The importance of using DNA profiles within livestock farming

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Advances in molecular technology, especially in genomics, have unveiled a whole new set of tools to utilise within the farming industry. With the simple act of collecting and submitting tail hairs, the consumer now has access to pedigree information, disease conditions and even growth and breeding potential of his/her animals. But, what does it mean to have an animal genetically tested and what good is it to the consumer?

DNA can be seen as the blueprint of any living organism. This blueprint consists of only 4 letters that are repeated millions of millions of times in different combinations and sequences throughout the genome/blueprint. Ultimately, it is the various combinations of these 4 letters that give the body instructions: how to grow a tail in a particular area or how to make horns grow out from the top of the head.

Genetic markers serve as the fundamental tool for genomics. The first genetic markers used in the livestock industry were blood typing in the 1960s. The technology has since progressed to the use of microsatellites in the 1990s and is slowly but surely moving towards the standard use of single nucleotide polymorphisms (SNPs) in future. Microsatellites are widely used today in various livestock species for parent and individual identification and verification. To generate the profile for any organism, we need to look at areas in the blueprint that abide by the following two rules:

- there should be enough variation within the marker to be able to distinguish one individual from another and secondly;
- The marker may not be under selection pressure. In other words, the organisms may not change the composition of the marker, from one generation to the next, in order to adapt better to the environment. Ultimately, we want to be able to trace back which copy of the marker came from mother, and which copy of the marker originated from the father.

The markers must therefore differ enough from each other so that we can distinguish between individuals, but in their uniqueness, they may not further alter themselves. The mother's marker must remain the same within her uniqueness so that we can see this same marker in her offspring and confirm that it is descended from her.

The sequences we refer to as "markers" are actually simple repetitions of a few letters at a specific place in the blueprint. For example: the sequence at a specific location in the blueprint is: **ACAG**. This **ACAG** repeats itself 5 times in that particular area of the blueprint. Individual A, at that particular location within its genome will be: **A**CAG**A**CAG**A**CAG**A**CAG**A**CAG. In another individual, in the same region of the blueprint, for example, this **ACAG** is repeated only 3 times; and is the individual at that particular area in the blueprint is: **A**CAG**A**CAG**A**CAG (see Figure 1).

Marker for Individual A:
Sequence repeated 5X

ACAGACAGACAGACAGACAG

Marker for Individual B:
Sequence repeated 3X

ACAGACAGACAG

Figure 1: Illustration of the differences between the same microsatellite marker for two individuals.

The number of times that **ACAG** repeats itself will remain constant from one generation to the next. Individual B, with 3 repeats, will produce offspring that also have only 3 repeats of ACAG at the specific location in the blueprint. Parentage is therefore determined by comparing the number of repetitions at each marker of the predicted mother and father with the number of repetitions seen within the calf's profile (Figure 2).

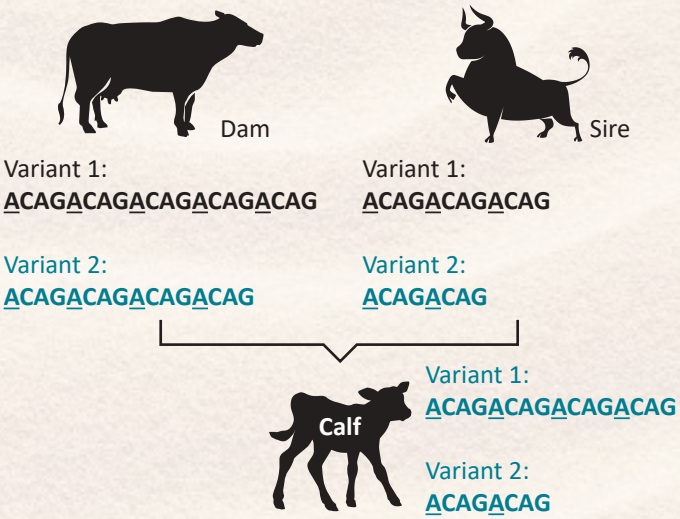

Figure 2: Illustration of parentage determination using a microsatellite marker.

A single marker, by itself, will not be able to pinpoint the parents of a calf out of possible hundreds, but when

several of these markers are used for analysis, it becomes a powerful tool to create unique profiles for each animal which can then be utilised to confirm parentage. For a parental pair to be verified, both mother and father must each contribute one copy of the calf's marker to that profile (Table 1). The calf in the table below is, for example, *121* and *139* at marker **BM2113**. The mother has the following copies available: *121* and *137*. The sire has two copies of *139* available. The calf thus inherits his *121* from the mother, and his *139* from the father. For a parentage to be declared valid, we must therefore be able to see for each marker within the calf's profile, which copy was inherited from the mother and which copy was inherited from the sire.

Table 1: Example of parentage verification for a calf. For a valid parentage, the calf must have a copy contribution from both the mother and the father at each of the markers. Maternal contributions are shown in grey. Paternal contributions are indicated in teal.

