

DNA-Based Technologies

Alison Van Eenennaam, University of California-Davis

Biotechnology is defined as technology based on biology. From this definition, it is obvious that animal breeders have been practicing biotechnology for many years. For example, traditional selection techniques involve using observations on the physical attributes and biological characteristics of the animal to select the parents of the next generation. One only needs to look at the amazing variety of dog breeds to realize the influence that breeders can have on the appearance and characteristics of animals from a single species. Genetic improvement through selection has been an important contributor to the dramatic advances in agricultural productivity that have been achieved in recent times (Dekkers and Hospital, 2002).

During the past century, several new technologies have been incorporated into programs aimed at accelerating the rate of the genetic improvement of livestock. These include artificial insemination (AI), sire testing programs that use data from thousands of offspring, the use of hormones to control the female reproductive cycle so as to allow for synchronization and superovulation, and embryo transfer. Prior to their eventual widespread adoption, some of these new technologies (e.g., AI) were initially controversial, and their introduction met with some resistance. In the past decade, applied DNA-based technologies have become available as a tool that livestock producers can use to aid in making their selection decisions.

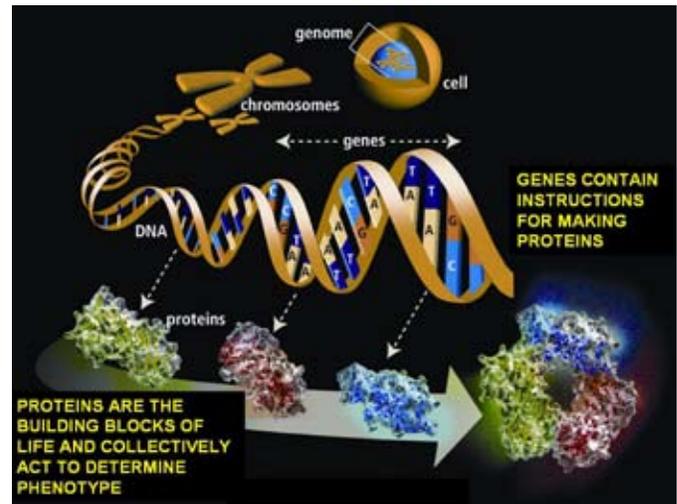
The intent of this chapter is to provide the necessary background to allow for an understanding of DNA-based technologies and to develop a set of guidelines to allow producers to evaluate the costs and benefits associated with incorporating DNA-based biotechnologies into their production systems.

What Is DNA?

Living organisms are made up of cells, and located on the inside of each cell is deoxyribonucleic acid (DNA). DNA is made up of pairs of four nucleotides abbreviated as “A,” “C,” “G,” and “T” (Figure 1). The entire genetic makeup, or *genome*, of an organism is stored in one or more chromosomes located inside each cell. DNA has two important functions; first, it transmits genetic information during reproduction, and, second, it continually spells out the identity and the rate of assembly of proteins. *Proteins* are essential to the structure and function of plants and animals. A *gene* is a distinct sequence of DNA that contains all of the instructions for making a protein. It is possible for the DNA sequence that makes up a gene or “*locus*” to differ between individuals. These alternative DNA sequences or forms of a gene are called *alleles*, and they can result in differences in the amount or type of protein being produced by that gene among different individual animals. This can affect the performance or appearance of animals that carry different alleles.

Alleles can be *recessive*, meaning that an animal must inherit the same allele (i.e., the same sequence) from both parents before there is an effect on performance or appearance; *additive*, mean-

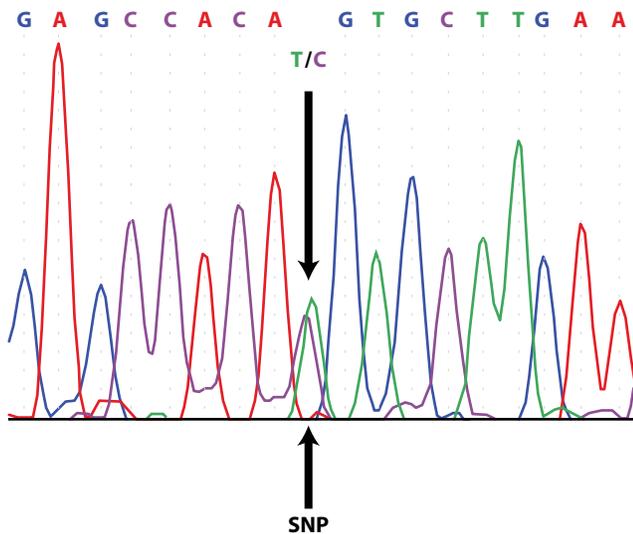
Figure 1. DNA (deoxyribonucleic acid) contains the instructions for making proteins. Differences in the nucleotide sequence of a gene’s DNA can influence the type or amount of protein that is made, and this can have an effect on the observed performance of an animal. Original graphic obtained from the U.S. Department of Energy Human Genome Program, <http://www.doegenomes.org>.



ing that an animal inheriting different alleles from each parent has an observed value or phenotype that is intermediate between animals carrying identical copies of the two alternative alleles; or *dominant*, meaning that the presence of one allele is sufficient to result in an effect on the trait or attribute of interest. Gender-determination is a well-known example of a simple trait where the presence of the dominant Y-chromosome dictates maleness.

