

Increasing PEDIGREE ACCURACY with DNA Parent Verification

Accurately recording pedigree information is one of the most fundamental jobs of a beef seedstock producer. Amongst other things, accurate pedigree information is fundamental for the maintenance of an accurate herd book by registered breed societies, it plays a crucial role in enabling the calculation of Estimated Breeding Values (EBVs) from genetic evaluation programs such as BREEDPLAN, and allows seedstock producers to meet their duty of care to provide the most accurate information as possible on their sale animals (e.g. bulls, semen or embryos).

Even with a concerted effort to record accurate pedigrees, just how accurate is the pedigree information in the average seedstock herd? Many situations can compromise the accuracy of pedigree information, including:

1. In a naturally joined single sire joining mob there is always a possibility of another bull (known or unknown) mixing with the mob at some stage.
2. Artificial breeding technologies can add another source of error. If the backup bull is put in soon after the AI program there will not be a clear break in calving between the AI calves and calves sired by the backup bull.
3. In multiple sire joining programs, individual sire identification is impossible without the assistance of sire verification through DNA testing.
4. "Mothering up" cows and calves may be compromised by mismothering, particularly in first calf heifers.
5. Human error when recording the mating

details either from natural or artificial breeding programs.

DNA parentage verification offers seedstock herds with a tool for accurately determining animal parentage and reducing pedigree errors that may otherwise occur.

DNA PARENTAGE VERIFICATION IN PRACTICE

An interesting case study into how easily errors can occur in pedigree recording even in a herd following best practice guidelines, was demonstrated in the Beef Information Nucleus Progeny Test Program conducted by the Australian Limousin Breeders Society. To ensure accuracy of sire information, the sires for all calves from the first joining in 2011 were checked by DNA profiling and verification. The parentage verification highlighted a number of pedigree recording errors including:

- The wrong straw of semen was used or the sire recorded incorrectly for three calves during the AI program.
- Four calves were incorrectly assigned to the back up bull whereas they were actually sired by the AI sire.
- Human error occurred during the data entry for another three calves.
- Two calves born on the same day had clearly swapped mothers at birth.

In total, 12 of the 227 calves (5.3%) were given incorrect sires when the pedigree information was collected. Considering the Limousin Progeny Test Program was following best practice guidelines, it is probably indicative

of the lower level of pedigree errors occurring across the registered cattle industry and highlights the benefit of DNA parentage verification.

HOW DOES DNA PARENTAGE VERIFICATION WORK?

There are currently two types of DNA tests for parentage verification available in Australia; microsatellite testing, which has been used for a number of years and is still a highly accurate test, and Single Nucleotide Polymorphism (SNP) testing, a new technology which has more recently become available as an option for parentage verification.

In general, parentage verification is based upon the detection of genetically inherited markers present in a calves DNA. Calves receive one chromosome from each of their parents, and so genetic markers are passed down from parent to offspring. Both microsatellite and SNP parentage verification are based on exclusion, proving that the animal is not the offspring of a sire or dam rather than proving it is. For example if a marker is present in a calf but not in the nominated parents, then the calf is excluded as an offspring of that mating (Figure 1). As a result no test is 100% accurate at positively identifying an animal's parentage. However 99.9% of incorrect matings will be detected by DNA parentage analysis.

MICROSATELLITE MARKERS

A microsatellite marker is a repeat of a particular base sequence over and over, for example CACACACA, which current tests utilising a panel of multiple microsatellites.

For example, the current microsatellite test offered by the Animal Genetics Lab (AGL) at the University of Queensland generally utilises a microsatellite marker panel of up to 21 markers, including the 12 internationally standardised markers recommended by the International Society for Animal Genetics (ISAG). A further 11 markers are available where necessary to resolve difficult parentage cases (maximum of 32 markers.). Microsatellite DNA markers have been used for nearly twenty years and remain an accurate method of parentage verification. Parentage verification tests using microsatellites are currently available in Australia through two laboratories being

the AGL at the University of Queensland and Zoetis Genetics.

SINGLE NUCLEOTIDE POLYMORPHISM MARKERS

Single Nucleotide Polymorphisms (SNPs) are markers which identify a difference in a single nucleotide base pair. As an example, the SNP parentage verification test offered by the AGL uses a panel of ~150 SNPs, including the 100 internationally standardised ISAG SNPs.

Many breed societies have or have indicated that they will be transitioning from the use of microsatellite markers to SNP markers for parentage verification in the future. The major benefit of SNP based parentage verification over microsatellites is that it is more compatible with use of DNA technology for other purposes.

