

DNA technology has developed rapidly in the past decade and now has a variety of applications. For beef cattle genetic improvement, the primary areas of application are pedigree validation, parentage determination, and gene-based (genotypic) selection. Individual and parentage verification are now routine practices, while gene-based selection is in the early stages of development. This chapter describes current uses of DNA technology and provide an overview of applications currently under development.

### **Types of DNA Markers**

Analytical techniques to differentiate DNA of individuals or populations require genetic markers, which are defined as identifiable DNA segments that differ in nucleotide sequence from one individual to the next. Two types of markers may be used: microsatellites and single nucleotide polymorphisms (SNPs). Both create uniquely identifiable DNA patterns that may be used to follow the transmission of specific chromosomal regions from parents to progeny.

Microsatellite markers are segments of chromosomal DNA that include a variable number of repeated two to six nucleotide base sequences. Such markers are interspersed throughout the genome and are generally found in non-coding regions. These repetitive regions are subject to additions and subtractions in the number of tandem repeats of the basic two to six nucleotide segment, and this creates uniquely identifiable alleles at each site within the genome at which the particular microsatellite is found. Microsatellites routinely have been used in parentage analysis, because the multiple alleles generally found at each locus make them highly informative. They have provided the basis for individual and parentage identification in humans, dogs, cattle, and many other species. The approximately 2,100 microsatellite loci that have been identified and mapped in cattle are referenced at the following web address:

<http://locus.jouy.inra.fr/cgi-bin/lqbc/mapping/bovmap/Bovmap/main.pl>)

Single nucleotide polymorphisms are the other marker. As the name implies, they are a change (mutation) from the specific nucleotide originally present in a particular location in an individual to a different nucleotide at that same site and can be transmitted from parent to offspring, just like any other gene. Across evolutionary time, thousands of SNPs have been created by mutation such that they now can be found every 100 to 300 bases throughout the genome. Over 2.8 million SNPs have been placed in the human SNP database (<http://www3.ncbi.nlm.nih.gov/SNP/>), and a similar number are likely to exist in cattle. Because SNPs are widely distributed, it is likely that any gene of economic importance is located closely adjacent to several of them that can be used to mark its presence.

SNP markers promise to be increasingly useful in the future for developing high-resolution maps. They are becoming a popular marker because of their high throughput capability and potentially low cost. With the availability of whole genome sequences, SNPs that are dispersed across all chromosomes have been identified, presenting important advantages as markers for genome analysis.

Some SNPs are located within the coding region of a gene and can affect the structure and

function of a protein. This type of variation may be directly responsible for differences among individuals in phenotypic merit for economically important traits. Other SNPs occur either “upstream” or “downstream” of the coding region of a gene but may influence the regulation of gene expression. Others occur in locations that do not interfere with the structure or production of a protein. SNPs have the advantage that they are less likely to undergo a spontaneous mutation than microsatellites; thus they are inherited with greater stability.

### **DNA Collection**

DNA is found in every nucleated cell in the body. It has been extracted from semen, muscle, fat, white blood cells found in blood and milk, skin, and epithelial cells collected from saliva. Minute amounts of tissue, such as a single drop of blood or several mucosal cells, are all that are required for routine DNA analysis. Common collection methods include a drop of blood blotted on a paper that is dried, covered, and stored at room temperature, ear tag systems that deposit a tissue sample in an enclosed container with bar code identification, and hair follicles. Techniques have been developed that allow for rapid release of DNA from cells and immediate analysis of the samples.

### **Uses of DNA Technologies**

DNA-based technologies have been used for animal identification or source verification, parentage verification or identification, diagnosis of genetic diseases, genotyping for simply inherited traits, and marker-assisted selection. Animal identification and parentage verification have been used for a number of years and the techniques for analysis are well developed. Genotypes for some genetic diseases can be identified by DNA analysis, and many others are under study. Marker-assisted selection is still in the research and development phase but will likely become an important technology in the future.

Whether a particular use of DNA technology is practical, useful, and cost-effective in beef cattle improvement depends upon the individual operation and the role of the cattle producer (e.g., seedstock, multiplier, or commercial) and whether he/she will retain calf ownership or contribute to a partnership program like an alliance. Producers are encouraged to evaluate the potential benefits of the individual technologies and incorporate them when they are likely to improve profitability of an operation.

Animal identification and source verification are becoming increasingly important around the world. There are standard procedures that match specific marker genotypes from birth with those taken later in life or matching harvested product back to an original source. Either microsatellites or SNPs can be used effectively to compare DNA samples, and the probability can be calculated that the two samples are from the same source. DNA remains constant in the tissues of an animal throughout life; it cannot be altered. Thus, it is the ideal basis for animal identification.

DNA technology is commonly used for parentage verification and identification. Parentage verification provides pedigree assurance and has been used to guarantee the parentage of embryo transfer calves and of registered bulls used in AI programs. Breed associations have used this technology to guarantee the integrity of pedigree information and provide

assurance to buyers. DNA samples from the sire, dam, and progeny are compared at a number of marker loci to determine the probability that the calf was in fact produced by a mating between its assumed parents

In many commercial enterprises, dams are matched to progeny at birth, but the sire is unknown. In such herds, parentage identification analysis assigns sires to calves by comparing the genotype of the progeny at a number of marker loci to genotypes of a panel of potential sires. Bulls are excluded until only one bull remains that has a genotype consistent with the genotype of the calf. To correctly match calves with sires, therefore, DNA samples must be included from all potential sires.