Recently scientists have started to identify regions of DNA that influence production traits. They have used the techniques of molecular biology and quantitative genetics to find differences in the DNA sequence in these regions. Tests have been developed to identify these subtle sequence differences and so identify whether an animal is carrying a segment of DNA that is positively or negatively associated with the trait of interest. These different forms of a genetic marker are known as DNA-marker alleles. There are several types of genetic markers. *Microsatellites* are stretches of DNA that consist of tandem repeats of a simple sequence of nucleotides (e.g., “AC” repeated 15 times in succession). The tandem repeats tend to vary in number such that it is unlikely two individuals will have the same number of repeats. To date, the molecular markers used to determine parentage have primarily utilized microsatellite markers. Another type of genetic marker is referred to as a *single nucleotide polymorphism* or SNP (referred to as “snip”), where alleles differ from each other by the sequence of only a single nucleotide base pair. SNP genetic tests focus on detecting precise single nucleotide base pair differences among the three billion nucleotide base pairs that make up the bovine genome (Figure 2).

Figure 2. A section of DNA output generated by a DNA sequencer. At the indicated site, this individual inherited a “T” nucleotide from one parent and a “C” nucleotide from the other parent. This site represents a single nucleotide polymorphism. *Original graphic obtained from Michael Heaton, USDA, ARS, Meat Animal Research Center (MARC). Used with permission.*



Genotyping is the term that is used to describe the process of using laboratory methods to determine which DNA-marker alleles an individual animal carries, usually at one particular gene or location (locus) in the genome. The *genotype* identifies the marker alleles an animal carries. Because an animal gets one allele of each gene from its sire and one allele of each gene from its dam, it can only carry two alleles of any given marker locus or gene. If an animal gets the same marker allele from each parent, it is referred to as *homozygous* (e.g., “**” or “TT” or “140, 140”), or it may inherit different alleles from each parent in which case it is referred to as *heterozygous*. (e.g., “*-” or “TC” or “144, 136”). DNA testing can be used to distinguish between animals carrying different marker alleles, and this information can also be used for tracking parentage.

Most of the economically relevant traits for cattle production (birth weight, weaning weight, growth, reproduction, milk production, carcass quality, etc.) are *complex traits* controlled by the protein products of many genes, and they are additionally influenced by the production environment. The protein produced by different alleles of genes may influence the observed performance or *phenotype* of the animal carrying those alleles. When an animal has an Expected Progeny Difference (EPD) above the base year average for a certain trait, what that means is that the animal has inherited a higher than average proportion of alleles for genes that favorably affect the trait. In other words, selection based on EPD results in an increase in the number of favorable alleles an animal has, without knowing which specific genes are involved.

This contrasts with DNA-based selection where knowledge of which chromosomal locations are associated with improvement in a given trait is the basis of the genetic test(s), and selection is focused on known “marker alleles” at those loci to make genetic improvement in the trait. It should be noted that traditional EPD-based selection methods inherently tend to increase the frequency of alleles of genes that have major beneficial effects on selected traits.

Parentage Analysis

Commercial herds using multiple-sire breeding pastures often have no way of identifying the paternity of the calves. DNA markers can be used to assign calves to their individual sires based on the inheritance of markers. Sires pass on only one of the two marker alleles that they carry for each gene. If a calf does not have a marker allele in common with a sire at a particular gene, then that sire is excluded as being the parent of that calf. Paternity “identification” involves examining each calf’s genotype at multiple different gene loci and excluding as potential sires those bulls that do not share common alleles with the calf. Because paternity identification is a process of excluding potential sires on the basis of their genotype, it is therefore important that DNA from all possible sires be included in paternity tests. While parents can be excluded using this process, results cannot be used to “prove” parentage. Parentage testing identifies individuals that, due to a specific combination of marker alleles, could qualify as a parent for a particular offspring. Paternity testing is complicated by genetic relationships between the bulls. If bulls are closely related, then they are more likely to carry the same marker alleles. Consequently, it will be more difficult to definitively make paternity assignments on closely related bulls in a multiple-sire breeding pasture. Forming multiple-sire groups for each pasture from unrelated animals, i.e., putting full brothers in with different groups of cows, will help to minimize this problem. If there is only one potential sire for a calf (e.g., an AI calf), then paternity can be “assigned” by confirming that the calf’s genotype shares a marker allele in common with the alleged sire at all of the genetic loci that are tested.

Example:

Genotype	Bull A 140,140	Bull B 134,146	Bull C 152,140	Bull D 152,140
----------	-------------------	-------------------	-------------------	-------------------

A calf with the genotype “134,140” could have received one allele from any of these bulls, and so none of these bulls can be excluded as the possible sire.

A calf with genotype “134,148” could not have been sired by Bulls A, C, or D and must have received the “134” allele from Bull B, and by a process of elimination, the “148” allele must have come from its dam.

