Theory and application of genome-based approaches to improve the quality and value of beef[1]

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Abstract: *The science of genome research in livestock has been the focus of substantial worldwide efforts over the last decade, resulting in the development of a genetic map for cattle and its use to identify chromosomal segments carrying genes affecting production traits. Variations in individual genes having a major impact on phenotypes in cattle, such as alleles causing double-muscling and black/red coat colour, have been identified in the process, but the majority of the loci remain unknown except for their approximate position in the cattle genome and the relative impact of variation on the trait. Approaches to fulfil the promise of genome research, resulting in DNA-based tests of genetic merit for important production traits, have been slow to develop. In this article, the authors review the theory of genome-based approaches and potential avenues of application. Application to meat quality and value is specifically discussed, but the general principles are similar for production traits such as reproduction and animal health. A substantial amount of the discussion centres on describing practical limitations on the use of genomic data and the currently available avenues for application, aspects that have previously received inadequate attention.*

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There has been significant interest in genetic selection to improve beef quality and carcass composition. However, there has been little or no genetic selection for many meat quality traits due to the expense and length of time required for progeny testing. The lengthy time interval required for selection, breeding, calving, slaughter and evaluation of the trait of interest makes it extremely difficult to conduct progeny testing for beef palatability. In many cases, by the time the effect of a given sire on beef tenderness could be known, that sire has expired or has become genetically obsolete with regard to other economically important traits.

Clearly, tools are needed to facilitate genetic improvement of meat quality and carcass composition. The scientists at the US Meat Animal Research Center, as well as others, are engaged in the development of tools that should facilitate genetic improvement of meat quality, carcass composition and production efficiency. There are several ways in which one can implement genomic information including knock-outs, transgenics, marker-assisted selection, functional genomics, etc. Our goals are to develop tools that can be used by the meat animal-producing industries to identify animals that excel in efficient production of high quality red meat. We do not

believe, and hence it is not the focus of our group, that the use of transgenic or knock-out approaches to alter the genetic make-up of meat-producing animals will be acceptable to most consumers. Furthermore, we contend that there is sufficient, naturally occurring genetic variation among modern day cattle to make possible adjustments in virtually all important production traits to meet a variety of production objectives. Therefore, our goals are to develop marker-assisted selection and functional genomics tools. The objectives of this manuscript are to: (1) describe the economically important beef traits and how they can be measured; (2) describe how marker-assisted selection and functional genomics can be used to breed animals that excel in expression of economically important traits; (3) describe how industry can implement these methods; and (4) determine realistic expectations from this area of science for the beef industry. The use of molecular markers offers great potential to improve the efficiency of animal breeding, but it is dependent on appropriate methods to discover naturally occurring variation and systems to apply the technology in an industry setting.

We will first present the process of genome research in livestock, starting with the definition and collection of relevant phenotypes, proceeding with the structure of resource populations and detection of loci affecting these phenotypes, and concluding with the development of genetic markers for tracking functional alleles. We will then propose strategies for implementation of genomic research in the beef cattle industry. Finally, we will discuss the promise, limitations and obstacles to application of the results in actual selection of livestock.

Economically important phenotypes and their measurement

A critical step in genome research is the definition of relevant phenotypes for analysis. For an extreme example, one might use a simple model in which the phenotype measured is profit per animal. Using this idea, any genetic variation, acting on average daily gain, carcass merit or a number of other variables, might be detected by using genetic markers in a herd for which the only phenotype collected is return on investment. This example serves to illustrate the need for careful definition of phenotype, since the impact of non-genetic factors on profit is obvious, ie the variation in the price of cattle is likely to be larger than the contribution of individual animal characteristics in determination of profit. To use a more realistic example, the amount of fat on the animal is an important characteristic and impacts on efficiency of production, but in this case the method of measurement can have a significant impact on the results. Is it more appropriate to use trimmed carcass weight *versus* live weight, or fat depth at some point along the carcass, or is a complete description of fat content at each depot required? A common approach is to use fat depth, but accurate definition of phenotype requires a standardized system for data collection, since depth can vary from point to point along the same carcass. Like carcass fat, most traits have many known components. For instance, it is known that tenderness is affected by *post mortem* proteolysis, sarcomere length and connective tissue. Clearly, correct identification of genetic variation requires the development of techniques that accurately measure important animal characteristics, are highly reproducible, inexpensive, and sufficient to describe the phenotype. In the following sections we describe a set of phenotypes and their measurement, which we have applied in the search for quantitative trait loci (QTL) affecting carcass traits.