DNA typing can be used as a progeny testing tool by assigning calves to their individual sires based on inheritance of markers. For traits easily measured in the bull (like weaning and yearling weights and growth rate), this additional information may add little accuracy to the estimate of genetic merit of a sire. However, for those traits that cannot be measured directly on the bull (carcass or milk production traits for example), parentage verification could provide additional information for genetic evaluation. Marketing cattle through strategic alliances and retained ownership programs provide commercial cattle producers incentives for improving the genetic merit of bulls for postweaning traits. DNA parentage identification technology will allow producers to identify bulls not suited to their marketing or management program.

Blood-type markers traditionally have been used for parentage verification and are reliable for most parentage disputes. However, with only 12 blood-group systems available to determine the genetic profile of an individual animal, this method is limited in parentage disputes involving closely related individuals or in large mating groups. DNA markers provide a virtually unlimited number of genetic determinants that can be used to follow the transmission of specific chromosomal segments between parents and progeny. The International Society of Animal Genetics (ISAG) has identified a panel of markers recommended for this purpose and provides a laboratory certification program (guidelines available at <http://www.wisc.edu/animalsci/isag/guidelines.html>).

A parentage identification kit is available from Applied Biosystems that utilizes a panel of 11 ISAG microsatellite markers (StockMarks II), and Celera AgGen has developed a 10 microsatellite marker kit especially designed for high throughput analysis (AgGen Panel III). Using allele frequency data from 10 divergent breeds, simulation work was conducted to determine the power of these kits to correctly discriminate among potential parents of calves.

When a potential and the actual parent were unrelated and the genotype of the other parent (e.g., the dam) was known, the probability always exceeded .99 that either kit would exclude the incorrect parent. When one parent (e.g., the dam) was not known and there was only one potential sire, probabilities of correct exclusion ranged from .95 to .99. These technologies thus provide a highly accurate method for identifying parentage across a wide array of breeds.

In the future, SNP markers could replace microsatellite markers for parentage identification if warranted by costs. Across existing cattle breeds, a panel of 70 to 100 SNP markers would be needed to assign parentage at the same level of discrimination as the StockMarks II or AgGen Panel III kits described above.

### **Marker Assisted Selection**

Genetic markers can be used to identify transmission of specific genes from parents to progeny. In some cases, a marker will identify a change in DNA nucleotide sequence that causes a unique phenotype of interest. In such cases it can be used directly in selection decisions. Examples would be diagnostic tests for recessive, single-gene effects such as bovine leukocyte adhesion deficiency, double muscling, and the recessive allele responsible for red coat color. Many of these tests have been patented, so to use the test, a royalty must be paid to the owner of the patent. Such tests have been invaluable in identifying animals (carriers) that are heterozygous for recessive genes. When available, they are quicker and much more economical than a progeny testing program to identify carriers of the mutant gene.

In other cases, the marker will not be located physically within a gene of economic importance but will be close to it on the chromosome. Once a marker of this sort has been identified and validated, it can serve as an indicator for the presence or absence of a quantitative trait locus or QTL in an individual. (A QTL is defined as a gene having a measurable influence on the phenotype of a measured, usually economically important trait.) Marker-assisted selection may be used within a family that contains two or more alleles at such QTL, one preferred over the others. The genetic marker can be used to determine whether progeny are likely to have inherited the favorable form.

Marker-assisted selection requires a three-phase development and testing program. First, in a preliminary screening procedure locations of QTL within the genome must be identified, using widely distributed markers in a reference population of known family structure. In this initial screening, the impact of marker loci genotypes on phenotypes for the economically important trait also is estimated. (If the QTL accounts for little of the variation seen in the trait, it will not be cost effective to use the test in a selection program; traditional selection will be more cost effective.) QTL locations must then be refined by identifying flanking markers on each side of the QTL. These flanking markers must be in close proximity to ensure high accuracy in identifying the presence of the favorable allele in candidates for selection. In the second phase, the presence and phenotypic impact of QTLs must be verified or validated in commercially relevant populations. Only after the location and effects of the QTL are validated can the third phase be undertaken, commercial application of marker assisted selection in the population at large.

SNP maps provide an opportunity to create a unique tool for identifying QTLs without relying on within family studies. Because SNPs are found in greater frequency than microsatellite markers, a map of SNP markers could be created of such a density that QTLs could be identified across a commercial population without its family structure being known. The QTLs could be identified directly in the population of interest, and all QTLs influencing a

trait could be evaluated in a single analysis. These types of studies are called association studies and have been used to identify disease causing variations in humans.

SNP diagnostic markers may be used in the future to identify animals with genetic potentials appropriate to meet specific management requirements and market specifications. For example, if DNA analysis predicts that a group of animals has the genetic potential to achieve marbling standards for choice and prime beef, producers could manage them to develop high marbling and market them on a quality grid pricing program. Likewise, if a group of cattle had outstanding predicted genetic merit for lean meat production but low predicted genetic merit for propensity to marble, they could be aggressively implanted, fed fewer days, and marketed on a red-meat grid pricing system. In the past, breed or crossbred group has been the primary determinant for predicting feedlot performance, quality grade, and yield grade. Although breeds do differ for these traits, there also is substantial within breed variation. Genotyping animals as they enter the feedlot could help to sort them into the most profitable management and marketing groups.

In the future, producers are likely to have a multitude of genetic markers available to them. It remains to be seen how they will be used in genetic improvement programs. Markers may improve the accuracy of selection of individual animals when combined with classical methods of genetic evaluation, but the increase in accuracy may not be worth the cost. For easily measured and highly heritable traits, markers probably will not provide any economic advantage over traditional estimates of genetic merit. Even for traits that are not easily measured in the animal, unless there is a set of markers that accounts for a substantial portion of genetic differences, marker-assisted selection may not be predictive of overall genetic merit.