DNA parentage and profiling testing

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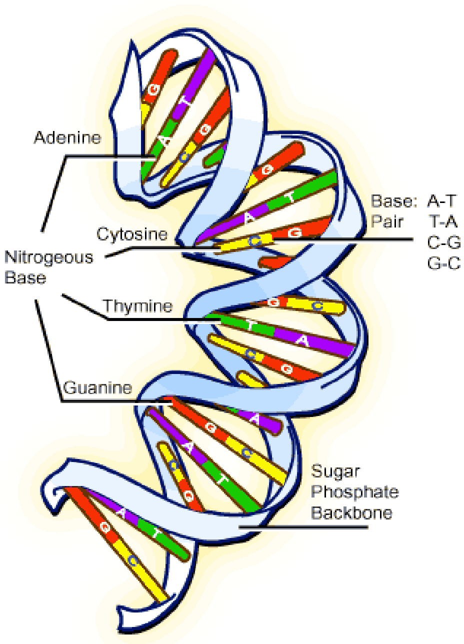
What is DNA, chromosomes, genes and genetic markers?

All living organisms have DNA. To picture DNA the easiest is to imagine a spiral staircase. The staircase consists of steps and handlebars. In the case of DNA the handlebars are less important and simply the backbone of the molecule and the important part is the steps, or bases. There are only four bases in DNA: A, T, C and G and they are found in pairs. A and T ALWAYS pair up and C and G ALWAYS pair up. Therefore a "step" in the DNA will be a base pair or A-T / C-G.

Figure 1: A DNA molecule



DNA is packaged in chromosomes and each species has a specific number of chromosomes. For example, dogs have 78 chromosomes and humans 46, all are in duplicate. Each animal inherits one copy of each chromosome from each parent, hence the duplications. Thus a chromosome is thus a string of A, T, C, G base pairs arranged in a specific order.

To explain the difference between genes and genetic markers the easiest way is to imagine the DNA or the chromosome as the N1 highway from Messina to Cape Town. On the N1 you will pass through areas where there are towns and areas of nothing or farms. Consider the towns as genes: it is a stretch of road (the DNA) that is arranged in a specific sequence (first the butcher, then the chemist, then the hairdresser…) and has a function (people live there). Now imagine a rock or a hill as a marker: it is something that you know is always at the same spot, in the same sequence but it has no function.

