

Genotyping Results explained

DNA testing has proved to be a powerful tool in verifying the parental links in cattle. It relies on the fact that within cattle DNA (and that of all animals) there are specific markers, known as microsatellites, which create uniquely identifiable DNA patterns that can be used to follow the transmission of DNA from parents to progeny.

Almost everyone is familiar with the idea that DNA is made up from a sequence of just four nucleotides, designated by the codes A, C, G and T. Most people also recognise that the nucleotide sequence varies, thereby creating the differences between animals of different species. It also results in differences between different individuals of the same species.

Microsatellite markers are specific and well documented segments of chromosomal DNA comprising a two nucleotide base sequence (eg. AT), repeated a set number of times (i.e. ATATATATAT..... and so on). The number of repeats differs in different individuals so that, for example, one individual may have the 2 letter code repeated 40 times, and another 46 times. This difference provides the basis for establishing a parental test.

Importantly, these markers occur in several places (loci) throughout the genome and are generally found in non-coding regions. They are also subject to additions and subtractions in the number of tandem repeats of the basic two nucleotide segment, and this creates uniquely identifiable alleles at each site within the genome at which the particular microsatellite is located.

Since DNA can be extracted from blood, hair, tissue or semen, it is relatively easy for a vet to provide a sample to the lab for testing. For BCMS applications, hair and blood are the preferred sample types. The only stipulation is that the hair must be collected from the withers and be clean and dry. It must be pulled out, not cut, and have follicles attached. Hair can be stored in a fridge or freezer for several days before testing. The blood must be taken into a glass or plastic tube lined with heparin or EDTA (to prevent clotting) and be sent directly to the testing laboratory. It can be stored for a few days in a fridge, but it must not be frozen.

At Reading Scientific Services Ltd (RSSL), one of only a few laboratories approved by BCMS to carry out this testing, samples are logged immediately on receipt into a Laboratory Information Management System (LIMS) to track them through the process. They are given unique identifiers to make the system fully auditable and traceable.

At the testing stage, DNA is first extracted from the samples, and then the markers that are of interest are copied many times to create sufficient numbers to be detected.

The detection is based on a very sensitive instrument that can accurately size the fragments created by the copying process. The DNA is then normalised to control samples so that results obtained in any other laboratory can be compared (International Standard Fragment Sizes).

Despite the fact that all loci are based on a 2 letter repeat, not all numbers are even (part of the normalisation process). However, for each locus all numbers must be either odd or even, not a combination of odd and even. Each animal will have 2 numbers at each marker Locus (see figures 1a and 1b), with one "number" being inherited from the dam and one from the sire, in any order. By

comparing the numbers from a parent and progeny, it is possible to interpret whether that progeny came from that parent.

Hence, if a calf has the numbers 81/89 and the parent in question has the numbers 81/83, then it would be possible that the parent is the correct parent of that calf (because the parent has passed on 81 to the calf). If the parent had the numbers 85/97, then it could not be the parent of that calf. That said, since DNA does not always copy exactly between generations and for each locus, there will always be a certain number of variations in any population and so it is important to study more than one locus. In fact, the International Society for Animal Genetics (ISAG) has recommended certain loci for this type of comparison, with the clear understanding that there will always be a chance that at some loci, mutations may occur between generations and so the numbers between true parents and progeny may not match at every loci. It is therefore important to study as many loci as possible and allow for some mutations. ISAG state that for true parental matches, parents and progeny must match at at least 9 loci, hence RSSL usually tests at 11. If they match at any fewer than 9, they are not considered to be parent and progeny.

Two examples are shown in figure 1a and 1b. The former illustrates the case where there is a poor match between calf and presumed parent, resulting in a negative result, and the latter, a case where there is a good match resulting in a positive result.

As the BCMS figures already reveal, when backed by supporting DNA evidence, the percentage of successful cattle passport appeals is close to 100%. With that kind of success rate, farmers with a legitimate claim for appealing a late application can be sure to receive a sympathetic hearing from BCMS. Hence there is every possibility that more appeals will be lodged in future, and CIR (2007) will continue to have an impact well into 2008 and beyond.

Figure 1a.

Locus	Sequence length in 'parent'	Locus	Sequence length in calf	match
TGLA227	81/97	TGLA227	81/95	Yes 81
BM2113	133/137	BM2113	127/135	No
TGLA53	160/162	TGLA53	160/162	Yes 160 and 162
ETH10	217/219	ETH10	219/219	Yes 219
SPS115	248/252	SPS115	256/256	No
TGLA126	123/123	TGLA126	115/117	No
TGLA122	143/151	TGLA122	151/151	Yes 151
INRA23	208/212	INRA23	200/214	No
ETH3	117/119	ETH3	117/119	Yes 117 and 119
ETH225	140/148	ETH225	140/152	Yes 140
BM1824	180/188	BM1824	178/188	Yes 188

Therefore, they match at 7 out of 11 loci – therefore the parent is excluded as a parent of this offspring.

Figure 1b.

Locus	Sequence length in 'parent'	Locus	Sequence length in calf	match
TGLA227	89/89	TGLA227	89/91	Yes 89
BM2113	133/135	BM2113	131/133	Yes 133
TGLA53	162/176	TGLA53	154/176	Yes 176
ETH10	217/219	ETH10	217/219	Yes 217 and 219
SPS115	250/250	SPS115	250/252	Yes 250
TGLA126	115/115	TGLA126	115/115	Yes 115
TGLA122	143/143	TGLA122	143/153	Yes 143
INRA23	206/214	INRA23	200/214	Yes 214
ETH3	117/119	ETH3	117/127	Yes 117
ETH225	146/148	ETH225	148/148	Yes 148
BM1824	178/182	BM1824	178/180	Yes 178

Therefore, they match at 11 out of 11 loci – therefore the parent qualifies as a parent of this offspring.