A calf with genotype “130,152” could have been sired by either Bull C or Bull D. The fact that these two bulls have the same genotype at this particular marker locus means that more loci will have to be tested to exclude one of these bulls as the sire. If these bulls are closely related such that they have the same genotype at many marker loci, then it will require more loci testing to uniquely assign one of the bulls as the sire of the calf.

Uses of parentage testing include identifying the sire(s) of outstanding or poorly performing calves and ascertaining whether one particular bull is routinely siring progeny that require calving assistance. The costs of DNA analysis can be minimized by sampling and DNA testing only a targeted subsample of the calves (e.g., calves that have to be pulled at calving or the top 10% of carcass quality animals) and the herd bulls.

More extensive sampling of the entire calf crop can allow for a determination of the proportion of the calf crop attributable to each bull in the herd. It is generally assumed that each bull contributes equally to the calf crop. However, studies have shown that some bulls sire more than their “fair share” of the progeny, while other bulls sire none of the progeny (Figure 3, Holroyd et al. 2002). Matching individual sires with the performance records of their entire calf crop also provides the data required to develop within-herd EPD for herd sires.

Matching individual sires with the performance records of their entire calf crop also provides the data required to develop within-herd EPD for herd sires. This may be particularly important in the case of postmortem traits such as carcass quality where progeny testing is the most accurate way to determine the genetic value of a bull. As with any new technology, the value associated with the parentage information must be estimated to ensure that it outweighs the expense of collecting and analyzing the DNA samples (currently ~\$10-35 per DNA sample submitted, although this cost is predicted to decrease markedly in the future).

Marker-Assisted Selection (MAS)

Marker-Assisted Selection (MAS) is the process of using the results of DNA-marker tests to assist in the selection of individuals to become the parents in the next generation of a genetic improvement program. That is, instead of using only a traditional or EPD selection program to increase the proportion of favorable alleles for the genes that affect a certain trait, specific DNA tests are used to assist in the selection of those favorable alleles. Genotyping allows for the accurate detection of specific DNA variations that have been associated with measurable effects on complex traits. It is important to remember that markers

for complex traits are associated with only those genes that are located in close proximity to the marker and do not identify favorable alleles for all the other genes that are associated with the trait. Selecting an animal that carries favorable alleles of a marker, which is the allele that is associated with a positive impact on the trait of interest, can result in an improvement in the observed phenotype for that trait. Although complex traits are influenced by a number of genes, the mode of inheritance of each genetic marker is simple. An animal gets one marker allele from its sire and one marker allele from its dam. The alleles of the marked genes, as well as the numerous other “unmarked” genes, and the production environment will determine an animal’s phenotype (e.g., weaning weight, marbling, etc.). EPD estimate the breeding value of all the genes (both “marked” and “unmarked”) that contribute toward a given trait; therefore, when EPD exist for a given trait, they should always be considered in selection decisions, even when marker data are available.

Potential benefits from marker-assisted selection are greatest for traits that:

- have low heritability (i.e., traits where an individual’s measured value is a poor predictor of breeding value due to the large environmental influences on the observed value).
- are difficult or expensive to measure (e.g., disease resistance).
- cannot be measured until after the animal has already contributed to the next generation (e.g., reproduction or longevity).
- are currently not selected for because they are not routinely measured (e.g., tenderness).
- are genetically correlated with a trait that you do not want to increase (e.g., a marker that is associated with increased marbling but that is not also associated with those genes that increase backfat thickness).

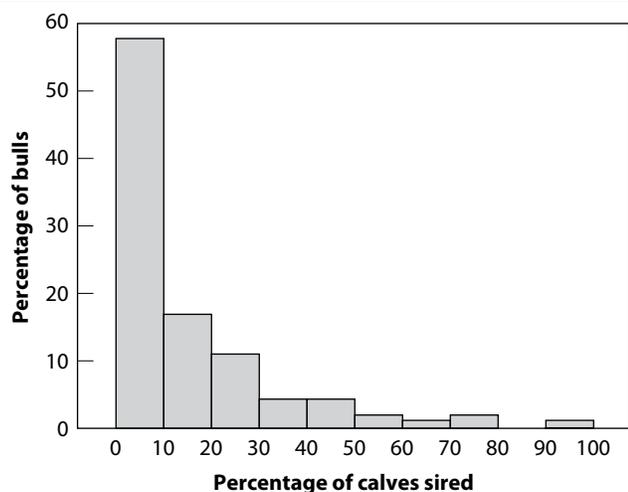
The following categories of traits are ordered according to those most likely to benefit from marker-assisted selection to those least likely to benefit:

1. simply inherited genetic defects,
2. carcass quality and palatability attributes,
3. fertility and reproductive efficiency,
4. carcass quantity and yield,
5. milk production and maternal ability,
6. growth, birth weight, and calving ease.

This ranking is due to a combination of considerations including: 1) relative difficulty in collecting performance data, 2) relative magnitude of the heritability and phenotypic variation observed in the traits, 3) current amount of performance information available, and 4) when performance data become available in the life cycle.

