DNA-Based Biotechnologies

Alison Van Eenennaam, University of California-Davis

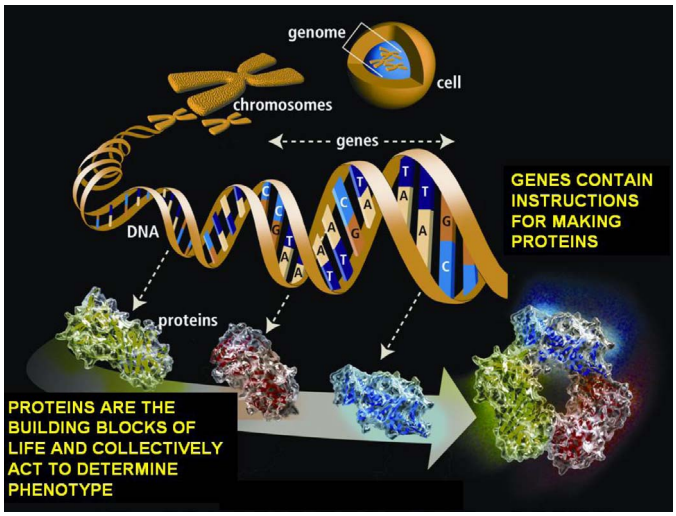
Biotechnology is defined as technology based on biology. From this definition, it is obvious that animal breeders have been practicing biotechnology for many years. For example, traditional selection techniques involve using observations on the physical attributes and biological characteristics of animals 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, but are not limited to, 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 create an understanding of DNA-based technologies, and to discuss some of the recent developments and future applications in cattle production systems.

What is DNA ?

Living organisms are made up of cells, and located inside each cell is deoxyribonucleic acid, or **DNA** for short. 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 between generations 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, **additive** meaning that the effect is proportional to the number of an allelic variants inherited by the animal (i.e. carrying two copies of a particular allele produces double the effect of carrying one copy), or **dominant,** meaning that the presence of one allele is sufficient to result in an effect on the trait or attribute of interest. Gender-

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.*

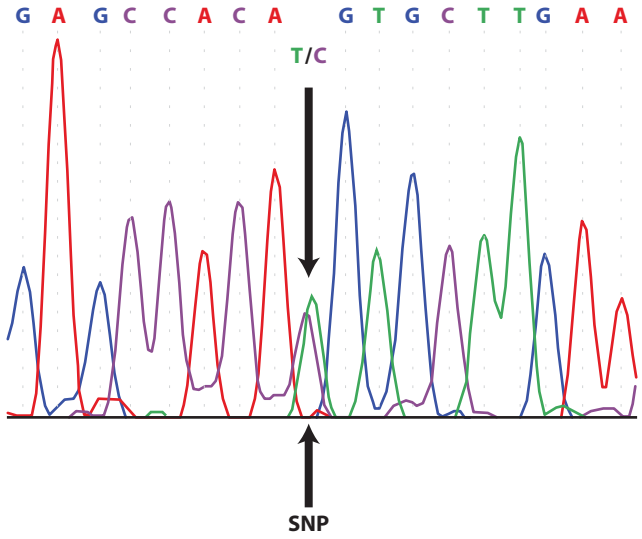


determination is a well-known example of a simple trait where the presence of the dominant Y-chromosome dictates maleness.

Scientists have started to identify regions in chromosomal sequence 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 a 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 DNA 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).

Genotyping refers to 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

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.*



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 also 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. The genetic component of phenotypic variation is the result of DNA sequence differences between chromosomes of individuals. When an animal has an EPD above the base year average for a certain trait, it means the animal has inherited a higher than average proportion of alleles for genes that favorably affect the trait. In other words, selection based on EPDs results in an increase in the average number of favorable alleles an animal can pass on to its offspring, without knowing which specific genes are involved. This contrasts with DNA-based selection which is based on the use of genotyping to identify animals carrying specific DNA variants that are known to be associated with the trait of interest. It should be noted that traditional EPD-based selection methods inherently tend to increase the frequency of DNA markers associated with the alleles of genes that have beneficial effects on selected traits.

Parentage Analysis

Commercial herds using multiple-sire breeding pastures often have no way of identifying the paternity of 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 locus. If a calf does not have a marker allele in common with a sire at a particular locus, then that sire is excluded as being the parent of that calf. Paternity "identification" involves examining each calf's genotype at multiple 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 A.I. 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. Although microsatellites have typically been the marker of choice for paternity analysis, the use of SNP markers is becoming more common for a number of reasons including their abundance, high potential for automation, low genotyping error rates, and ease of standardization between laboratories (Figure 2).

