

EQUINE PARENTAGE & ANIMAL GENETIC SERVICES CENTRE

Instructions for the taking of hair samples for DNA typing

Background

The required sample for DNA typing is mane or tail hair to be taken in accordance with the following instructions.

Hair Sampling Instructions

When: Hair samples can be tested at any age. Contact your breed society as they may have specific requirements re timing of branding and registration.

Where From: Either mane or tail hairs are suitable – in some foals, mane hair may be very fine and likely to break in the pulling process – in these cases, tail hair may be more suitable.

How: When taking the hair sample it is **absolutely essential that most hairs have intact hair roots**. Thus hairs must be pulled out **NOT CUT**. Grasp a tuft of 20-30 hairs as close to the skin as possible (wrap around finger or comb) and pull evenly and slowly in a peeling motion away from the skin – samples obtained in this way should have at least half of the hairs with intact hair follicles – these can be seen as small bulbs. If tail hair is taken, some samples may require trimming to fit into the sample envelopes – PLEASE ensure the actual hair samples sent contains the hair roots

Despatch of samples to the laboratory

Hair samples are to be placed in the pre-printed envelopes (standard plain business 223 x 112mm) provided for TBs by NZTR, and for SBs by HRNZ. Other breeds follow your societies recommendations – always use a **paper** envelope, with the following basic identification information completed on the outside of the envelope (YOB, Dam, Sire, Sex and Brands, and if applicable bar code label for microchip inserted).

Hair samples can be kept in a cool dry place for up to one week prior to despatch to the laboratory. Send samples at least weekly by courier or fast post. Please do not send the individual hair sample envelopes through the post.

Address to send samples:

Dr J I Cahill
Equine Parentage & Animal Genetic Services Centre
Drysdale Drive
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EQUINE DNA TESTING FOR BREED REGISTRIES

The use of DNA technology is something most of us are familiar with – mostly thanks to its uses in human forensics. But its applications in the equine world, including DNA profiling for identification and parentage, screening tests for coat colour and some inherited diseases, are growing all the time. Of particular importance to all equine breed registries, regardless of size, is the production of DNA genotypes or profiles for individual horses, and the use of these profiles in verifying parentage of foals before registration.

Here are some basic genetic concepts to help you understand the following information on DNA genotyping.

Chromosomes – long double stranded molecules of DNA, present in the nucleus of every cell in the body, containing all of the information of inheritance. Chromosomes come in pairs – one from each parent.

DNA (deoxyribonucleic acid) – molecules made up of millions of the four basic repeating units (nucleotides or bases); A (adenine), T (thymine), G (guanine) and C (cytosine). Only 10% of DNA sequence codes for genes – the function of the 90% of non-coding DNA is unknown.

Genes – specific lengths/sites on the DNA, containing the information for inherited traits, coded by the order of the four bases. Genes and gene markers also come in pairs – known as loci.

Microsatellites – DNA markers which are present in the 90% of non-coding DNA, come in pairs, one from each parent.

Allele – form of a gene or genetic marker at its specific locus. There are two alleles at each locus – one from each parent.

Parentage verification

A scientifically based method for verifying parentage is crucial to any breeding programme, to ensure that pedigree information, upon which breeding decisions are made, is absolutely accurate. Reliance on standard methods such as observation, manual identification and record-keeping produces a surprisingly high level of errors. Even in the 'gold standard' of pedigrees, the Thoroughbred Stud Book, established in 1791 by Wetherby and Sons, recent genetic studies tracing back to the 30 founder thoroughbred mares showed that up to 50% of maternal lines contained DNA they could not possess if the pedigrees were accurate.

While it has been standard in New Zealand for some years that all thoroughbred and standard bred foals undergo DNA genotyping and parentage verification before they can be registered, it is equally important for all breed societies that their pedigree keeping uses an accurate scientifically based method of parentage verification.

How is a genotype produced in the laboratory and then used to verify parentage for the foal?

This technology is the same as that used in human forensics and parentage testing.

1. The first step in any form of genetic testing involves isolating DNA from the individual involved. In horses the sample of choice for DNA testing is hair— specifically the hair follicle cells attached to hair pulled from the mane or tail.

2. DNA is extracted from the hair follicle cells, and it contains the entire DNA sequence from all of the chromosomes of that horse.

3. From this DNA 17 specific gene markers, microsatellites, are tested to produce the DNA profile or genotype for that individual. At each of the 17 microsatellite markers there is a pair of alleles, one from each parent.

4. The two alleles of the foal are compared with those from the dam and from the sire, for each of the 17 markers tested, to ensure that one of each pair has indeed come from the dam and one from the sire.

Steps 1-3 result in the production of the individual animals DNA profile, which can then be used for identification or for use in parentage verification.

Step 4 is the process of parentage verification, and this test is more than 99.9% accurate.

Below is an example of a parentage report for 5 of the 17 microsatellites.

The abbreviations across the top row are the names of the microsatellite markers.

In the columns below are the 2 alleles present for that marker in the foal, the dam and the sire.

	HTG4	ASB2	ASB17	HTG10	LEX33
Foal	KK	KR	NR	LM	MQ
Dam	KO	PR	LN	LL	OQ
Sire	KL	KP	RR	MN	LM

For each marker you can see that one allele has come from the dam and one from the sire. For example, for the microsatellite ASB2 the foal has the alleles K and R. R has come from the dam and K from the sire. For parentage to be verified there can be no mismatches at any of the 17 markers.

Equine parentage laboratories worldwide use the same base set of 12 markers, under the recommendation of the International Society of Animal Genetics, so that exchange of information can take place between laboratories and breed societies in different countries. This means that horses don't have to be retested when they travel internationally, and when imported semen is used it can be accompanied by a DNA profile for use in parentage reporting for any offspring.

Sometimes the results produced are not as expected— there will always be surprises when dealing with horses! Some of the scenarios we see where there are mismatches between foal and/or dam and sire include mares swapping foals in the paddock, and stallions that should not have been with a particular mare using all sorts of devious means (sometimes seemingly impossible) to mate with said mare. Then there are the cases involving human error - mares can be misidentified, transcription errors can occur filling out the paperwork, and hair samples can be put in the wrong envelopes. But with DNA technology and some old fashioned detective work we can now reach a conclusion on the vast majority of these difficult cases.

It must be remembered that we can only produce parentage reports for a foal if we have the DNA profiles for the dam and sire on the database. For this reason it is extremely important to test all breeding stock (or possible breeding stock) as soon as possible. Every year we see a number of cases where foals can not be registered because the dam has died before she has been DNA typed.

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