Recently genetic tests for DNA markers associated with simple traits such as coat color, simply inherited genetic defects, as well as complex product quality traits such as marbling and tenderness, have become commercially available. Genetic tests for simple traits that are controlled by one gene are able to accurately assess whether an animal is a “carrier” (i.e., heterozygous) or will “breed true” (homozygous) for the marker alleles that result in a certain phenotype (red versus black). That is because there is little or

Figure 3. Frequency distribution of percentage of calves sired by percentage of bulls. Of 235 bulls mated, 58% individually sired 10% or fewer calves in each of their respective mating groups with 6% not siring any calves. In contrast, 14% sired over 30% of the calves in each of the respective mating groups. *Original graphic reprinted from Animal Reproduction Science, 71, Holroyd, R.G.; Doogan, V.J.; De Faveri, J.; Fordyce, G.; McGowan, M.R.; Bertram, J.D.; Vankan, D.M.; Fitzpatrick, L.A.; Jayawardhana, G.A.; Miller, R.G., Bull selection and use in northern Australia. 4. Calf output and predictors of fertility of bulls in multiple-sire herds, pages 67-79. (2002), with permission from Elsevier.*



no environmental influence on simple traits like coat color, and usually a single gene is responsible for the phenotype. However, in the case of complex traits, each marker is only associated with one of the genes that contributes toward the phenotype. Both “marked” and “unmarked” genes, in conjunction with the production setting, will determine whether an animal marbles or has tender meat. It may be hard to understand why a well-proven bull with a high EPD for a certain trait can be found to carry no copies of a marker allele that has been positively associated with that trait. This can occur if the bull inherited a higher than average proportion of “unmarked” alleles that favorably affect the trait.

To be able to estimate the value of a marker to your breeding program, it is useful to know what proportion of the variation in the trait of interest is attributable to the favorable form of the DNA-marker allele. Remember that heritability is defined as the proportion of phenotypic variability that is accounted for by the additive genetic variability. Even if a marker explains half of the additive genetic variance, if the trait that it influences has a low heritability, e.g. 10%, then that marker will only account for $50\% \times 10\% = 5\%$ of the phenotypic variation for that trait. It is also important to know the frequency of the marker alleles in your herd, and whether the effect of the marker is recessive, codominant (additive), or dominant.

If all of the animals in a given breed carry two copies, or no copies, of a marker allele, then no genetic progress can be achieved by using marker-assisted selection for that marker as it accounts for none of the genetic variability seen for the trait in that herd. In the case of a herd carrying no copies of a given marker allele, bringing in an outside bull carrying two copies of the marker would be a way to rapidly introduce a desirable marker allele into the herd. Phenotypic progress will be evident in the first generation if the marker is dominant or codominant. If the trait is recessive, such that both alleles have to be present to see an effect, a second generation of crossing a homozygous bull with females carrying one copy of the favorable allele will be required to see a phenotypic response in the proportion (i.e., one in two, or 50%) of resultant offspring that are homozygous for the marker-allele. The frequency of marker alleles in a herd can be approximated by the gene frequencies of marker alleles in different breeds, although they may not accurately reflect the localized frequencies found in a specific herd.

Currently there are no requirements that must be fulfilled for a company to market a DNA-marker test for cattle producers. The National Beef Cattle Evaluation Consortium (NBCEC) has been working with testing companies to independently validate the various genetic tests by attempting to replicate the company's claims on commercial resource populations. The NBCEC provides DNA to the testing company, who is then responsible for genotyping the samples for the marker test and sending the test results back to NBCEC. The NBCEC then compares the genotyping data to the values for the trait(s) that were observed for the animals in the resource populations. Results are available at the Web site <http://www.nbcec.org>. Independent validation of commercialized DNA tests, comparing the performance of animals with and without the marker, should be an important consideration when evaluating the likely benefit of including marker(s) that have been associated with a given trait in a genetic selection program.

It is likely that the use of MAS will increase exponentially as the industry evaluates and integrates the data from the bovine genome sequencing project (see discussion below). Over time, it is possible that different markers will be associated with many of the genes that control complex production traits. This approach has the potential to bring about great genetic progress in traits that are difficult to measure such as disease resistance and product quality attributes such as tenderness. In the future, it is likely that there will be too many tests available for breeders to make breeding decisions based on the results of individual DNA test results. Each marker will need to be incorporated into genetic evaluations using a weighting that is based on the proportion of the additive genetic variance attributable to the marker allele associated with each genetic locus. It is also likely that the various sources of information (pedigree, phenotypes, and DNA test information) will be combined into one value, a “DNA-adjusted EPD.” Some breed associations have already begun to incorporate DNA-marker test information into their EPD calculations. The challenge will be to ensure that the value associated with marker-derived genetic progress outweighs the expense of collecting and compiling the DNA-marker information.

