

DNA Testing and Marker Assisted Selection

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Introduction

Beef cattle breeders have heard for years that DNA testing is coming and that it will change the way they breed cattle. At long last, the time is here when DNA testing for economic traits is available, albeit in a very immature form. Breeders must decide whether to use the technology, and if so, how to use it. Breed associations must decide what role they will play in the adoption of this technology.

DNA testing has a number of potential applications in cattle breeding, including parentage testing, tests for genetic diseases or defects, and tests for qualitatively inherited traits such as color or horns. However, most economically important production and end-product traits are influenced by several or many genes. The individual genes that influence such traits are known as quantitative trait loci (QTL). The identity of these genes may be known, but in many cases only the general location of the QTL on a chromosome is known. This presentation will focus on tests for quantitative traits.

Benefits of DNA Testing

DNA testing can make evaluations available shortly after birth, or even at the embryo stage. This is an important advantage for traits that can only be measured after the age at which selection decisions are normally made (or postmortem).

It should provide greater information from each phenotype that is measured. This is especially important for traits that are expensive to measure or sex-limited.

It should provide greater opportunity to select for traits with antagonistic genetic relationships (e.g., birth weight and growth rate).

Current Status of DNA Testing in Beef Cattle

As recently as four years ago, there were no commercial DNA tests for quantitative traits in beef cattle, but today there are at least seven companies performing or marketing such tests. The tests currently available include at least the following:

- GeneStar Marbling (thyroglobulin) – Genetic Solutions/Bovigen
- GeneStar Tenderness 2 (calpastatin and 1 SNP in μ -calpain) – Genetic Solutions/Bovigen
- TenderGENE (2 SNP in μ -calpain) – Frontier Beef Systems/GeneSeek

- IGENITY L (leptin) – Merial/Quantum Genetics
- MMIG Mu-Calpain Tender (2 SNP in μ -calpain) – MMI Genomics

The names of the genes upon which these tests are based are listed in parentheses, followed by the name of the company that markets and/or performs the test. Single nucleotide polymorphisms (SNP) are locations in the genome at which differences in sequence occur. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. A description of most of the above tests (by the company marketing each of them) is provided in the proceedings of the Genetic Prediction Workshop held in December, 2003. Those proceedings are available online at:

http://www.beefimprovement.org/gp_proceedings.pdf.

Hopefully, the list of commercial DNA tests will continue to grow rapidly. A list of available tests is maintained by Alison Van Eenennaam (University of California, Davis) at <http://animalscience.ucdavis.edu/animalbiotech/Biotechnology/MAS/index.htm>.

Most of the current DNA tests are offered by only one testing company. It is anticipated that a greater number of non-proprietary tests will be offered by multiple companies in the future. Nonetheless, breeders that wish to evaluate their cattle as thoroughly as possible must currently send samples to several DNA service labs and this situation is expected to continue. Other breeders will seek to use one company that offers the best (albeit not complete) suite of tests relative to price. In any case, breeders need independent information with which to make decisions about their use of DNA tests.

Independent Characterization of DNA Tests

Considerable information about a DNA test is required in order to decide whether to use it or not. Some of the required information may seem technical, but breeders are becoming more familiar with it as they gain experience using DNA tests.

Some of the information required to decide whether or not to use a DNA test could only be provided by the company that is providing the testing service. However, other information can be provided by an independent institution using standard resource populations with phenotypes for the desired traits in cooperation with the testing company. This is currently being done through the

National Beef Cattle Evaluation Consortium (NBCEC). The NBCEC provides DNA to the testing company, which runs the test on the DNA and sends the test results back to the NBCEC. The NBCEC then analyzes the data and reports the results publicly.

Ideally, the reports will include not only information on the individual test, but also its interactions with other DNA tests. This is important both for selecting tests and for inclusion of the results in National Cattle Evaluation (NCE).

Independent characterization of commercialized DNA tests provides better information from which to decide which tests to include in NCE. Furthermore, it should enable DNA testing companies to market tests more effectively and with greater confidence. The process also generates information (such as the effect of the test) that is needed in order for DNA testing data to be included in NCE.

