing mutation. Such a marker will be a hereditary DNA variation which is not the cause of the condition but is located close on the chromosome to the mutation and is thus co-inherited with it. Sometimes a test is developed using the linked marker to indicate the likely presence or absence of the disease causing mutation in a test animal. This is known as a *linked marker* test. Linked marker tests have limitations and are typically used to indicate which test animals either are likely to have the disease-causing mutation OR are likely to be free of the disease-causing mutation. The reliability of a linked-marker test is dependent on a number of factors including the genetic distance that the marker is from the disease-causing mutation. Once the actual causal mutation for the condition is identified a mutation detection test can be developed.

Ideally for each hereditary eye disease for which a test is commercially available there should be a peer-reviewed publication to allow the validity of the claim that the DNA variance that is being tested for causes the disease in question, or in the case of a linked marker test is very closely linked to the disease location. The publication should describe the mutation and preferably provide supporting evidence that the DNA variant does cause the disease and is not a non-pathogenic polymorphism. Unfortunately not all putative disease-causing mutations are published at the time that the test is introduced.

Well-designed and carefully conducted DNA-based tests should be very specific in identifying the presence or absence of the causal mutation. Most potential mistakes result from human error such as mislabeling of the collected blood or cheek swab samples.

## **Laboratories offering DNA tests**

Utilizing a well-established laboratory is recommended. ECVO does not recommend specific laboratories. Some of the laboratories offering tests are listed on the web site but this should not be taken as an endorsement from the ECVO. Some laboratories will test submitted DNA samples for a series of DNA mutations known to cause disease in one or more breeds of dog. Therefore animals may be tested for a condition that is not known to be present in that particular breed.

## **Samples for DNA-based tests**

It is important to follow the sample collection and handling instructions for the laboratory running the DNA test. Although DNA can be obtained from many different types of sample (blood, cheek swab, hair plucking, semen sample etc) the test being run may be optimized for the quality of DNA obtained from one source (typically blood) and the laboratory may not wish to utilize another DNA source (such as cheek swabs). Accurate labeling of the sample is critical (particularly if multiple animals are being

sampled) and steps should be taken to avoid contamination (particularly important if cheek swabs are being used).

Permanent identification of the animal being tested is also optimal to avoid misrepresentation of the status of a breeding animal. However when cheek swabs are collected and submitted for testing by the owner of the animal there is no veterinary control to confirm that a submitted sample originated from a specific animal.

### **Considerations for DNA Testing**

The largest number of DNA tests for hereditary eye diseases are for various forms of progressive retinal atrophy (PRA). PRA is a genetically heterogeneous condition; mutations in many different genes can lead to the clinical signs that we categorize as “PRA”. Some genetic mutations that cause PRA are found across multiple breeds of dog, for example the PRCD mutation causes PRA in many different breeds of dog. Other gene mutations are specific to one breed of dog. It is known that in some breeds of dog multiple different PRA-causing gene mutations are segregating. Because each DNA test is specific for a single gene mutation it is possible for a dog to be cleared for the form of PRA that was tested for and yet still develop PRA due to a different gene mutation. This can be confusing for owners and breeders if they do not understand that each test is specific for only one form of PRA.

Some laboratories offer testing for a barrage of different tests, some of which are not even known to occur in the breeds being tested.

### **DNA-based tests enable carriers of recessive conditions to be used in breeding programs**

The aims for genetic testing are to firstly prevent the production of more animals affected with the hereditary disease and secondly to eliminate the mutant allele from the population. If the mutant allele is at a high frequency in a population complete avoidance of breeding from carrier animals as well as affected animals could mean that the gene pool is seriously limited. Limiting the gene pool in such a way could mean that other hereditary diseases that are present in the population may become more prevalent. Additionally there may be carrier animals that have particularly good features that breeders would wish to maintain in their lines. To achieve the first aim of not producing any more affected animals carriers can still be used for breeding but only if they are bred with an animal that is homozygous normal for the mutated gene. Fifty percent of offspring from a carrier to normal mating will be expected to also be carriers. Such selective breeding allows for the gradual elimination of the defective gene over the period of a few generations while avoiding limitation of the breeding gene pool and maintaining desirable features of carrier animals for future generations.