Questions for Evaluating Marker Tests

Questions to ask when evaluating a new DNA-based genetic marker test:

1. How big of an effect does the marker have on the trait of interest?
2. What are the frequencies of the marker alleles in your breed and or herd?
3. Is the marker allele dominant, codominant (additive), or recessive?
4. Has the effect of the marker been independently validated or published in a peer-reviewed journal?
5. Has marker information already been incorporated into the EPD? If it is incorporated into the EPD, then ignore the actual marker information and use the DNA-adjusted EPD to make selection decisions, as the marker information is already built into the EPD calculation.

Whether to use DNA-based marker-assisted selection in a breeding program is the most important question for producers and one that is not easily answered, as it will differ for every producer based on the production system, costs for obtaining the genetic information, and marketing considerations. The following questions should be asked when evaluating the use of marker-assisted selection in a breeding program:

1. **Will marker-assisted selection make you money?** For marker-assisted selection to be profitable, the increased economic returns from greater genetic gain as a result of using the markers must outweigh the cost of genotyping. Producers need to consider how they are being financially compensated for DNA testing.
2. **What impact does increasing the frequency of the marker allele have on the trait of interest in your herd?** The genetic gain that can be achieved by using marker-assisted selection depends on the amount of additive genetic variation that is accounted for by the marker, and marker data should be accordingly weighted. If the marker accounts for only a small

proportion of the additive genetic variability for a trait, then little genetic improvement will be made by exclusively focusing on increasing the frequency of the marker. Likewise, if all of the animals in a given breed are homozygous (carry two copies of a given marker), then no genetic progress can be achieved by using marker-assisted selection, as the marker accounts for none of the genetic variability seen for the trait in that breed.

3. **Is it a single gene test, or are there results from more than one gene?** The results from DNA-based marker tests can be reported in many ways. Single gene tests may be reported as “**” meaning that the animal is homozygous for the preferred allele of that gene.

They may also be reported as the actual SNP analyzed in the test, e.g., “TT”. It is then important to know which form of the marker (i.e., what nucleotide) has been associated with a positive effect on the trait of interest (see next section). Some of the tests are reporting on analyses that have been done at two different locations in the genome. For example, *TenderGENE* reports on the results from two different SNPs located in one gene, while GeneStar *Tenderness 2* reports the results of SNPs in two different, independent genes. The results are presented as multiple stars, where each star represents one favorable allele. Ideally, tests that include multiple genes or SNP locations will quantify the relative effect of each loci on the trait of interest. Results should distinguish between a two-star animal that is homozygous at one gene and carries no copies of the desirable allele (i.e., the star allele) at the other gene, and a two-star animal that is heterozygous at both genes. Irrespective of how many markers become available for each trait, it is important to remember that every individual receives one marker allele from each parent, and therefore it is not possible for an animal to ever have more than two favorable alleles for any given marker locus.

4. **What form of the marker do you want for your herd and production environment?** The “best” marker allele may differ depending on the environment. If a marker is associated with increased milk production, then using a homozygous bull may be desirable for a beef producer with highly productive irrigated pasture, while a bull carrying no copies of that marker may be better suited to a range cow-calf operation in a dry environment with limited feed resources. Likewise, some tests are recommended only for use in certain breeds of cattle. For example, one of the μ -calpain tenderness SNPs (530) is only recommended for use in cattle without Brahman influence.
5. **What are you giving up to use animals that are carrying the marker of interest?** Selection usually focuses on more than one trait. It is important not to narrow down the set of animals eligible for selection based solely on their genotype for a marker. Selecting from a smaller set of animals that carry the marker could eliminate animals with high EPD for other economically relevant traits. This will decrease the intensity of selection, and hence genetic progress, that is being made for these other traits. Additionally, special care should be taken to ensure that selection for the marker does not negatively affect genetic improvement in other traits of economic importance. Despite the trend to label commercial DNA tests as having an influence on only one trait, it is unlikely that any gene affects only one single trait.

Example:

Consider the following two scenarios where you are choosing between two bulls. One carries two copies of a marker allele that is associated in a positive way with a trait that you are interested in improving, while the other bull carries no copies of the marker allele.

Two full brothers produced by embryo transfer that have identical, low-accuracy EPD based on their pedigree data.

This is a simple choice, and it would clearly be the animal carrying two copies of the marker allele. The DNA test tells you with a fair degree of certainty that one bull is carrying two “good” alleles for one of the genes associated with the trait of interest. Subsequent progeny testing may prove the other bull superior based as a result of chance inheritance of good alleles for the many other genes associated with the trait, but the markers providesome definitive information to enhance your chances of choosing the better of the two bulls at an early age.

Two well-proven bulls have identical, high-accuracy EPD based on progeny testing.

This is a more difficult scenario. The marker test tells you that the bull with the two copies will transmit a favorable form of the gene associated with the marker to all of his progeny. If the marker allele accounts for a large proportion of the additive genetic variance, then using him as a herd sire will ensure that all of his calves get this desirable form of the gene. Using this bull may make sense if your herd has a low frequency of the marker allele. However, if your herd already has a high frequency of the marker-linked allele, then using the bull that carries desirable alleles of all of the other genes that contribute to trait, as evidenced by an EPD equal to the homozygous marker bull’s EPD, will likely accelerate genetic progress more rapidly by bringing in new sources of genetic variation.