Successful implementation of independent characterization requires the cooperation of a number of groups. Breed associations, independent ranching operations, and/or research institutions need to provide DNA and phenotypes on appropriate groups of animals. The DNA testing companies need to provide the testing services. A research institution needs to conduct the data analysis. Finally, none of this is likely to happen regularly unless the breeders provide motivation and encouragement for it. Breeders should recognize that it is important for this information (even the more technical aspects of it) to be available, because much of it will be necessary in order to include DNA test data in NCE.

Guidelines for Use of DNA Testing

It would help if the information provided by the independent characterization process was presented in a standard format so that comparable information was available for each DNA test. The format for this “label” could be included in the BIF Guidelines.

A subcommittee of the Emerging Technologies committee has been formed to write a section of the BIF Guidelines dealing with DNA testing. The guidelines are likely to cover a wide range of topics including terminology, independent characterization, which animals should be tested, collection/storage of tissues, reporting of results, the role of breed associations in the process, inclusion of the results in NCE, and most importantly, use of the information by breeders.

Traits Emphasized in DNA Testing

All of the DNA tests listed above are associated primarily with meat quality traits. There are undoubtedly tests related to carcass composition in the development pipeline. For good reason, most interest in DNA testing is focused on traits that are difficult or expensive to measure;

EPDs are very effective for traits that are routinely measured prior to selection.

Considerable efforts are underway to develop tests related to feed efficiency, reproductive efficiency, and disease resistance. Such tests are challenging to develop, for exactly the same reasons that they are difficult to improve using conventional means. DNA testing is probably our best hope for improving such traits, but it should not be expected to happen immediately.

We are all learning together about the application of DNA testing to cattle breeding. One of the big challenges is that phenotypes are scarce for the primary traits influenced by currently available tests. This makes it impractical to do large-scale evaluations from field data of the performance of the tests or to address questions such as whether the tests perform the same in all breeds or whether some tests need to be enhanced in order to describe genetic variation that exists in certain lines of cattle.

A potential benefit of DNA testing, which has perhaps received too little emphasis, is selection for sets of traits that have antagonistic genetic relationships. Perhaps we should put some emphasis on developing and using DNA tests for genes that influence growth rate without changing birth weight. Those phenotypes are readily available and that is an important, but somewhat challenging objective of current breeding programs. We would almost certainly learn a great deal about fundamental aspects of DNA testing in beef cattle that could be applied to DNA tests for traits with fewer phenotypes.

It is unfortunate that DNA tests tend to be labeled as influencing one particular trait. This reinforces the common misconception that there is a one-to-one relationship between genes and traits. Quantitative traits (which include most economically important traits in cattle) are, by definition, influenced by at least several genes and most genes influence a variety of traits. The latter is a cause of genetic correlations and large volumes of data supporting the existence of these have been amassed.

How Should Breeders Use Information from DNA Testing?

The availability of DNA testing will bring, along with all of the advantages, misuse of information, especially in the early years when only a few DNA tests are available. We have heard much discussion of the evils of “Single-Trait Selection.” Breeders must now face the temptation of “Single-Gene Selection,” which may have far greater consequences.

For example, a bull with one of the top EPDs in his breed for a trait, had the least desirable, but most common, genotype (test result) for a DNA test for one of the genes affecting the trait. Semen sales on this bull dropped off sharply following the release of the test result. Apparently, breeders decided that they could not use bulls with the less

favorable allele (form) of this gene, a prime example of “single-gene selection.” This is understandable, but is not good use of DNA test information for several reasons:

