DNA Markers: Explanation of Validation and Utilization

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After years of research, development, and lofty promises, DNA testing for beef cattle is here. Yet there is confusion about how to interpret and use the results from DNA marker tests. This is perhaps not surprising given that genotyping companies are all offering unique products through different reporting systems, and we are all learning together about how to apply DNA testing to cattle breeding. The purpose of this article is to clarify the meaning of some new terms that are being used in conjunction with DNA testing, discuss the concept of marker validation, and outline the different applications for products that are currently being offered by commercial genotyping companies. (Words in boldface are defined in the glossary in the accompanying box.)

DNA marker is a term used to refer to a specific DNA variation among individuals that has been found to be associated with a certain characteristic (e.g., increased tenderness). These different DNA or genetic variants are known as **alleles**. **DNA marker testing** or genotyping determines which alleles an animal is carrying for a DNA marker(s). The use of this genotype information from DNA marker tests associated with **simple traits** is relatively straight forward. Such traits are often controlled by a single gene, and a marker allele associated with that gene can perfectly predict the phenotype of that trait (the physical attributes of an animal). DNA tests for simple traits have been on the market for several years and include those for certain diseases, such as DUMPS (Deficiency of Uridine Monophosphate Synthase) and BLAD (Bovine Leukocyte Adhesion Deficiency)**,** coat color, and horned status. However, most economically relevant traits (ERT) are **complex**, meaning they are controlled by many genes and are also influenced by the environment. Examples of complex traits include growth traits, carcass characteristics and reproductive performance. Any single DNA marker is associated with only one of the many genes that control complex traits.

Research to find DNA markers that are associated with complex traits often begins with a **discovery population** or research herd where cattle have been measured for a number of traits of interest. These animals are extensively genotyped using a large number of markers, and then studies are performed to see if there is any association between alleles at these markers and phenotypes for traits of interest. Once an association has been identified, **validation** studies on independent populations are necessary to ensure that the association is real. That is, other groups of animals, independent of the original discovery population, are tested to see if the relationships identified in the discovery herd holds true for other animals. Validation studies can be **internal validations** performed by the DNA companies to further assess the efficacy of their tests or **independent validations** performed by a third party.

Validation is necessary because false associations between markers and the trait of interest can arise if breed composition or pedigree information is omitted from the discovery population analysis, perhaps because such information is unavailable as may often be the case in data from commercial cattle populations. This can lead to false associations that are due to population **stratification**. For example, if a discovery population was made up of Angus and Brahman cattle, i.e., breeds that differ in their allele frequencies at many markers, it might appear that all marker alleles that have a high frequency in Brahman cattle are associated with having long ears and a hump, even though some of them are not genetically associated or linked with the genes that actually control those traits. Similar false associations can also result from ignoring

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pedigree. It is also true that an association in one particular experimental study could occur by chance alone, and the verification of previous results has always been one of the prime motivators driving scientists to repeat experiments. Markers that cannot be validated have no value as tools for **marker-assisted selection.**

When marker discovery is performed by public sector scientists, results are typically reported in the **scientific literature**. This gives other researchers the opportunity to examine the methods used by the researcher and evaluate the evidence being presented in support of a marker, and even replicate the experiment or use the markers in their own research. However, marker discovery and the development of DNA-marker tests are increasingly being carried out by the private sector. There are no regulations guiding the development and marketing of DNA tests for beef cattle, and companies have little incentive to disclose their markers or discovery process in the scientific literature lest they provide their competitors with valuable proprietary information. Consequently, DNA marker tests can come to market with little independent review of their efficacy.

The National Beef Cattle Evaluation Consortium (NBCEC) Advisory Council, made up of ten representatives from industry stakeholder organizations including the Beef Improvement Federation, the National Cattlemen's Beef Association, and the U.S. Beef Breeds Council, were concerned about the reliability of DNA tests being marketed to beef cattle producers and asked the NBCEC to develop a process to verify that commercial DNA tests worked in accordance with company claims. This resulted in the development of the NBCEC commercial test validation process. This independent validation process was designed to evaluate the associations between genetic tests and traits as claimed by commercial genotyping companies. Most of the initial DNA tests being commercialized for use in beef cattle were for carcass characteristics, and DNA and phenotypes from the NCBA Carcass Merit Project (CMP) reference cattle populations were used for the validation. These populations represent a cross-section of commercial cattle sired by prominent bulls from various breeds raised under different management practices and environmental conditions. Such diversity of breeds and environments is likely to be typical of commercial applications of DNA testing.