Seedstock breeders need to be particularly careful not to inappropriately discriminate against bulls that have well-ranked, high-accuracy EPD but that are found to carry no markers associated with a given trait. They represent a valuable source of alleles for all of the unmarked genes associated with the trait of interest. Offspring that inherit both the marker-allele from their dam and desirable alleles of unmarked genes from high-rank EPD bulls carrying no copies of the marker are likely to inherit the greatest number of favorable alleles for both the unmarked and marked genes that affect the trait of interest.

6. **Could good progress in that trait be achieved without the expense of marker-assisted selection?** Markers are most useful for traits that are not routinely recorded (have no phenotypic measurement data) and for individuals that have low accuracy EPD. Also, as trait heritability increases, the benefit due to marker information decreases as it becomes easier to select superior animals based on performance records.

Once a decision has been made to use marker-assisted selection, the actual application of the technology is fairly straightforward. DNA samples should be collected from all animals to be tested. Common collection methods include a drop of blood blotted on paper (make sure to let the sample dry well before storing), ear tag systems that deposit a tissue sample in an enclosed container with bar code identification, semen, or hair samples (including the DNA-rich follicle or root). To increase the frequency of a marker that is positively associated with the trait of

interest, select for animals that are carrying one or two copies of the marker and against those carrying no copies of the marker. All of the offspring from a parent carrying two copies of the marker (homozygous) will inherit a copy of the marker from that parent. In a typical herd, selection for homozygous sires will probably be the most rapid way to increase the frequency of the marker, although this may severely limit your choice of sires and hinder progress in other traits. Marker-assisted pre-selection of young sires with equivalent EPD is an excellent way to rapidly increase the proportion of animals carrying a specific genetic marker and increase the frequency of that marker allele in the population.

Web Sites of U.S. Companies Providing Genotyping Services for Beef Cattle

(current as of 1/2006)

A listing of available tests is maintained at the following web address <http://animalscience.ucdavis.edu/animalbiotech/Bio-technology/MAS/index.htm>.

- <http://www.bovigensolutions.com>
Parentage, GeneSTAR marbling, GeneSTAR tenderness 2
- http://www.dna.com/products_services/bovine_id.html
Coat color, tenderness, parentage, identity tracking
- <http://www.geneticvisions.net>
Coat color, Prolactin (CMP), BLAD, Citrullinemia, DUMPS, Kappa-Casein, Beta-lactoglobulin, Complex Vertebral Malformation
- <http://www.genmarkag.com>
Parentage, coat color, BLAD, Citrullinemia, MSUD, Kappa-Casein, Beta-lactoglobulin, AlphaS1-casein, Piedmontese Myostatin
- <http://www.igenity.com>
IGENITY™ L (leptin), Parentage, TenderGENE tenderness, DoubleBLACK coat color
- <http://www.immgen.com>
Parentage, Complex Vertebral Malformation (CVM), BLAD, DUMPS, Kappa-Casein, Beta-lactoglobulin, Pompe's disease
- <http://www.metamorphixinc.com>
Parentage, coat color, polled/horned
- <http://www.viagen.com/>
Breed identification, animal identification

Figure 4. The cow that is the source of DNA for sequencing the bovine genome. L1 Dominette 01449 stands with her calf on the rangeland of the Agricultural Research Service's Fort Keogh Livestock and Range Research Laboratory at Miles City, Montana.



Original photo taken by Michael MacNeil, USDA, ARS, Miles City, Montana. Used with permission.

Future Directions

Bovine Genome Sequencing Project

Plans to sequence and describe the genome of the cow were announced in December of 2003. The \$53 million Bovine Genome Sequencing Project is a collaboration among the National Human Genome Research Institute (NHGRI), which is part of the National Institutes of Health (NIH); USDA; the state of Texas; Genome Canada; the Commonwealth Scientific and Industrial Research Organization of Australia; and Agritech Investments Ltd., (a subsidiary of Meat New Zealand), Dairy Insight Inc., and AgResearch Ltd., all of New Zealand. A first version of the bovine genome sequence has been deposited into free public databases for use by researchers around the globe. The animal that is the source of the DNA being sequenced is a Hereford cow named L1 Dominette 01449 (Figure 4). Having access to the complete bovine genome sequence will accelerate the discovery of markers, especially SNPs. Ideally, this will allow for the development of a set of DNA-based markers that will account for a substantial portion of the genetic variation for economically important traits. It is likely that whole genome association studies, where thousands of evenly distributed SNP markers are associated with phenotypes from thousands of cattle, will become an increasingly important tool for the identification of specific regions in the cattle genome that are associated with desirable beef traits.