- Applying this much selection pressure to one gene, greatly reduces the selection intensity that can be applied to the other genes that affect this trait and others, especially when the frequency of the desirable allele is low. Selection is more efficient when applied to all genes simultaneously, in proportion to the size of effects of the genes and the relative economic importance of the traits.
- Few animals have two copies of the desired allele. Restricting the choice of herd sires to only those with the desired genotype of the “single gene” would put the breed through a bottleneck that would reduce the effective population size and increase inbreeding. When tests for more genes become available, very few animals will have the desired genotype at each of ten, or perhaps even 100, genes that we might test for.
- Given that the bull’s EPD is very high in accuracy (presumably due to numerous progeny with phenotypes), a DNA test result should not greatly influence our estimate of his overall genetic merit for the trait. This may sound counterintuitive, but it is an important point. His EPD estimates his total genetic merit at all genes that influence the trait. The DNA test predicts his genetic merit at one of those genes. Therefore, an unfavorable DNA test result should be interpreted to mean that he is probably even better at the other genes affecting the trait than we would have guessed without the DNA test. Consequently, our estimate of the high accuracy bull’s breeding value is

not influenced much by his DNA test. The DNA test does suggest that we might want to breed the bull’s daughters to sires with the desired genotype. DNA testing is most useful for individuals that would otherwise have low accuracy genetic evaluations. There is little opportunity for change in the evaluation of an animal with a high accuracy EPD.

In summary, DNA tests should not be used as all-or-none selection criteria, but rather should be used as one of several sources of information upon which selection is based.

Use of DNA Test Results in National Cattle Evaluation

For the foreseeable future, DNA tests will only account for some of the genetics of any trait; we will still need EPDs. One vision of the future is illustrated in Figure 1, where phenotypes and DNA tests on the individual and its relatives are combined, through NCE, to produce marker-adjusted EPDs, upon which selection decisions can be made. The methods used in NCE will have to be enhanced to accommodate DNA testing.

At recent BIF meetings, there has been considerable discussion of the optimum allocation of selection pressure to the various traits that are evaluated. The problem can be partitioned into a biological component and an economic component. The economic component involves determining the relative economic value of each economically relevant trait (ERT), which is usually done by modeling the relationships between the ERT and some measure of profit for a particular set of circumstances. The biological

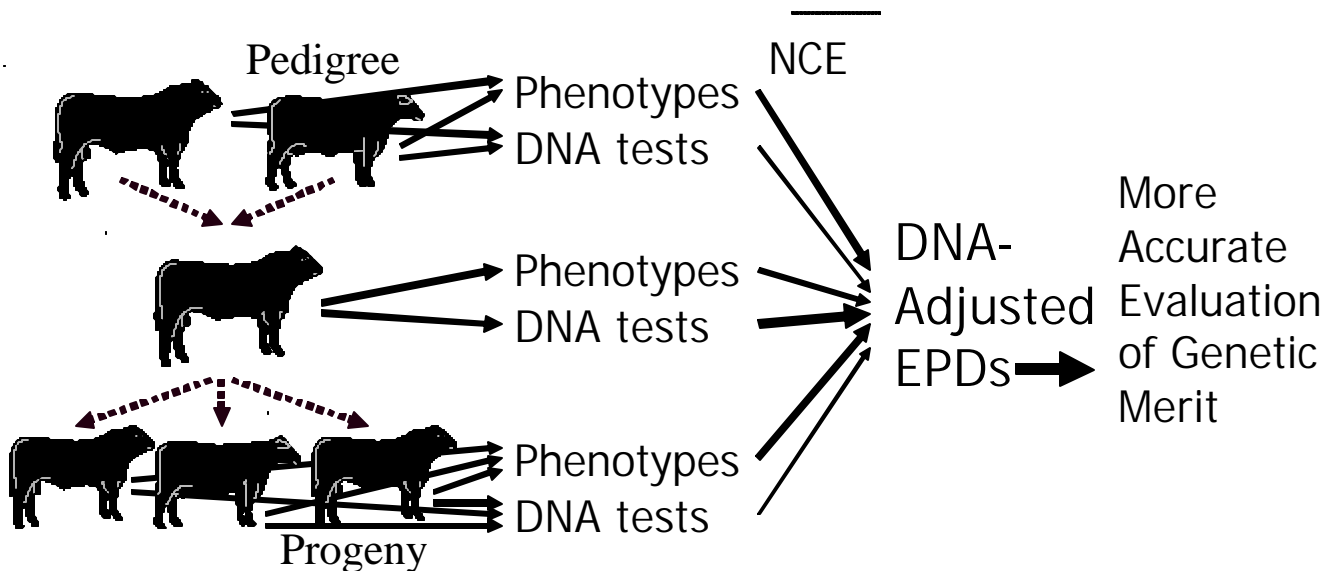


Figure 1. A Vision of NCE in the Future

component involves estimating the genetic relationships between the indicator traits (those that are actually measured) and the ERT (the traits we would ideally measure, if it were practical to do so).