Example. How does parentage assignment work?

	Bull A	Bull B	Bull C	Bull D
Genotype	A/A, C/C	A/T, C/G	T/T, G/G	T/T, C/C

A calf with the genotype "A/T, C/G" could have received one allele from any of these bulls and so none of these bulls can be excluded as the possible sire. Additional markers would need to be used to uniquely assign one of the bulls as the sire of the calf.

A calf with genotype "A/A, C/C" could not have been sired by Bulls C or D, but could have been sired by either Bull A or B.

A calf with genotype "T/T, G/G" could not have been sired by Bulls A or D, but could have been sired by Bull B or C.

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. To identify the sire(s) of a select group of calves (e.g. calves that have difficult births or top 10% of carcass quality animals) the costs of DNA analysis are minimized by sampling and DNA testing the herd bulls and only a targeted subsample of the calves. Yet another use of parentage testing would be to identify which sire is responsible for contribution of a genetic defect. 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; Van Eenennaam et al. 2007b).

Matching individual sires with the performance records of their entire calf crop also provides the data required to develop

within-herd EPDs for herd sires (Van Eenennaam et al., 2007b). The use of progeny testing to develop within-herd EPDs for herd sires on economically-relevant traits has the potential to generate value by improving the response to selection for targeted traits.

In practice it is preferable to collect DNA samples from all potential sires at the beginning of the breeding season. It is also important to try to keep young sires and mature bulls in separate breeding pastures as dominant mature bulls will tend to keep young bulls from siring any calves (see Figure 3).

Missing identification of sires can occur for a variety of reasons (neighboring bulls jumping the fence, precocious bull calves, or inadvertent omission of sire(s) from sample collection). Missing sire DNA samples when using DNA marker-based parentage for genetic evaluation decreases the rate of genetic gain. The frequency of sire misassignment can be minimized by using a powerful marker panel; or by simple management practices that include: dividing large herds into smaller multiple-sire breeding groups with fewer sires while maintaining the same bull:female ratio; sorting bulls into sire groups with divergent genotypes; and minimizing relatedness among bulls. It is also important to try to keep young sires and mature bulls in separate breeding pastures as dominant mature bulls will tend to keep young bulls from siring any calves.

The return on investment that results from such progeny testing has been found to be greatly influenced by the cost of genotyping (Pollak, 2005). New SNP genotyping platforms continue to drive down the cost to generate SNP genotypes, and the future will undoubtedly see the introduction of less expensive genotyping assays using high resolution SNP parentage panels. As with any new technology, the value associated with the parentage information must be estimated to determine if it outweighs the expense of collecting and genotyping the DNA samples.

Marker-Assisted Selection (MAS)

Marker-**A**ssisted **S**election (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. Selection may be based on test results associated with simple traits such as coat color, horned status, or simply inherited genetic defects. Such traits are determined by the inheritance of specific alleles at known genes and so tests are able to accurately assess whether an animal is a "carrier" (i.e. heterozygous) or will "breed true" (homozygous) for that trait (e.g. red versus black).

The test for Arthrogryposis Multiplex (AM) is an example of this type of test. The genetic test for this recessive lethal genetic defect also known as "curly calf," identifies an animal as a carrier of the AM mutation (AM**C**) or a non-carrier (AM**F**), meaning that an animal that has been determined to be free of the AM mutation. Of course, the genotype of an AM affected (AM**A**) animal is obvious on the basis of its appearance and lethality. Irrespective of its pedigree, an animal that has been tested and found to be a non-carrier (AMF) did not inherit the mutation and will not carry or transmit this genetic defect to its progeny. If a cow has an AM calf, it means that the cow is a carrier of the AM mutation and that the sire she was bred to also carries the AM mutation.

Example. Determining the proportion of offspring that will inherit a genetic defect.

From a breeding standpoint there are several possible scenarios when considering the inheritance of a recessive genetic defect. In the case of AM, if both parents are carriers (AMC), then there is a one in four chance of producing a dead AMA calf, a one in two chance of having a normal-appearing AM carrier (AMC) calf, and a one in four chance of having a normal AM free (AMF) calf.

AMC x AMC = ¼ affected (AMA):
½ normal-appearing carrier (AMC): ¼ AM free (AMF)

If only one parent is a carrier, then all of the offspring will be normal appearing, but half of them will be carriers (AMC).

