

MARKER-ASSISTED SELECTION

Adapted from 'DNA Based Technologies' by Allison Van Eenennaam, University of California-Davis

Marker-Assisted Selection (MAS) is the process of using the results of DNA-marker tests to assist in the selection of individuals to become the parents in the next generation of a genetic improvement program. That is, instead of using only a traditional or EPD selection program to increase the proportion of favorable alleles for the genes that affect a certain trait, specific DNA tests are used to assist in the selection of those favorable alleles. Genotyping allows for the accurate detection of specific DNA variations that have been associated with measurable effects on complex traits. It is important to remember that markers for complex traits are associated with only those genes that are located in close proximity to the marker and do not identify favorable alleles for all the other genes that are associated with the trait. Selecting an animal that carries favorable alleles of a marker, which is the allele that is associated with a positive impact on the trait of interest, can result in an improvement in the observed phenotype for that trait. Although complex traits are influenced by a number of genes, the mode of inheritance of each genetic marker is simple. An animal gets one marker allele from its sire and one marker allele from its dam. The alleles of the marked genes, as well as the numerous other "unmarked" genes, and the production environment will determine an animal's phenotype (e.g., weaning weight, marbling, etc.). EPD estimate the breeding value of all the genes (both "marked" and "unmarked") that contribute toward a given trait; therefore, when EPD exist for a given trait, they should always be considered in selection decisions, even when marker data are available.

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

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

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

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

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

Recently genetic tests for DNA markers associated with simple traits such as coat color, simply inherited genetic defects, as well as complex product quality traits such as marbling and tenderness, have become commercially available. Genetic tests for simple traits that are controlled by one gene are able to accurately assess whether an animal is a "carrier" (i.e., heterozygous) or will "breed true" (homozygous) for the marker alleles that result in a certain phenotype (red versus black). That is because there is little or no environmental influence on simple traits like coat color, and usually a single gene is responsible for the phenotype. However, in the case of complex traits, each marker is only associated with one of the genes that contributes toward the phenotype.

Both "marked" and "unmarked" genes, in conjunction with the production setting, will determine whether an animal marbles or has tender meat. It may be hard to understand why a well-proven bull with a high EPD for a certain trait can be found to carry no copies of a marker allele that has been positively associated with that trait. This can occur if the bull inherited a higher than average proportion of "unmarked" alleles that favorably affect the trait.

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

If all of the animals in a given breed carry two copies, or no copies, of a marker allele, then no genetic progress can be achieved by using marker-assisted selection for that marker as it accounts for none of the genetic variability

seen for the trait in that herd. In the case of a herd carrying no copies of a given marker allele, bringing in an outside bull carrying two copies of the marker would be a way to rapidly introduce a desirable marker allele into the herd. Phenotypic progress will be evident in the first generation if the marker is dominant or codominant.

If the trait is recessive, such that both alleles have to be present to see an effect, a second generation of crossing a homozygous bull with females carrying one copy of the favorable allele will be required to see a phenotypic response in the proportion (i.e., one in two, or 50%) of resultant offspring that are homozygous for the marker-allele. The frequency of marker alleles in a herd can be approximated by the gene frequencies of marker alleles in different breeds, although they may not accurately reflect the localized frequencies found in a specific herd.

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

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

Questions for Evaluating Marker Tests

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

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

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

- 1. Will marker-assisted selection make you money?** For marker-assisted selection to be profitable, the increased economic returns from greater genetic gain as a result of using the markers must outweigh the cost of genotyping. Producers need to consider how they are being financially compensated for DNA testing.
- 2. What impact does increasing the frequency of the marker allele have on the trait of interest in your herd?** The genetic gain that can be achieved by using marker-assisted selection depends on the amount of additive genetic variation that is accounted for by the marker, and marker data should be accordingly weighted. If the marker accounts for only a small proportion of the additive genetic variability for a trait, then little genetic improvement will be made by exclusively focusing on increasing the frequency of the marker. Likewise, if all of the animals in a given breed are homozygous (carry two copies of a given marker), then no genetic progress can be achieved by using marker-assisted selection, as the marker accounts for none of the genetic variability seen for the trait in that breed.
- 3. Is it a single gene test, or are there results from more than one gene?** The results from DNA-based marker tests can be reported in many ways. Single gene tests may be reported as "*", meaning that the animal is homozygous for the preferred allele of that gene. They may also be reported as the actual SNP analyzed in the test, e.g., "TT". It is then important to know which form of the marker (i.e., what nucleotide) has been associated with a positive effect on the trait of interest (see next section). Some of the tests are reporting on analyses that

have been done at two different locations in the genome. For example, *Tender-GENE* reports on the results from two different SNPs located in one gene, while *GeneStar Tenderness 2* reports the results of SNPs in two different, independent genes. The results are presented as multiple stars, where each star represents one favorable allele. Ideally, tests that include multiple genes or SNP locations will quantify the relative effect of each loci on the trait of interest. Results should distinguish between a two-star animal that is homozygous at one gene and carries no copies of the desirable allele (i.e., the star allele) at the other gene, and a two-star animal that is heterozygous at both genes. Irrespective of how many markers become available for each trait, it is important to remember that every individual receives one marker allele from each parent, and therefore it is not possible for an animal to ever have more than two favorable alleles for any given marker locus.

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

Once a decision has been made to use marker-assisted selection, the actual application of the technology is fairly straightforward. DNA samples should be collected from all animals to be tested. Common collection methods include a drop of blood blotted on paper (make sure to let the sample dry well before storing), ear tag systems that deposit a tissue sample in an enclosed container with bar code identification, semen, or hair samples (including the DNA-rich follicle or root). To increase the frequency of a marker that is positively associated with the trait of interest, select for animals that are carrying one or two copies of the marker and against those carrying no copies of the marker. All of the offspring from a parent carrying two copies of the marker (homozygous) will inherit a copy of the marker from that parent. In a typical herd, selection for homozygous sires will probably be the most rapid way to increase the frequency of the marker, although this may severely limit your choice of sires and hinder progress in other traits. Marker-assisted pre-selection of young sires with equivalent EPD is an excellent way to rapidly increase

Example:

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

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

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

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

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

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

the proportion of animals carrying a specific genetic marker and increase the frequency of that marker allele in the population.

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

Web Resources on Animal Biotechnology

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