If DNA testing technology is successful, there will be too many tests available for breeders to make breeding decisions based on raw test results. The relative emphasis on each gene will need to be weighted by its effect and the relative importance of the trait(s). Most DNA tests will be related to several traits. This adds a new dimension to the problem of allocating selection pressure. The biological component of the allocation problem is expanded to include relationships among DNA test results, as well as, indicator traits and ERT. Fortunately, the DNA test results do not need to enter into the economic component, which is sufficiently complicated without them. Therefore, inclusion of DNA test results in the NCE process should be an effective means to put the appropriate degree of emphasis on each DNA test.

It has been suggested that EPDs could be externally adjusted for DNA tests by simply adding or subtracting fixed amounts to or from the EPDs, depending on the DNA test results. This approach, referred to as the “myth of additivity,” is illustrated in Figure 2. However, DNA test results affect low accuracy EPDs much more than they do high accuracy EPDs; therefore, the adjustment factors would have to be “shrunk” by an amount depending on the accuracy of the EPD to be adjusted. Furthermore, DNA test results affect the evaluation of progeny of heterozygous (have two different forms of a gene) parents more than they do the progeny of homozygous (have two identical copies of a gene) parents. Therefore, any “adjustment factors” that might be developed for DNA tests would not be generally applicable and would make the process of using DNA test

results unnecessarily complicated. Simultaneous analysis of DNA test results and phenotypes, resulting in “DNA-adjusted EPDs” as illustrated in Figure 1, will be the most effective means to include DNA test results in the NCE process. This will not happen overnight, but it is an objective of the National Beef Cattle Evaluation Consortium.

Which Animals Should be Tested?

When a breeder or breed association decides to begin using a DNA test, the next logical question is “which animals?” A good place to start is usually the influential sires in the herd or breed. This will allow the frequencies of the various test alleles in the population to be estimated and also provides the most information about which untested animals are most likely to have the desired allele. For sires that have either no EPDs or low accuracy EPDs for the traits associated with the DNA test, the test will provide some information about the genetic merit of sires for those traits. For sires that have high accuracy EPDs for the traits associated with the test, the test results should have little influence on the evaluation of genetic merit of the sires, but could be used to validate or estimate the effect of the test within the breed of interest.

The next set of animals to consider testing would be herd sire and donor prospects. Selection among these candidates has a large effect on genetic progress, but they typically have lower accuracy EPDs, especially for traits that require progeny testing or that are measured later in life. Therefore, the improved accuracy of evaluation that DNA testing could provide could be very beneficial. However, it is important that the DNA test results be used only to influence decisions among animals that would otherwise be