AMC x AMF = ½ normal-appearing carrier (AMC):
½ AM free (AMF)

Naturally-occurring recessive genetic defects are common in all species, and only become evident when certain lines of cattle are used very heavily, such that both cows and bulls have common ancestors in their pedigree, thereby allowing a rare genetic defect to become homozygous in their offspring. The widespread use of the superior carcass-trait bull Precision 1680, an AM carrier (AMC), increased the probability of this bull showing up on both sides of many Angus pedigrees, and this uncovered the presence of the recessive lethal AM mutation.

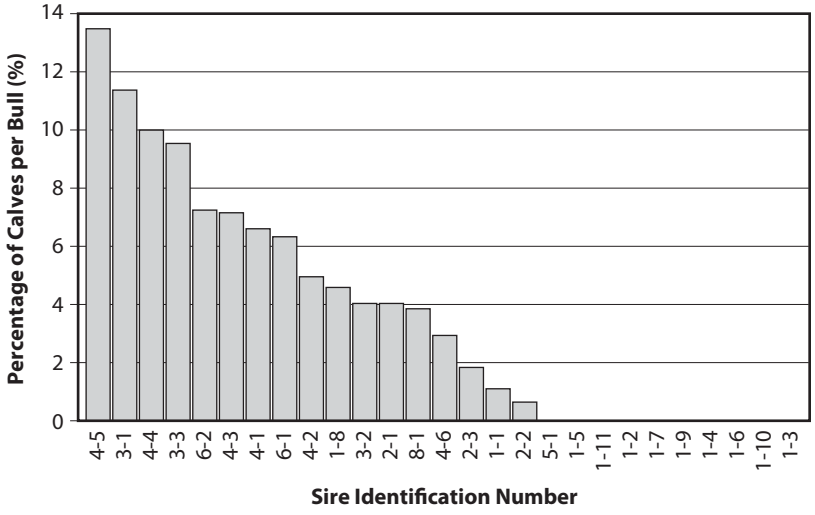


Figure 3. Calf output of 27 herd bulls of varying ages in a single multiple-sire breeding pasture. Five of the 27 herd sires produced over 50% of the calves. The leading digit of the sire identification number denotes the age of the bull at the time of breeding, and it can be seen that of the ten natural-service herd bulls that sired no progeny, nine were yearlings. *Modified from Journal of Animal Science, 85, Van Eenennaam, A. L.; R. L. Weaber; D. J. Drake; M. C. T. Penedo; R. L. Quaas; D. J. Garrick; E. J. Pollak. DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting., pages 3159-3169. (2007), with permission from American Society of Animal Science.*

The rapid development of a commercial DNA test for this genetic defect by Dr. Jonathan Beever and colleagues over a period of approximately 4 months was made possible by the availability of the bovine genome sequence. It represents one of the most compelling examples of the power and utility of this sequence information for the cattle industry. In the absence of a DNA test, there would have been no way to determine the AM-status of animals with affected pedigrees, and in the process of proactively eliminating potential carriers, many AMF animals would have been needlessly culled. It is likely that the bovine genome information will accelerate the development of DNA tests for other genetic defects as they become evident in the population.

MAS also holds great promise for selection based on complex production traits, both those that are in existing genetic evaluation programs, and those for which no genetic merit estimate currently exists. In order of greatest to least degree of benefit, the following categories of traits are likely to benefit the most from marker-assisted selection:

Greatest	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,
Least	6. growth traits and birth weight.

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) amount of performance information available, and 4) when performance data become available in the life cycle.

The first commercial test for a quantitative production trait in beef cattle was a single marker test for marbling (Barendse et al., 2001). This was soon followed by other tests involving a small number (1-3) of markers associated with marbling (Buchanan et al., 2002) and tenderness (Casas et al., 2006; Schenkel et al., 2006). Early methods of marker discovery focused on finding SNP markers in regions of the genome that were experimentally known to have a relatively large effect on the trait of interest. Rarely are DNA markers the actual DNA sequence causing the effect, rather markers are closely situated or "linked" to the causative sequence. Markers therefore flag the location of sequences that directly have an effect on the trait (Figure 4).

However, it is important to understand that any one marker will identify the alleles for only one of the many genes that are associated with complex traits. Put another way, any single marker is only going to account for a fraction of the genetic variation associated with a complex trait. This is distinct from the situation for simple traits (e.g. coat color, horned status, lethal recessive mutations) where one or two markers may be sufficient to accurately predict an animal's phenotype and carrier status. Conflicting reports about some of these first commercially-available markers (Barendse et al., 2005; Casas et al., 2005), and the recognized occurrence of well-proven bulls with a high EPD for a given trait but carrying two copies of the "wrong" (unfavorable) marker allele for that trait made some producers understandably wary of investing in DNA-based testing. Genetic tests for complex traits are likely to require hundreds or even thousands of markers to effectively track all of the genes influencing complex traits.