Three commercially available DNA marker tests for carcass quality traits have been independently validated by the NBCEC as of 8/31/2007. Results from these analyses are publicly-accessible at the following website (http://www.nbcec.org). GeneSTAR® Quality Grade is a DNA genetic marker panel test comprised of four markers that have been associated with an increase in marbling score and quality grade (% choice and prime) in company trials. Favorable forms of these markers (★) were associated with improved quality grade in an NBCEC validation study.

GeneSTAR® Tenderness is a DNA genetic marker panel test comprised of three markers that have been associated with tenderness. Favorable forms of these markers (★) have been associated with increased tenderness in company trials, and this finding was validated by the NBCEC. The tenderness component of the IGENITY® Profile involves three markers that have been associated with tenderness. Favorable forms of these markers have been associated with increased tenderness in company trials, and this finding was validated by the NBCEC. Animals are scored on a scale of 1-10, with 10 being the most favorable for the trait. Two markers (Calpain 4751 and Calpain 316) are common between the tenderness tests offered by these two companies.

The NBCEC also independently evaluated the association between marker alleles located in the leptin gene and the growth hormone receptor gene and various carcass traits. Effects for the leptin markers on carcass traits could not be validated. The growth hormone receptor gene marker had significant effects on yield grade and ribeye area but not on marbling, carcass weight, or fat thickness. The results of these analyses can be found in the "Ancillary Results" tab of the NBCEC website. Tests using these markers are not currently being offered commercially for marker-assisted selection**.**

Several multiple-marker tests have recently become commercially available. MMI Genomics offers two multiple-marker tests, one associated with tenderness and one with marbling. The company has performed an internal validation of these tests using the CMP Angus population. IGENITY also offers multiple-marker tests for percent choice and marbling score. The NBCEC is currently collaborating with these two companies to do an independent validation of these multiple-marker tests, and results from these validation studies will be placed on the NBCEC website (http://www.nbcec.org) as they become available.

Some companies are offering marker panels for **marker-assisted management** (MAM). MAM is a relatively new term that refers to the process of using DNA marker information to make decisions on how to manage a particular animal or a specific group of animals. An example of markers for MAM is some of the carcass composition components of the IGENITY® Profile product. This genetic profile includes information from multiple markers in genes related to various components of carcass composition including quality grade, yield grade, fat thickness, marbling, hot carcass weight, and ribeye area. The company website clarifies how the carcass composition information should be used, stating that "*IGENITY carcass composition is a tool designed for marker-assisted management. Results from this analysis are only relevant for procurement, management, and as a marketing tool for feeder calves intended for finishing and harvest. IGENITY carcass composition results should not be used for selection or breeding decisions*." The results of such tests can be used to sort cattle, such as when a group enters the feedlot, to assist in the management of that group of cattle.

The NBCEC does not currently validate DNA tests that are intended for applications other than genetic evaluation and selection. MAM markers may or may not work for within-breed selection, but until they have been independently validated for this application, the NBCEC cannot recommend they be used for genetic selection within breed. However, it may be time for the beef industry to consider developing a process for the independent validation of DNA tests intended for MAM.

The NBCEC validation process was developed at the request of their Advisory Council to independently confirm a genetic association between marker tests and their targeted trait. It was felt that producers would be reluctant to invest in unproven markers, and validating the markers was seen as a way to decrease the risk associated with investing in this technology. The validation process is a work in progress, and it will likely evolve as more markers associated with a variety of traits that were not measured in the CMP reference populations enter the marketplace. To address this need, the NBCEC is working to develop populations with suitable phenotypes for future validation studies. The widespread adoption of marker-assisted selection in the beef industry will likely depend upon the development of seamless methods to utilize DNA marker information in National Cattle Evaluation (NCE) programs to enable the generation of "DNA marker-assisted EPD" with improved accuracies. Independent validation studies provide a way to generate the information (e.g., size of the marker effect) that will be required to

incorporate the results from DNA tests into the NCE. Therefore, it is anticipated that only DNA tests that have passed the independent validation process will be used in calculating EPD. Seedstock producers should consider this as they make purchasing decisions regarding DNA tests.