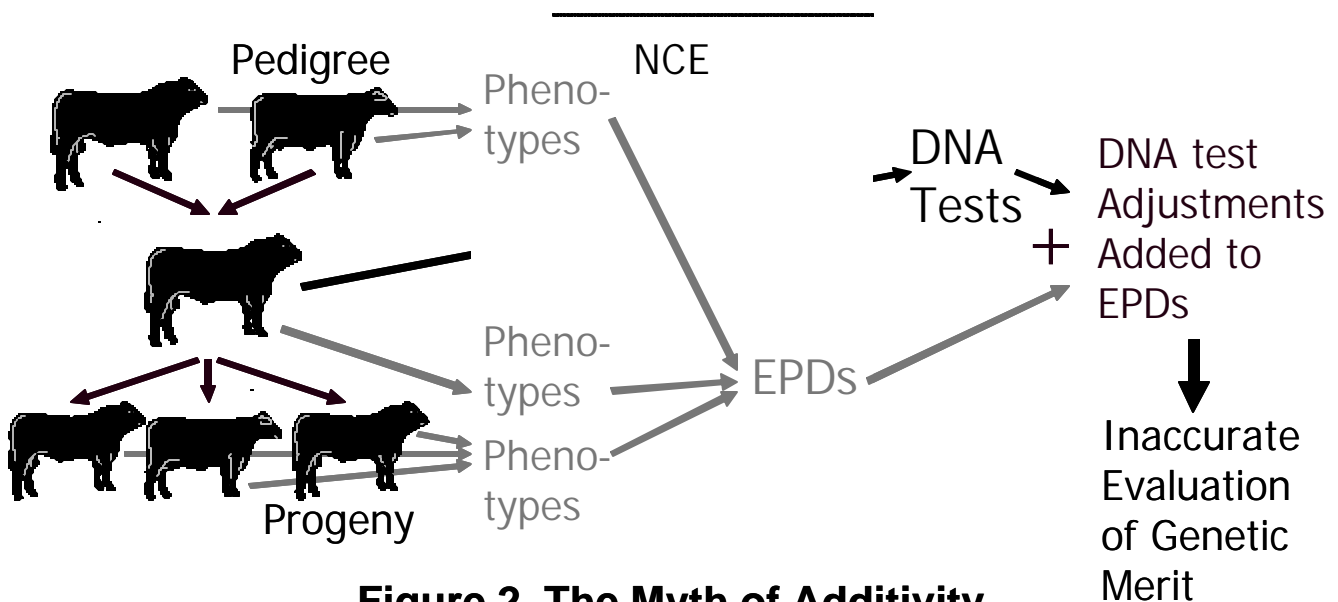


Figure 2. The Myth of Additivity

candidates for selection. The DNA test results should not be used as any kind of "litmus test" that animals must pass before being considered further.

A natural extension to testing herd sire and donor prospects could be testing all candidates to become replacement females, but this would involve testing considerably more animals. In some situations, it might be beneficial to test some or all of the bulls offered for sale. The ideal situation is that it would become cost-effective to test all of the calves produced. How far down this priority list breeders can afford to go will depend on the cost of testing. The cost of testing should decrease as the number of animals tested goes up, but the number tested may not increase sufficiently until the cost goes down. It is likely to require a coordinated effort from testing laboratories, breeders, and breed associations to move beyond this impasse.

Strategies for selecting animals to test also depend of the frequency of the favorable allele. Tests with a high frequency of the favorable allele have the desirable property that breeders will like the results that they get most of the time. However, tests with a low frequency of the favorable allele actually offer greater opportunity for genetic improvement. Breeders must test more animals to find one with the result they are looking for, but the very fact that they are rare can add considerable value to those animals that do have two copies of the desired allele.

For tests with a low frequency of the favorable allele, a reasonable testing strategy may be to first screen influential sires, and then to test descendants of those sires that have at least one copy of the desired allele.

What Should the Role of Breed Associations Be?

Breed associations can play an important role in encouraging the flow of DNA testing information into NCE and reporting the resulting DNA-adjusted EPDs back to the breeders. They will need to provide education on how to use this technology effectively and on how not to misuse it.

Selective reporting of DNA test data is likely to be a much greater problem for NCE than selective reporting of phenotypes is. Therefore, it would help greatly if the breed associations required that all DNA test data be submitted directly to them for use in NCE. However, this would require the cooperation of the DNA testing companies and it might decrease the submission of test data to NCE. As testing companies begin to offer "panels" of tests, breed associations should consider policies that, if one test in a panel is reported, all tests in the panel must be reported.

Breed associations may also participate in negotiating contracts for DNA testing and storage to protect the interests of their members. For example, who owns DNA or tissue that is left over after a DNA test is performed? What

happens to stored tissue samples if the testing and(or) storage company goes out of business?