Example. Making selection decisions based on DNA marker test results.

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 favorable marker allele.

1. Two full brothers produced by embryo transfer that have identical, low-accuracy EPDs 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 high degree of certainty that one bull is carrying two favorable alleles for one of the genes associated with the trait of interest. Subsequent progeny testing may prove the other bull superior based on the chance inheritance of "good" alleles for the many other genes associated with the trait, but the markers provide some definitive information to enhance your chances of choosing the better of the two bulls at an early age.

2. Two well-proven bulls have identical, high-accuracy EPDs 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 favorable marker 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 EPDs but that are found to carry no favorable alleles of a single marker associated with a given trait, especially if such bulls are relatively common or have desirable EPDs for other traits. These bulls 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.

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

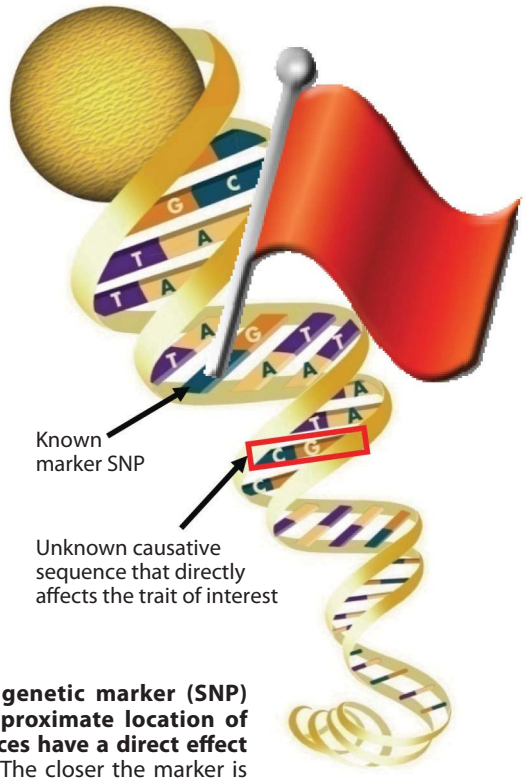


Figure 4. A genetic marker (SNP) flags the approximate location of DNA sequences have a direct effect on the trait. The closer the marker is to the causative locus the more likely it is that they will be inherited together and so the marker acts as a proxy for the causative sequence. If the marker is a long way from the causative sequence then it may become uncoupled from the sequence, and so selecting for the marker will no longer lead to genetic improvement for the trait of interest.

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 EPDs 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.

Marker-Assisted Management (MAM)

Marker-Assisted Management (MAM) is the process of using the results of DNA-marker testing to predict the future phenotype of the animal being tested and sort individual cattle into management groups that are most likely to achieve specific end points (e.g. Quality grade Choice or better). The word "assisted" implies that markers can be used in conjunction with other information on the individual animal such as breed composition, age, weight, condition score, and ultrasound measurements, to assist in sorting animals into groups that can then be managed in a uniform manner to target a specific performance goal or market.

It is possible for a test to be useful for MAM but not for MAS. For example, if all of the animals in a given breed carry two copies (fixed), or no copies, of a marker allele, then that marker will be of no use for within-breed MAS as the marker accounts for none of the genetic variability seen for the trait in that breed, even though that marker may be associated with a big effect on the trait in breeds where it is not fixed. In cattle of unknown origin or mixed breeds however, marker frequencies may be of use in sorting animals with similar genetic backgrounds into management groups. For example, if a set of markers was fixed in *Bos taurus* cattle and absent in *Bos indicus* cattle, then the allele frequencies of these markers would give some indication as to the proportion of *Bos taurus* influence in a mixed population of cattle. This information may be of use to help sort animals into more uniform groups that target a specific market or end point.

Validation

Prior to moving genetic markers from discovery populations to commercialization, it is important to validate their purported effects on the trait of interest in a different population, and assess them for correlated responses in associated traits (Barendse, 2005). As mentioned previously, genetic markers are usually closely associated or "linked" to the DNA sequence that is actually having an effect on the trait of interest (Figure 4). However, the relationship between the marker and the causative sequence may differ among breeds, and even between subpopulations within a breed. For one breed, a marker might be linked to the DNA sequence causing the desirable effect on the trait, whereas in other breeds there may be no effect of that marker on the trait or the opposite might be true such that the marker flags the "bad" sequence. The predictive value of a DNA test decreases (that is it does not "work" as well) when markers are incorrectly associated with the trait of interest in a given breed or animal. Therefore, once an association has been found between a DNA marker and a trait in a discovery population, that association needs to be validated in a different population. This validation will be most effective when the validation population is representative of the population where the test will ultimately be used.