GLOSSARY

Allele: Different forms or variants of a DNA marker are known as alleles. Each animal inherits two alleles of each DNA marker, one from its sire and one from its dam. These two alleles can be identical (making the animal homozygous), or different (making the animal heterozygous) for any given DNA marker.

Complex traits: Traits that are controlled by many genes that interact with each other and environmental conditions to result in the observed phenotype. Most traits of economic importance such as growth, reproduction and carcass composition are complex traits.

Discovery population: The population of cattle that was used to find an association between a DNA marker and the trait of interest.

DNA marker (aka genetic marker): Specific DNA location(s) where variation among individuals has been found to be associated with a positive or negative effect on the trait of interest.

DNA marker testing (aka genotyping): A test to determine which DNA sequence variant(s) an animal is carrying for DNA marker(s). Different forms of a DNA marker are known as alleles.

Independent validation: A validation study to verify an association between prospective marker(s) and the trait of interest carried out by a third-party with no financial interest in the outcome.

Internal validation: A validation study performed or sponsored by the company that is developing the DNA-marker test. It is carried out on groups of cattle that are distinct from the discovery population that was used to identify the association between DNA marker(s) and the trait of interest.

Marker-assisted management (MAM): 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 (eg. 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.

Marker-assisted selection (MAS): The process of using the results of DNA-marker testing to predict the genetic merit of the animal being tested and assist in the selection of individuals to become parents in the next generation. The word "assisted" implies that the selection is also influenced by other sources of information, such as animal's observed performance and EPD.

Scientific literature: Journal articles, books or book chapters that have been through a peer-review process. Peer review is the process of subjecting an author's scholarly work to the anonymous scrutiny of others who are experts in the field. The peer-review process aims to make authors meet the standards of their discipline and of science in general. Passing the peer-review process is often considered in the scientific community to be a certification of validity.

Simple traits: Traits that are controlled by one or a small number of genes and that are not greatly influenced, if at all, by environmental conditions, e.g., gender, horns, coat color.

Stratification: Population stratification refers to differences in DNA-marker allele frequencies that occur due to breed ancestry rather than the association of certain alleles with specific trait phenotypes.

Validation: A study conducted to confirm a reported association between DNA-marker alleles and the trait of interest. Validation studies play an important role in ensuring that potential markers have a real association with the trait of interest in commercial cattle populations.

WHAT TESTS ARE OUT THERE?

U.S. Companies Providing Genotyping Services for Beef Cattle *(current as of 8/2007)*
A listing of available tests is maintained at the following web address
http://animalscience.ucdavis.edu/animalbiotech/Biotechnology/MAS/index.htm.

- Biogenetic Services (http://www.biogeneticservices.com)
    - Freemartin, coat color, leptin, meat quality, BSE resistance, Johne's disease
- Bovigen (http://www.bovigen.com)
    - **GeneSTAR® Quality Grade*, GeneSTAR® Tenderness*,** GeneSTAR® BLACK
- Genetic Visions (http://www.geneticvisions.net)
    - Coat color, BLAD, Citrullinemia, DUMPS, Kappa-Casein, Beta-lactoglobulin, Complex Vertebral Malformation (CVM), Calpain 316/530, Freemartin
- GenMark (http://www.genmarkag.com/cattle_tests.php)
    - Coat color, BLAD, Citrullinemia, Kappa-Casein, Beta-lactoglobulin, Alpha S1 Casein, Piedmontese myostatin, Freemartin
- GeneSeek (http://www.geneseek.com/prod_cattle.php)
    - Seek-Black, Seek-Tender, bovine viral diarrhea (BVD-PI)
- Igenity (http://us.igenity.com)
    - IGENITY® Profile which includes carcass composition components (**tenderness**, quality grade, yield grade, fat thickness, marbling, hot carcass weight, and ribeye area), maternal milk yield, coat color, horned-polled, bovine viral diarrhea (BVD-PI)
- MMI Genomics (http://www.metamorphixinc.com)
    - Tru-Marbling™, Tru-Tenderness™ , MMIG Homozygous Black, polled/horned
- Repro Tec Inc. (http://www.reprotec.us)
    - Fertility Associated Antigen (FAA)
- Viagen (http://www.viagen.com)
    - Breed identification (AnguSure™, Inducator™)

* Tests in boldface are those that have been independently validated by the NBCEC