Marker	Calf	Mother	Sire
BM1824	180/180	180/182	180/180
BM2113	121/139	121/137	139/139
ETH10	211/211	211/217	211/211
ETH225	151/154	150/154	140/151
ETH3	121/125	117/121	117/125
INRA23	208/214	208/208	214/214
SPS115	248/254	248/254	248/248
TGLA122	137/149	137/137	143/149
TGLA126	115/115	115/115	115/117
TGLA227	87/97	87/93	77/97
TGLA53	154/166	154/172	166/176

If a mismatch is found, the parentage is declared invalid. Table 2 demonstrates mismatches between the calf and the nominated parents. At marker **BM1824**, the calf is *180* and *190*. The *180* could have been inherited from the mother. However, if we look for the calf's *190* within the father's profile, we will see he does not have it available. The nominated father therefore falls out on this marker as a possible parent. At marker **INRA23**, the calf is *208* and *216*. The calf could have inherited the *208* from the mother. The calf now needs *216* from the father. However, the father only has *214* within his profile, he therefore falls out on this marker as possible parent.

Table 2: Example of mismatches during parentage verification for a calf. For a valid parentage, the calf must have a contribution from both the mother and the father at each of the markers.

Marker	Calf	Mother	Sire
BM1824	180/190	180/182	182/182
BM2113	121/139	121/137	139/139
ETH10	211/215	209/217	211/211
ETH225	151/154	150/154	140/151
ETH3	121/125	117/121	117/125
INRA23	208/216	208/208	214/214
SPS115	248/254	248/254	248/248
TGLA122	137/149	137/137	143/149
TGLA126	115/115	115/115	115/117
TGLA227	87/97	87/93	77/97
TGLA53	154/166	154/172	166/176

The main function of the generated DNA profiles is parentage and identity verification. It is important to verify parenting for the following reasons:

- More **accurate breeding selections**. New trends in animal production systems tend to encourage producers to produce a larger number of animals per farm in response to environmental constraints. In this type of setup, several animals are bred together on the same day or give birth on the same day, which can lead to pedigree recording errors.
- **Genetic improvement** of the population due to more accurate economic breeding values - by incorporating the correct parents' data in their calculations.
- Identification of bulls that over and underperform in the stud.
- Identification of problem bulls in the stud - those that lead to calving problems or mutations in the group.
- Enables you to sell animals at a higher price due to pedigree verification.

Given the need for high quality standards in all uses of molecular data, laboratories offering microsatellite and/or SNP-based parentage analysis services, including parenting verification or animal identification verification, are encouraged to meet the basic accreditation requirements. This includes evidence of the minimum internal management quality assurance standards as well as participation in the international ring test (comparison) offered by the International Society for Animal Genetics (ISAG).

The purpose of the ISAG comparative testing is to ensure that laboratories that offer genotyping services maintain a high and comparable standard as well as to establish rules for how kinship testing should be done and the nomenclature that should be used during reporting. When laboratories all follow the same nomenclature and rules when it comes to creating profiles, it enables laboratories around the world to exchange profiles with each other which can then be used for parentage and identity verification. If laboratories follow the same rules and procedures for creating DNA profiles, the profiles should always be comparable to

each other, regardless of where the profile was originally created. These ring tests take place every two years, with a service laboratory designated for each species per testing cycle. This service laboratory collects 21 samples representative of the different breeds within the species and extracts sufficient DNA to send to each participating laboratory. Each laboratory participating in these tests receives the same set of samples to test, with the profile of 1 of the samples disclosed to the laboratories. This profile then serves as a reference for how the laboratory should score the profiles of the remaining 20 samples. At the end of the testing period, the participating laboratories submit their results, which are then evaluated by the service laboratory. The laboratory is then assigned a rank (Table 3), based on how accurate their submitted profiles were and whether they were able to interpret the parentage verification questions correctly.

Table 3: Rankings during participation in ISAG ring tests.

Genotyping Accuracy **Total number of participating laboratories: 60**	
Ranking	**% Laboratories within the ranking**
1: 98 – 100% accuracy	70
2: 95 – 97,9% accuracy	10
3: 90 – 94,9% accuracy	7
4: 80 – 89,9% accuracy	8
5: 80% accuracy	5

Laboratories then receive a certificate that can be sent to clients in order to demonstrate their genotyping competence to their clients. However, laboratories that do not regularly participate in these tests run the risk that their profiles will not be comparable to those of other laboratories.

Accurate parentage verification is a key component in taking a farm's herd improvement plan to the next level. One of the biggest challenges for farmers during calving season, is the time required to consistently monitor and accurately record calving details for a sustained period. Parentage verification is also especially important when multiple bull matings are used within the stud. Not only does it highlight problem pairings and good matches, but DNA testing can make calving time much easier to manage, because you can verify parentage with certainty, without having to be there to record every birth.