The U.S. National Beef Cattle Evaluation Consortium (NBCEC) has been involved in the process of independently validating commercial DNA tests for quantitative beef quality traits since their first appearance on the U.S. market in the early 2000s (validation results are posted at www.NBCEC.org; Accessed 3/09/10). The term "having validated" was originally defined as finding a significant association "between genetic tests and traits as claimed by the commercial genotyping company based on phenotypes and genotypes derived from reference cattle populations" (Van Eenennaam et al., 2007a). Validation is a critical activity to test the strength of support for the genotyping company's published claims based on independent data. This process sometimes revealed that tests did not perform as expected, and in certain cases companies chose to withdraw those tests from commercialization.

During the past decade, the DNA testing industry matured from single gene tests to panels involving an ever-increasing number of markers with purported effects on multiple traits and/or in specific cattle populations. As marker panels grew in size and there were increasing intellectual property concerns regarding disclosure of the specific marker loci involved in a genetic test, validation moved from testing the effect of individual loci towards testing a single marker score, sometimes called a molecular breeding value (MBV), based on a panel of SNP markers.

The NBCEC and DNA testing companies sometimes struggled to find appropriately-phenotyped populations that were not involved in the discovery process for validation studies. Additionally, results from different validation populations genotyped with the same SNP panel were often inconsistent with respect to the significance of the association between the test and the trait(s),

and sometimes even with respect to the direction of the association (i.e. the test predicted the worst animals, not the best). This complicated the interpretation of validation results, and created confusion as to whether "validation" meant a test "worked" (i.e. was significantly associated with the trait) in one or more of the test populations, or had simply been tested by an independent third party.

At the current time the data that are reported on the NBCEC validation website include the direction of the effect ("b" value; regression coefficient), and the significance ("p" value; associations are typically considered significant if $p < 0.05$) of that effect. A positive regression coefficient means that the test was associated with the trait in a positive way, i.e. one unit of test increase was associated with an increase of (1 x regression coefficient) unit of the trait.

Example. If two animals have a DNA-based tenderness score that differs by 2 units and the regression coefficient of phenotype on the genetic score is 0.3, then it would be predicted that there would be a (2 x .3) = 0.6 lb difference in Warner Bratzler Shear force between steaks derived from these two animals.

A common criticism of the currently-available DNA tests for quantitative traits in beef cattle is that their ability to predict genetic merit is limited. The accuracy of a DNA test at predicting the true genetic merit of an animal is primarily driven by the proportion of additive genetic variation accounted for by the DNA test. Current estimates suggest this proportion is generally low (0-0.10) in existing tests, although this number is not readily available for all tests. The exception is tenderness DNA tests where available estimates for the proportion of genetic variation range from .016-0.299 (http://www.beefcrc.com.au/Aus-Beef-DNA-results; Accessed 3/09/10). Over time it is envisioned that genetic tests will have many more markers which will be associated with the majority of important genes influencing a trait. A January 2010 press release announced the availability of greater than 50,000 marker DNA test for Angus cattle (http://www.pfizeranimalgenetics.com/Pages/HD50KRelease.aspx, Accessed 3/09/10). It is hoped that in the future DNA tests will be highly predictive of the true genetic value of an animal. Future NBCEC validations will report the accuracy and proportion of genetic variation accounted for by DNA tests. Obtaining estimates of these values is an important step in moving the focus of validation from whether a test "works", towards developing the information that will be needed to incorporate DNA testing into cattle genetic evaluations. Publishing traditional EPDs and marker information separately, as is currently the case, is confusing and can lead to incorrect selection decisions when emphasis is placed on marker scores that predict only a small proportion of the genetic variation. Developing an approach to develop marker-assisted EPDs seems to be a logical next step in the implementation of DNA tests into national genetic evaluations. In fact some breed associations are already moving in that direction as indicated in a July 2009 press release (http://www.angus.org/Pub/Newsroom/Releases/AGI_Igenity_EPDs.html; Accessed 3/09/10).