It is inevitable that breed associations will have to deal with contradictory results (sometimes referred to as "non-Mendelian inheritances") of DNA tests between close relatives. Some simple examples are when a parent and offspring do not share an allele in common (e.g., parent is GG and offspring is CC) or when an offspring has an allele that neither of its parents have (both parents are GG and offspring is CG). These situations could be due to pedigree errors, errors in the genetic test results, sample labeling errors, or mutations. If there are only one or a few markers in common between the individuals, it will be difficult or impossible to distinguish between these alternatives.

When contradictory DNA test results occur, the best solution is to run a parentage panel on the individuals involved to determine whether the pedigree is correct. If the pedigree is correct, then it may be appropriate to retest (from new tissue samples) one or more of the animals involved in the non-Mendelian inheritance and/or relatives of those animals.

Sample misidentification is a problem that breed associations will have to deal with. Some cases will be detected through non-Mendelian inheritances and some will be detected through samples for the same animal being submitted to multiple testing companies that run some tests in common. Some cases will be detected as a result of multiple entities submitting samples for the same animal. It is possible that semen samples may be submitted for testing by entities that have no ownership in the bull.

Some breed associations have programs in which animals are randomly sampled for parentage verification. Such programs could be expanded to include tests for quantitative traits. Random re-sampling of animals for which DNA test results had previously been submitted could also be contemplated. Statistical methods to identify likely errors in pedigree (beyond exclusions) and in DNA test results are available. Sampling programs could involve testing of individuals (or their relatives) that are most likely to be in error (either pedigree or sample identity).

It should be possible to develop methods to identify instances in which selective reporting of DNA tests is likely to have occurred. Semi-random testing in such instances could be an effective means of mitigating the impact of selective reporting on NCE.

Increased use of DNA testing will provide increased opportunities for breed associations to be proactive in protecting the integrity of the data they record, but a number of new issues will need to be considered.

The data processing requirements for DNA testing data are likely to be substantial enough that it may not be practical for each association to expand its data processing software to handle such data. Instead, it may be more efficient for the breeds to work together to jointly contract out the data processing to one, or at most a few, organizations.

It may be beneficial for breed associations to collect sets of DNA on the most influential sires in the breed and fund DNA testing on those sires.

Breed associations should also ensure that DNA is collected and stored from animals in future progeny testing projects so that they can serve as resources to tie DNA test data to phenotypes so that DNA tests can be characterized within individual breeds and test effects can be estimated directly in NCE. The National Cattlemen's Beef Association Carcass Merit Project is a great start, but to be most effective, it should be followed up periodically with more current sires.

Expectations for the Future of DNA Testing

In the short run, DNA testing should not be expected to simplify cattle breeding. Selection decisions will be based on more pieces and types of information and breeders will have to decide which tests to run and which animals to test.

There is a common misperception that DNA tests will eliminate the need for phenotypes, especially for traits like tenderness that are expensive to measure. However, phenotypes will continue to be important. Although DNA testing can increase the amount of information that each phenotype contributes and thus reduce the number of phenotypes needed, DNA testing can not completely replace phenotypic data.

New tests will continue to be developed for the foreseeable future. DNA tests should not be considered

absolute or unchangeable. They should be expected to improve over time, just as EPDs have improved over time and will continue to improve.

We should assume that the cost per test will decrease over time due to improvements in technology and to greatly increased volume of DNA testing. Eventually, it should not cost much more to run a battery of many tests per animal than to run only one test per animal.

Conclusions

It will be a challenge for the beef industry to develop systems through which DNA testing data are shared sufficiently to allow their inclusion in NCE so that they can be used appropriately in selection decisions. This will require a direct benefit to whoever has to pay for the testing. However, it seems unlikely that the beef industry will be able to maintain market share over the long term without fully utilizing the information that can be provided by DNA testing. There are more challenges in using DNA testing effectively in beef cattle than in some other food species. Nonetheless, cattle breeders are making strides in implementing DNA testing and are making changes in traits, such as tenderness, that have been difficult to select for in the past. Undoubtedly, the way in which DNA testing is used by the beef industry will change over time, but the early adopters of the technology are likely to be in a better position to capitalize on that change.