SNP-Based Fingerprinting for Cattle

"SNP fingerprinting" may also play a role in individual animal identification (Figure 5). After an animal has been slaughtered, DNA remains a stable, identifiable component to track the origin of beef products. Genotyping 30 SNP loci that exhibit variability across all common beef breeds would be sufficient to uniquely identify 900,000 cattle (Heaton et al., 2002). The odds that two individuals coincidentally possess identical 30-SNP loci genotypes is less than one in a trillion! And 45 highly informative SNP loci are estimated to be sufficient to identify all of the cattle in the world (estimated to be approximately 1 billion). In the future, SNPs may also be used as a tool to counter inbreeding by maintaining genetic diversity at many sites on the genome and to allow for the transmission of beneficial alleles from rare breeds into commercial breeds of cattle.

Figure 5. SNPs may offer a permanent and traceable fingerprint for cattle and beef in the future.



Original graphic obtained from Michael Heaton, USDA, ARS, Meat Animal Research Center (MARC). Used with permission.

Cloning

The term “cloning” became infamous following the appearance of “Dolly the sheep,” the first mammal cloned from DNA derived from differentiated adult tissue, in 1997. In fact, cloning has been going on for a long time. Plant breeders have been using this technique to “clonally propagate” desirable plant lines for centuries.

Cloning is defined as making a genetic copy of an individual. Identical twins are clones, but more commonly the term is now used to refer to an individual that results from the transplantation of the DNA contained in a single cell of somatic tissue derived from an adult organism into an enucleated oocyte (an egg that has had its own DNA removed). This process is called somatic cell nuclear transfer (SNT) and has been successfully performed on many species including cattle (Figure 6).

It is important to note that prior to SNT, two other well-established procedures were available and used to make cattle clones. Splitting or bisecting embryos, a process in which the cells of a developing embryo are split in half and placed into empty zona (the protective egg coat around early embryos) prior to transfer into different recipient mothers, was commonly used in the 1980s. Likewise, cloning by nuclear transplantation from embryonic cells was developed in the 1970s and introduced into cattle breeding programs in the 1980s, well before the appearance of Dolly. From an animal breeding perspective, the importance of the SNT procedure that created Dolly is that it allows for the replication of adult animals with known attributes and highly accurate EPD based on pedigree, progeny, and their own performance records.

Although clones carry exactly the same genetic information in their DNA, they may still differ from each other, in much the same way as identical twins do not look or behave in exactly the same way. In fact, a recent study showed that SNT clones differ more from each other than do contemporary half-siblings (Lee et al., 2004). Clones do not share the same cytoplasmic inheritance of mitochondria from the donor egg, nor the same maternal environment, as they are often calved and raised by different animals. It is also important to remember that most traits of economic importance are greatly influenced by environmental factors, and so even identical twins may perform differently under varying environmental conditions. In the case of SNT, there is an additional complicating factor, and that is the requirement for “reprogramming” of the transferred nuclear DNA as it goes from directing the cellular activities of a somatic cell to directing the development of an entirely new embryo. Currently this process is not well understood, and there appears to be an increased rate of perinatal and postnatal loss and other abnormalities in SNT clones relative to offspring conceived in the traditional way. It may be that SNT clones differ from the original DNA-donor in the way that their nuclear genes are expressed. These problems are not seen universally in SNT cloned cattle, and there are reports of apparently healthy cattle that have gone on to conceive and have healthy calves (Pace et al., 2002; Lanza et al., 2001).

Studies comparing the performance of SNT and other types of dairy cattle clones to their full siblings found that there were no obvious differences in performance or milk composition (Norman and Walsh, 2004; Walsh et al., 2003). Although the performance records of SNT clones may be different from their DNA donor, as far as we currently know, they would be expected to have the same ability as their progenitor to transmit favorable

Figure 6. Two somatic cell nuclear transfer (SNT) cloned Holstein calves, Dot and Ditto.



Original photo taken by Alison Van Eenennaam, University of California-Davis. Used with permission.

alleles to their offspring. More research is required to determine if the offspring of SNT clones perform as well as would be expected based on the predicted genetic potential of the original DNA-donor animal.

Cloned animals may provide a “genetic insurance” policy in the case of extremely valuable animals or may produce several identical bulls in production environments where AI is not a feasible option. Clones could conceptually be used to reproduce a genotype that is particularly well suited to a given environment. The advantage of this approach is that a genotype that is proven to do especially well in a particular location could be maintained indefinitely, without the genetic shuffle that normally occurs every generation with conventional reproduction. However, the disadvantage of this approach is that it freezes genetic progress at one point in time. As there is no genetic variability in a population of clones, within-herd selection no longer offers an opportunity for genetic improvement. Additionally, the lack of genetic variability could render the herd vulnerable to a catastrophic disease outbreak or singularly ill suited to changes that may occur in the environment. Currently, the FDA continues to call for a voluntary prohibition of the marketing of milk or meat from SNT clones and their offspring until more data can be collected on the performance and food safety attributes of animals produced using this reproductive technology.

Genetic Engineering of Cattle

Genetic engineering is the process of stably incorporating a recombinant DNA sequence (i.e., a DNA sequence produced in a laboratory by joining pieces of DNA from different sources) into the genome of a living organism. What this means is that new genes, possibly derived from different species, can be directed to make novel proteins in genetically engineered organisms. Genetically engineered organisms are commonly referred to as “transgenic,” “genetically modified,” “GMO,” or simply “GE.” Genetic engineering has been successfully used to make transgenic cattle, although none have been approved for commercialization or entry into the U.S. marketplace. The Food and Drug Administration (FDA) is the agency responsible for regulating genetically engineered animals.