Web Sites of US Companies Providing Genotyping Services for Beef Cattle

(current as of 3/2010)

A listing of available tests is maintained at the following web address: http://animalscience.ucdavis.edu/animalbiotech/Biotechnology/Companies/index.htm

AgriGenomics, Inc. (http://www.agrigenomicsinc.com) Arthrogryposis Multiplex (AM), Tibial Hemimelia (TH), Pulmonary Hypoplasia with Anasarca (PHA), Black/Red Coat Color (CC), Dilution (DL), Idiopathic Epilepsy (IE), Arthrogryposis Multiplex (AM) or Curly Calf Syndrome analysis

Biogenetic Services (http://www.biogeneticservices.com) Parentage, freemartin, coat color, leptin, meat quality, BSE resistance, Johne's disease, Bovine Virus Diarrhea (BVD)

GeneSeek (http://www.geneseek.com) Arthrogryposis Multiplex (AM), Parentage, coat color, Seek-Black, Seek-Tender, Bovine viral diarrhea (BVD-PI), identity tracking, 50,000 SNP CHIP genotyping

Genetic Visions (http://www.geneticvisions.net) Coat color, Prolactin (CMP), BLAD, Citrullinemia, DUMPS, Kappa-Casein, Beta-lactoglobulin, Complex Vertebral Malformation (CVM), Calpain 316/530, Freemartin

Igenity (http://us.igenity.com) Arthrogryposis Multiplex (AM), Neuropathic Hydrocephalus (NH) , Coat Color Dilution (DL), Idiopathic Epilepsy (IE), Osteopetrosis (OS), Pulmonary Hypoplasia with Anasarca (PHA), and Tibial Hemimelia (TH), Parentage, Myostatin, Breed-specific horned/polled, BVD-PI diagnostic test, Igenity Profile Analysis (tenderness, marbling, quality grade, fat thickness, ribeye area, hot carcass weight, yield grade, heifer pregnancy rate, stayability, maternal calving ease, docility, residual feed intake, average daily gain), DoubleBLACK coat color, identity tracking

MMI Genomics (http://www.metamorphixinc.com) Arthrogryposis Multiplex (AM), Neuropathic Hydrocephalus (NH), Osteopetrosis (OS) or "marble bone disease", Parentage, Tru-Marbling™, Tru-Tenderness™, MMIG Homozygous Black, polled/horned

Pfizer Animal Genetics (previously Bovigen) (http://www.pfizeranimalgenetics.com) Arthrogryposis Multiplex (AM) or Curly Calf Syndrome analysis, Neuropathic Hydrocephalus (NH), Osteopetrosis (OS), Tibial Hemimelia, Pulmonary Hypoplasia with Anasarca, Idiopathic Epilepsy GeneSTAR® MVP™ (feed efficiency, marbling, tenderness), HD 50K for Angus (Calving ease direct, birth weight, weaning weight, average daily gain, dry matter intake, net feed intake, calving ease maternal, mature weight, milking ability, carcass weight, backfat thickness, ribeye area, marbling score, tenderness), GeneSTAR® Elite Tender, GeneSTAR® BLACK, parentage, identity tracking

Quantum Genetics (http://www.quantumgenetics.ca) Leptin

Repro Tec Inc. (http://www.reprotec.us) Fertility Associated Antigen (FAA)

Veterinary Genetics Laboratory (UC Davis) (http://www.vgl.ucdavis.edu) Parentage, freemartin, coat color, Dexter Cattle: Dexter Dun, Extension (Red/Black), Bulldog Dwarfism (Chondrodysplasia), freemartin karyotyping

Viagen (http://www.viagen.com) Breed identification (AnguSure™)

Whole Genome Selection

Recent developments in genotyping technologies and SNP discovery methods (Van Tassell et al., 2008) have led to the development of panels that allow a single DNA sample to be simultaneously genotyped for tens of thousands of SNPs (e.g. the 50,000 SNP bovine panel). It is hoped that cumulatively these markers will be associated with a large proportion of the genetic variation associated with various traits of importance to the beef cattle industry. This may pave the way for producers to select animals to become parents of the next generation based on breeding values calculated from DNA marker data, a process called whole genome selection (**WGS**) or genomic selection.

WGS is a form of marker-assisted selection (MAS) that uses thousands of markers that are distributed throughout the genome. With WGS, the approach is to genotype thousands of SNPs on animals that have phenotypes for a given trait, and then use these data to determine a prediction equation that predicts how well an unknown animal will perform for that trait based on its SNP genotype alone (Meuwissen et al., 2001). There are three populations required for WGS; a training population, a validation population, and the application or selection population (i.e. animals where the test will be applied to make selection decisions; (Goddard and Hayes, 2007). WGS effectively derives an EPD estimate for thousands of individual SNPs based on phenotypes in the training population. An overall measure of the merit of an animal is then obtained by summing the EPD estimates to generate a molecular breeding value (**MBV**). The accuracy of the prediction equation is then assessed by applying it to an independent group of animals that have been genotyped and measured for the trait to estimate the correlation between the MBV and the true breeding value. Ideally validation populations should have a similar genetic makeup to the application population where the prediction equation will be applied (Figure 5).