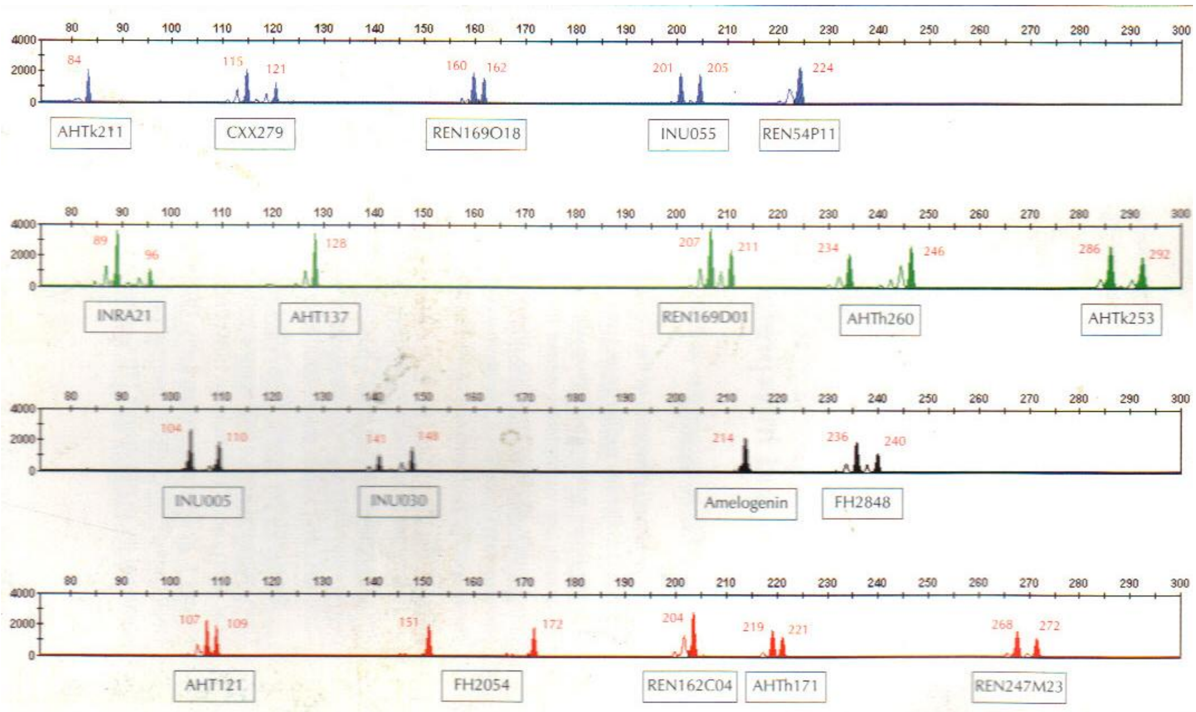
Thus a gene is a piece of DNA or sequence of base pairs that has a specific function. This function can be colour or a biochemical pathway or hormone production etc. The sequence of the base pairs is very important and needs to be in the exact order for the gene to function normally. If this sequence is broken we call it a mutation and we get "a change". This change can be positive e.g. a new colour variation or negative e.g. a genetic defect or disorder like PRA-prcd found amongst Yorkshire terriers. Genes are also found in pairs and each animal inherits one copy of each gene (allele) from each parent.

A genetic marker on the other hand is also a piece of DNA but it has no function. There are many different kinds of markers but the two mostly used in DNA profiling and parentage testing are microsatellite markers and SNP markers. SNP markers are one base pair that is exchanged for another base pair, e.g. one animal has an A at that specific spot and another animal has a C at that same spot. Thus there can be only two options or alleles. Microsatellites are the marker used most widely in parentage testing (and used by Inqaba biotec). These are pieces of DNA that are repeated e.g. ATCATCATCATCATC and the number of repeats (in this case 5 repeats of ATC) differ between individuals. Any one microsatellite marker can have up to 30 different repeats and this is what we call alleles. Similarly to genes, each animal has two alleles of each marker, inherited from each parent. Parents pass the number of repeats on to their puppies so a puppy and its parent must share at least one allele at each marker.

How does a DNA parentage and profile test work?

The laboratory test for DNA parentage verification and profiles are identical. The only difference is that the data of the possible parents and the offspring is compared in the parentage verification test. For canine DNA parentage and profiling 19 microsatellite markers are tested. The picture seen on the computer after the analysis is completed looks like the image in Figure 2.

Figure 2: A presentation of the microsatellite markers during the test



Most of the markers in Figure 2 have two peaks, or alleles. Markers AHTk211, AHT137, Amelogenin and REN162CO4 also have two alleles, but the peaks lie on top of each other so it isn't visible. This is the profile of the animal. Table 1 shows a couple of markers tested to generate the profile of the animal in Figure 2.