Genetic engineering could conceptually be used to improve production traits in cattle. It is unlikely that this will be implemented in the near future due in part to the fact that it is difficult to determine which proteins might be good candidates to positively influence these complex, multigenic traits. Additionally, genetic improvement for most production traits can be effectively achieved using traditional selection techniques, without the expense and time involved with the production and regulatory approval of genetically engineered cattle.

Genetic engineering might find a place in agricultural production as a way to change the nutritional attributes or improve the safety of animal products in ways that are not possible through traditional selection techniques. Such applications might include milk lacking allergenic proteins or containing viral antigens to vaccinate calves against disease, or beef optimized for human nutrition. Genetic engineering in conjunction with SNT cloning could also be used to remove or “knock out” certain proteins from the genome of cattle, such as the prion protein responsible for bovine spongiform encephalopathy (BSE).

The application of genetic engineering in cattle that is the most likely to be cost effective, at least in the near future, is the production of useful protein products, such as human hormones or blood proteins, in the milk of genetically engineered cows. Such animals would not be destined, or permitted, to enter the food supply. These “biopharming” applications have the potential to produce large amounts of human therapeutics at a relatively low cost relative to the current mammalian cell culture techniques. It remains to be seen whether any of these potential benefits are sufficient to outweigh the considerable time and expense involved in the development and approval of genetically engineered cattle.

DNA-based technologies are developing at a rapid pace. It is likely that these technologies will play a progressively more important role in beef production and marketing in the future. As the sequencing of the bovine genome continues, it is likely that the number of DNA-based marker tests will increase exponentially, and eventually “DNA-adjusted EPD” for different traits may be routinely calculated for breed associations as a part of the national cattle evaluation program. Although DNA-based markers are relatively new and alluring, they are not a silver bullet. For marker assisted selection to be profitable in the short term, the increased economic returns from greater genetic gains as a result of using markers must outweigh the costs (DNA sampling, genotyping) associated with obtaining the additional genetic information.

Web Resources on Animal Biotechnology

- <http://www.animalbiotechnology.org/>
Federation of Animal Science Societies Animal Biotechnology Web site
- <http://animalscience.ucdavis.edu/animalbiotech/>
UC-Davis Animal Genomics and Biotechnology Cooperative Extension Program

Literature Cited

- Dekkers J.C.M., and Hospital F. (2002) The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics* 3, 22-32.
- Heaton M.P., Harhay G.P., Bennett G.L., Stone R.T., Grosse W.M., Casas E., Keele J.W., Smith T.P., Chitko-McKown C.G., and Laegreid W.W. (2002) Selection and use of SNP markers for animal identification and paternity analysis in U.S. beef cattle. *Mamm Genome* 13, 272-281.
- Holroyd R.G., Doogan V.J., De Faveri J., Fordyce G., McGowan M.R., Bertram J.D., Vankan D.M., Fitzpatrick L.A., Jayawardhana G.A., and Miller R.G. (2002) Bull selection and use in northern Australia. 4. Calf output and predictors of fertility of bulls in multiple-sire herds. *Anim Reprod Sci* 71, 67-79.
- Lanza R.P., Cibelli J.B., Faber D., Sweeney R.W., Henderson B., Nevala W., West M.D., and Wettstein P.J. (2001) Cloned cattle can be healthy and normal. *Science* 294, 1893-1894.
- Lee R.S.F., Peterson A.J., Donnison M.J., Ravelich S., Ledgard A.M., Li N., Oliver J.E., Miller A.L., Tucker F.C., Breier B., and Wells D.N. (2004) Cloned cattle fetuses with the same nuclear genetics are more variable than contemporary half-siblings resulting from artificial insemination and exhibit fetal and placental growth deregulation even in the first trimester. *Biol Reprod* 70, 1-11.
- Norman H.D. and Walsh M.K. (2004) Performance of dairy cattle clones and evaluation of their milk composition. *Cloning and Stem Cells* 6, 157-164.
- Pace M.M., Augenstein M.L., Betthausen J.M., Childs L.A., Eilertsen K.J., Enos J.M., Forsberg E.J., Golueke P.J., Graber D.F., Kemper J.C., Koppang R.W., Lange G., Lesmeister T.L., Mallon K.S., Mell G.D., Misica P.M., Pfister-Genskow M., Strelchenko N.S., Voelker G.R., Watt S.R., and Bishop M.D. (2002) Ontogeny of cloned cattle to lactation. *Biol Reprod* 67, 334-339.
- Walsh M.K., Lucey J.A., Govindasamy-Lucey S., Pace M.M., and Bishop M.D. (2003) Comparison of milk produced by cows cloned by nuclear transfer with milk from non-cloned cows. *Cloning and Stem Cells* 5, 213-219.