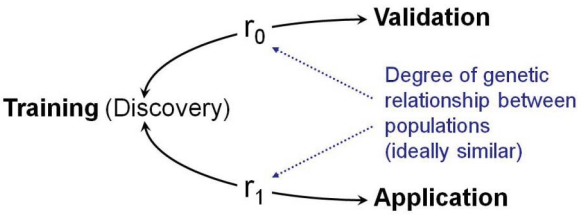


Figure 5. Populations involved in Whole Genome Selection. *Original graphic obtained from Mark Thallman, USDA, ARS, Meat Animal Research Center (MARC). Used with permission.*

The potential benefits of whole genome selection are likely to be greatest for traits that:

- have low heritability (reproductive traits).
- 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. stayability).
- are currently not selected for as they are not routinely measured (e.g. product composition, tenderness, or nutritional value).

It is envisioned that whole genome selection will accelerate genetic progress by increasing the accuracy of selection, and allowing selection decisions to be made at a younger age (Schaeffer, 2006). The prediction of breeding values at an early age removes many of the limitations of current phenotype-based breeding programs and provides a clear time advantage in developing genetic estimates for sex-limited traits, or traits that are not available until late in an animal's life, such as fertility or longevity. Additionally this approach may open the way to develop genetic predictions on difficult to measure economically-relevant traits, such as disease resistance and feed efficiency, which are not currently included in beef cattle genetic evaluations. It may also allow for selection on traits that have never been previously considered in genetic evaluations such as the compositional makeup and nutritional value of meat for human consumption.

SNP-based Fingerprinting for Cattle

"SNP fingerprinting" may also play a role in individual animal identification (Figure 6). After an animal has been slaughtered, DNA remains a stable, identifiable component to track the origin

Table 1. Possible progression of DNA testing technologies over the next decade.

2003	2008	2013	2020
Single marker/single trait tests	Multimarker tests become available for a small number of traits	Panels with 100-1000s of markers for multiple traits	Testing costs are low
Actual genotyping results reported	Results reported in a variety of formats although move towards reporting numeric scores	Results consistently reported in unit of the trait	Large SNP panel used by worldwide beef cattle community for a large number of traits
Low accuracy	Tests account for <10% additive genetic variation	DNA information starting to be routinely incorporated into genetic evaluation	Seamless submission of genotype data into national genetic evaluation schema
Limited adoption	No tie in between results and national genetic evaluation	DNA-based evaluations begin to improve accuracy of EPDs	EPDs available on many economically relevant traits
Technology oversold	No way to determine appropriate emphasis to place on test result	Larger numbers of genotyped populations start to become available for validation	DNA information greatly increases the accuracy of genetic evaluations
	Technology not in form producers could easily use		Industry routinely uses DNA information for herd management, and breeding decisions

Figure 6. 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.*

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 (Daetwyler et al., 2007), and to allow for the transmission of beneficial alleles from rare breeds into commercial breeds of cattle.

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 which has had its own DNA removed). This process is called somatic cell nuclear transfer or **"SCNT"** and has been successfully performed on many species including cattle (Figure 7). It is important to note that prior to SCNT, 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 SCNT procedure that created Dolly is that it allows for the replication of adult animals with known attributes and highly accurate EPDs based on pedigree, progeny, and their own performance records.

Figure 7. Two somatic cell nuclear transfer (SCNT) cloned Holstein calves, Dot and Ditto. *Original photo taken by Alison Van Eenennaam, UC Davis. Used with permission.*

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, it has been found that SCNT 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 SCNT 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 entire 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 SCNT clones relative to offspring conceived in the traditional way. It may be that SCNT clones differ from the original DNA-donor in the way that their nuclear genes are expressed. These problems are not seen universally in SCNT cloned cattle, and there are reports of apparently healthy cattle that have gone on to conceive and have healthy calves (Lanza et al., 2001; Pace et al., 2002). Studies comparing the performance of SCNT 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 SCNT 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 alleles to their offspring. More research is required to determine if the offspring of SCNT 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 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. On January 15th, 2008 the FDA published its final 968-page risk assessment on animal cloning which examined all existing data relevant to 1) the health of clones and their progeny, or 2) food consumption risks resulting from their edible products, and found that no unique food safety risks were identified in cloned animals. This report, which summarizes all available data on clones and their progeny, concludes that meat and milk products from cloned cattle, swine and goats, and the offspring of any species traditionally consumed as food, are as safe to eat as food from conventionally bred animals (http://www.fda.gov/cvm/CloneRiskAssessment_Final.htm; Accessed 3/09/10).