For example, if breeders choose to genotype their animals with a larger SNP panel for utilisation in future genetic evaluation for their breed (e.g. GeneSeek Genomic Profiler panel available through UQ AGL, including 20,000 SNP markers), the 150 SNP profile for parentage would automatically be collected, removing the need for duplicate DNA tests to be conducted.

Unfortunately, microsatellite profiles cannot be imputed or converted to a SNP profile equivalent (or vice versa), therefore animals that require parent verification via DNA, need to have the same DNA test as their parent(s).

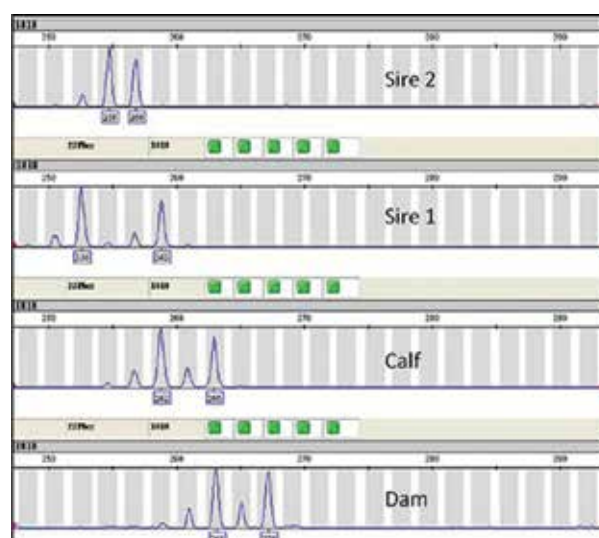


Figure 1. DNA testing for parent verification excludes potential parents based their DNA profile. In this simple representation based on microsatellite testing, Sire 2 is excluded as the calf's parent while sire 1 qualifies as the parent.

For example if the calf has a SNP profile, the sire also needs a SNP profile. If the sire only has a microsatellite profile, it needs to be re-genotyped to have a SNP profile available. Hence transitioning to SNP parentage verification can come at an additional cost to the seedstock breeder and as a result some breed societies have chosen to continue using microsatellite markers in the short term.

BREED SOCIETY REGULATIONS

In addition to the benefit of using DNA parentage verification to improve pedigree accuracy, most breed societies have registration requirements and regulations around DNA profiling and parent verification.

These will include minimum DNA parentage verification requirements and which type of profile they accept (e.g. microsatellite or SNP) both now and in the future. It will also include the process to follow to submit the sample (e.g. facilitated by the breed society or direct to the laboratory). If you are not aware of the regulations for your breed you are encouraged to contact your breed society directly for this information.

HOW TO GET YOUR CATTLE TESTED

The genotyping laboratories offering this service in Australia (e.g. Zoetis Genetics and AGL) generally use tail hair samples (hair roots attached) as a source of DNA, however they can use other samples such as blood, semen or tissue if required.

Most breed societies have regulations for DNA profiling which may include the preferred use of one or both of these laboratories. They will also have a process in place for collection and submission of the DNA sample for profiling and parent verification. This may include supplying the DNA sample collection kits and facilitating the submission of the kits to the laboratory. Or the breed society may prefer that you work with your preferred laboratory directly.

THE COST OF DNA PARENTAGE VERIFICATION

The cost of DNA profiling and parent verification will vary depending on the breed society, type of DNA sample, type of DNA test and laboratory being used. Contact either the DNA lab or your breed society for a price list.



Figure 2. Collecting a DNA Sample for DNA parent verification, pull 20-30 thick hairs from the brush of the animal's tail including the hair roots.

REFERENCES

University of Queensland Animal Genetics Lab (2014), "DNA Parentage Testing - Fact Sheet", accessed 19th of May 2014 from: <http://www.uq.edu.au/vetschool/content/animal-genetics-laboratory/dnaparentage-testing.pdf>

GENERAL GUIDELINES FOR SUBMITTING DNA FOR PARENTAGE VERIFICATION

1. Contact your Breed Society or the DNA lab for a DNA sample collection kit
2. Pull 20-30 thick hairs from the brush of the animal's tail including the hair roots.
3. Complete the required submissions forms
4. Mail the forms plus the DNA sample to your breed society or the genotyping laboratory. This depends on your breed society's arrangement with the genotyping laboratory
5. The results to be returned by email or mail. The results may also be supplied direct to you breed society. The turnaround time will depend on the laboratory and level of activity at the time of submission. As a general guide to turnaround time is 4 to 6 weeks.