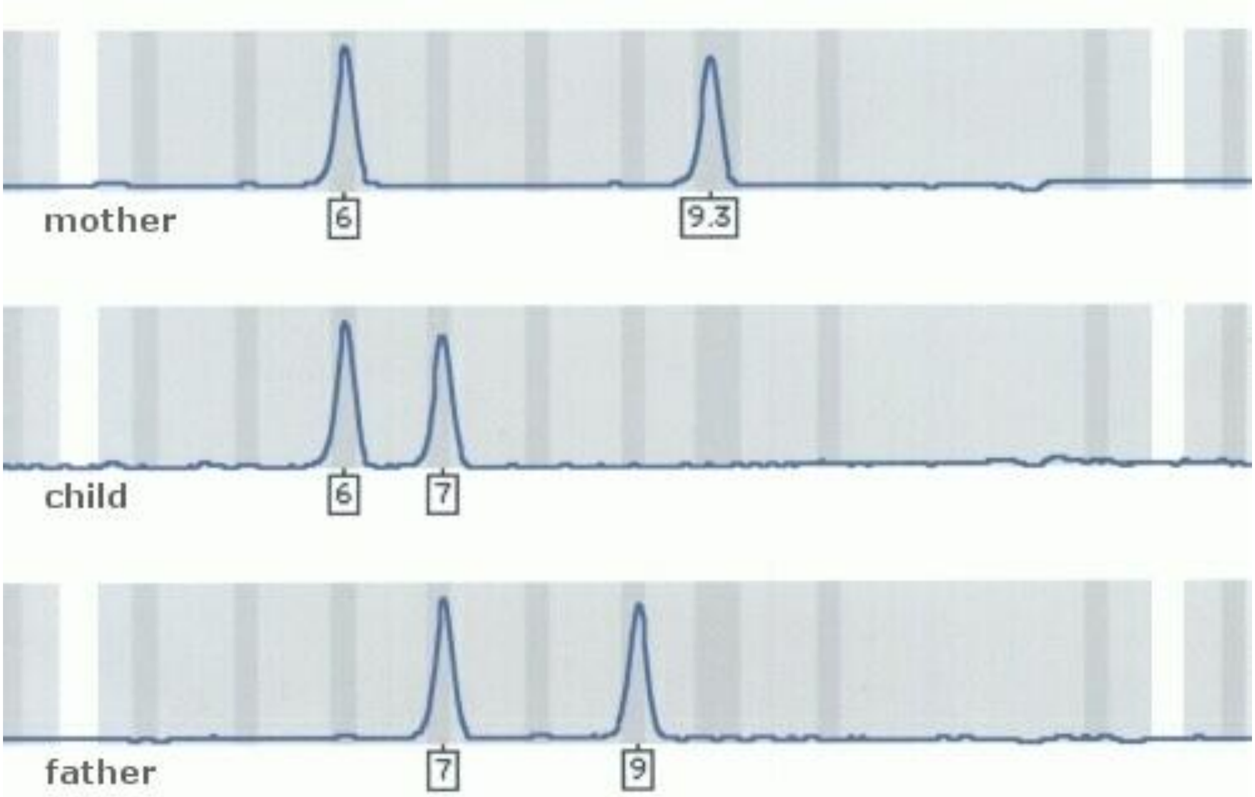
Table 1: A shortened version of the profile of Animal 1

Marker	Allele 1	Allele 1
AHT121	107	109
AHT137	128	128
AHTh171	219	221

The alleles 107/109 or 128/128 or 219/221 are the number of repeats (like the example above ATCATCATCATCATC) measured from a specific reference point.

When verifying parentage the profiles of the dam, puppy and all the possible sires are compared as shown in Figure 3.

Figure 3: Profiles of dam, sire and puppies



In Figure 3 the mother has two alleles 6 and 9.3, father 7 and 9 and the child 6 and 7. The child had to inherit allele 6 from the mother and allele 7 from the father. If the father had e.g. alleles 9 and 11 he could not be this child's father. Of course this conclusion cannot be made from one marker alone and the decision is made based on the comparison of all 19 markers.

Sometimes if the possible sires are related or if one of the sires is related to the dam it is very difficult to discriminate between sires. However if the dam is tested with the puppies this eliminates the problem in most cases.

What can I do with a DNA profile?

There is only one thing that you can do with a DNA profile: identification! It can't tell you the breed of the dog, it can't tell you which dog is the "best" to breed with, it can only tell you two things:

1. Which individual it is
2. Who is the parents of the individual

Price by inqaba biotec:

Number of dogs tested	**Cost (per dog) incl. VAT**
1 to 8	R342
9 or more	R250

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