Although cloning is not genetic engineering per se, there is a logical partnership between the two technologies. Cloning offers the opportunity to make genetically engineered or transgenic animals more efficiently from cultured somatic cells that have undergone precise, characterized modifications of the genome. The first genetically engineered mammalian clones were sheep born in 1997 carrying the coding sequences for human clotting factor IX, which is an important therapeutic for hemophiliacs (Schnieke et al., 1997). Cloning has also be used to generate genetically engineered cows that produce human polyclonal antibodies (Kuroiwa et al., 2002). It is envisioned that these unique cows will make it possible to create an efficient, safe, and steady supply of human polyclonal antibodies for the treatment of a variety of infectious human diseases and other ailments including organ transplant rejection, cancer and various autoimmune diseases, such as rheumatoid arthritis. Cloning also offers the possibility of producing animals from cultured cells that have had selected genes removed. This "gene knockout" technique, commonly used in research with mice and the subject of the 2007 Nobel Prize in medicine, enables selective inactivation of specific genes in livestock with applications for both agriculture and biomedicine. For example, cloning has been successfully used to produce cattle from cells lacking the gene for the prion protein responsible for mad cow disease (Kuroiwa et al., 2004).

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 US marketplace (Table 2). The Food and Drug Administration (FDA) is the agency responsible for regulating genetically engineered animals.

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 SCNT cloning could also be used to remove or "knock out" certain proteins from the genome of cattle. 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 difficulty in identifying genes that 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 organisms.

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. Several human therapeutic proteins have been produced in cattle (Salamone et al., 2006; van Berkel et al., 2002; Wang et al., 2008). The first human therapeutic protein,

Table 2. Existing and potential genetically engineered cattle applications for agriculture.

EXISTING TRANSGENIC CATTLE	**Target Gene**	**Approach**	**Reference**
BSE resistance	Prion	Knockout	(Richt et al., 2007a; Richt et al., 2007b)
Mastitis resistance	Lysostaphin	Transgene overexpression	(Wall et al., 2005)
Mastitis resistance	Lactoferrin	Transgene overexpression	(van Berkel et al., 2002)
Increase cheese yield from milk	β-casein, κ-casein	Clone/Transgene overexpression	(Brophy et al., 2003)
CONCEPTS UNDER DEVELOPMENT	**Target Gene**	**Approach**	**Reference**
Increased lean-muscle growth	Myostatin	RNAi /Knockout	(McPherron and Lee, 1997)
Suppressing infectious pathogens	RNA viruses (eg. foot and mouth, fowl plague, swine fever)	RNAi	(Clark and Whitelaw, 2003; Whitelaw and Sang, 2005)
Coronavirus-resistance	Aminopeptidase N	RNAi /Knockout	(Schwegmann-Wessels et al., 2002)
Low lactose milk	Lactase	Transgene overexpression	(Jost et al., 1999)
Low lactose milk	α-lactalbumin	RNAi /Knockout	(Stacey et al., 1995)
High omega-3 fatty acid milk	n-3 and n-6 fatty acid desaturase	Transgene overexpression	(Morimoto et al., 2005)
Resistance to Brucellosis	NRAMP1	Transgene overexpression	(Barthel et al., 2001)

Antithrombin III (ATryn®, GTC Biotherapeutics, Framingham, MA), derived from the milk of genetically engineered goats has been approved by the European Commission and the FDA for the treatment of patients with hereditary antithrombin deficiency. These "biopharming" applications have the potential to produce large amounts of human therapeutics at a 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.

Conclusion

DNA-based technologies are developing at a rapid pace. It is likely that these technologies will play a progressively important role in beef production and marketing in the future. DNA-based tests can be used for various purposes; for example selection and breeding decisions, feedlot sorting, pedigree verification, and as a marketing tool. Estimates of DNA test performance (e.g. proportion of genetic variation accounted for by a DNA test panel) and accuracy in representative populations will be required to evaluate their use for selection, and also for incorporation of DNA data into the existing genetic evaluation infrastructure. Whole genome selection has the potential to improve traits that are currently intractable (feedlot health, feed efficiency, palatability). As a result of experiments with the 50,000+ SNP chip in cattle, it is likely that the number and accuracy of DNA-based marker tests will increase in the coming years, and eventually "DNA-adjusted EPDs" will become a reality. In the meantime, however, the increased economic returns from using DNA-marker tests and ultimately incorporating them into the national cattle evaluations must outweigh the costs (DNA sampling, genotyping, phenotyping) associated with obtaining the additional genetic information.

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