DNA research for Picards

Here at the University of Missouri College of Veterinary Medicine, in the Animal Molecular Genetics Laboratory, we search for mutations causing inherited diseases in domestic animals (and a few wild species as well). Most of our work is with dogs, and we have discovered mutations responsible for many neurologic conditions, several eye diseases, and cardiac issues. In the past 18 months, we have been using a technique called Whole Geneome Sequencing (WGS) to search for mutations. With WGS, the entire genetic sequence – every protein, every gene, every chromosome – is generated on a single individual. That genetic sequence is aligned to a reference sequence, and also compared to the other WGS's we have run on other dogs. This creates a mountain of data, and identifies thousands of variations from the reference sequence. Then our task is to sort out which variations may be responsible for the disease we are targeting. When we believe we have found a likely candidate, other individuals with the same disease, plus normal relatives (parents, siblings, etc) are also tested to see if they do have the newly identified mutation, and determine if the genetic pattern seen fits the clinical information known for all the dogs used in the research.

Using WGS, we have identified 10 causative mutations so far, and many more are in progress. The mutations identified are responsible for Fanconi Syndrome (a kidney problem) in Basenjis, a juvenile Parkinson's syndrome in Kerry Blue Terriers and in Chinese Cresteds (2 different mutations), 2 forms of ataxia (inability to coordinate movement), one in Soft-Coated Wheaten Terriers, and the other in Jack/Parson/Russell Terriers and related breeds, 2 different forms of PRA in Basenjis and in Tibetan Terriers, and dilated cardiomyopathy in Standard Schnauzers.

In Berger Picards, we have completed a WGS from a Picard diagnosed with Canine Multifocal Retinopathy (CMR), and are searching the resulting sequence data to find the mutation for this eye disease. We have also sequenced a second Picard, as a result of request and generous donation by a private owner. At present, this dog does not have any heritable diseases diagnosed, but the sequence data is still very useful in the search for disease-causing mutations. The analysis process identifies locations in the genetic sequence where this dog is heterozygous – it has one form of the gene inherited from one parent that matches the reference sequence (considered the normal form of the gene), but a different form of the gene from the other parent (a mutation). Where this becomes interesting is some of these mutations could cause disease if a dog had 2 mutated copies of the gene. One example is a possible cardiac arrhythmia gene mutation currently being evaluated. It is also sometimes very useful to look at more than one WGS from the same breed, and look at what is the same in those dogs but different in the sequence from other breeds.

For each dog sequenced, the actual cost of generating the WGS is $5000. The current priority for most Picard fanciers seems to be PRA. There probably are at least 2 forms of PRA in this

breed – one with an onset around 2-3 years of age, and another with a later onset, generally 8-10 years of age. We have DNA samples from dogs with both forms of PRA, but welcome additional samples from affected dogs, plus their normal relatives (most useful are parents, grandparents, siblings, but any relative is of some use). These additional cases and normal related dogs are used when we are evaluating a possible mutation, so it is quite important to have DNA banked. It would make the most sense to target the early onset form first, as it has the greatest impact on the dog's life and owner's ability to enjoy the dog. If enough funds are raised to also sequence a dog with the late onset form, that can be done as funds become available.

It is important to realize that this is scientific research, not manufacturing widgets – so we cannot guarantee that we will identify the mutation as a result of going through this process. However, once we have WGS on a dog, that sequence data can be reviewed and re-analyzed with newer and better tools as they become available. Should we be unable to find a mutation with the initial analysis, we will continue to search, and sooner or later, the mutation can be identified. We are working on several other PRA's in other breeds, and the equivalent human disease is of great interest to many research teams, so there is a wealth of information and discovery happening with the genes associated with PRA's. We would not propose starting this research if we did not think there was a reasonable chance of finding the mutation – and there's no way to find it without going looking!

If there are any additional questions about the process or what we propose to do, we are happy to answer them. We look forward to working with Picard fanciers, wherever they may live, toward improved health, and a strong and diverse gene pool for the breed.

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