Tenderness

It is well established that meat tenderness is the most important trait in determining consumer satisfaction when eating beef. Flavour and juiciness also have a significant effect, but there is twice as much inherent variability in tenderness as in flavour or juiciness in US production systems. Furthermore, flavour and juiciness are influenced more by consumer preparation than by inherent variability between carcasses. Thus, there is much more opportunity to improve tenderness through genetic selection. The first step in a genomic approach is to identify an appropriate specific measure and system for collecting phenotypic data, in order to identify loci contributing to variation in meat tenderness. As we have seen, tenderness can be affected by a variety of factors, thus definition of the appropriate measure is not necessarily straightforward. It is possible for animals to possess genetics with variable effect on each of the components of tenderness, so that a particular carcass could theoretically produce tender meat as a result of an ideal genotype for sarcomere length in spite of an undesirable genotype for *post mortem* proteolysis. Thus, it is important to consider the possibility that a direct measure of tenderness may not be the best way to identify QTL affecting tenderness. However, a direct measure of tenderness should encompass the contributions of sarcomere length, *post mortem* proteolysis and connective tissue. In addition, it has been shown that most of the variation in tenderness of beef longissimus steaks is attributable to variation in the extent of *post mortem* proteolysis. Given that techniques to measure tenderness are much more repeatable and amenable to phenotyping of large numbers of animals than techniques to measure the biochemical components of tenderness, we have concluded that the best method of detecting QTL affecting tenderness is by making a direct measure of tenderness.

The 'gold standard' measurement for tenderness is the trained sensory panel tenderness rating. However, sensory panels are time-consuming and expensive to perform and are not amenable to the large numbers of observations that are required in experiments involving genomics. An alternative approach is to use an objective measure of the force necessary to cut through meat, termed 'shear force'. Numerous variations of shear force measurement have been used as an objective instrumental index of meat tenderness. Although some believe that shear force determination is a simple laboratory method and that anyone can collect such data, shear force, like any other laboratory method, requires attention to detail in order to obtain an accurate measurement. To be most useful, shear force should be measured at one or more *post mortem* intervals that correspond to the time *post mortem* when the meat will be consumed.

To date, Warner-Bratzler shear force (WBSF) remains the most widely used instrumental measure of meat tenderness. The origins of WBSF and the effects of numerous factors that influence WBSF values have been summarized by Wheeler *et al* (1997a). There are standardized procedures that should be followed to obtain accurate data, and shear force measurements that deviate from those that define WBSF should not be called WBSF. The factors that are most important for obtaining repeatable, accurate data include: standardizing the protocol; using consistent thawing procedures; using a cooking method that provides consistent results; using care in obtaining consistent diameter cores; coring parallel to the muscle fibre orientation so that shearing is perpendicular to the muscle fibres; and shearing at a standard crosshead speed (Wheeler *et al*, 1994, 1996a, 1997a and b, 1998).

We have recently developed 'slice shear force', which is a modification of Warner-Bratzler shear force (Shackelford *et al*, 1999a and b). Slice shear force is a simple, rapid, accurate technique, with the advantages that it is technically less difficult, more rapid, accurate and repeatable than WBSF. Slice shear force requires the removal of a 1 cm thick × 5 cm long slice parallel to the muscle fibres from the lateral end of the cooked longissimus from near the 12th rib region instead of the six 1.27 cm diameter round cores required for WBSF. It is easier to train technicians to obtain 'good' slices than it is to obtain 'good' cores, thus, slice shear force is less susceptible to operator error. In addition, the National Cattlemen's Beef Association Committee on Instrument Grading for Tenderness recommended that the industry adopt slice shear force as the standard measurement for meat tenderness.

Marbling

The importance of USDA quality grade in valuing and marketing beef is well established, not only in the USA, but also in the world market. Quality grade is determined by two factors estimated by trained USDA graders (USDA, 1997). The first factor is the age or maturity of the animal the carcass came from, which is primarily estimated by the degree of ossification of cartilage into bone and by lean colour in the longissimus at the 12th rib. The second factor is the degree of marbling. Marbling score is determined by the amount and distribution of intramuscular fat pieces in the longissimus at the 12th rib of the chilled carcass. The marbling score for a carcass is the highest marbling score from either of the two sides. In addition, or alternatively, chemical (ether-extractable) fat concentration of the longissimus (100 g in duplicate, raw and/or cooked) can be used as a measure of marbling. Whether or not this grading system accurately predicts consumer eating satisfaction, this phenotype represents an important production trait under current market conditions.

Carcass composition

The most precise and arguably the most repeatable method of assessing beef carcass composition is chemical analysis. However, assessment of chemical composition is very expensive because it requires that the cuts of beef, which could be sold to offset production costs or sampled for other experimental purposes (eg shear force analysis), must be ground and sampled. The next most precise method would be to determine yields of fully trimmed retail cuts and lean trim. Given the numbers of observations needed to detect QTL of moderate effect (hundreds or thousands), collection of whole carcass cutting yields is a practical impossibility. Thus, we (Shackelford *et al*, 1995) developed a procedure for estimation of carcass composition traits based on dissection of the wholesale rib. This technique allows accurate prediction of completely trimmed retail product yield, fat yield, bone yield, retail product weight, fat weight and bone weight. Also, this procedure leaves the boneless rib-eye roll containing longissimus muscle (5th through 12th ribs) intact. Therefore, the longissimus can be sampled for shear force or other analyses.

Additional considerations in phenotype collection

Definition of relevant phenotype and collection of high quality phenotypic data are not the only important aspects for a cattle genomics effort. It is imperative to have a system of checks and balances to ensure that the carcass or cut of meat on which the phenotype is determined is properly identified and, thus, matched to the biological sample (eg blood) that was the source of DNA for genotyping. For example, although we have elaborate procedures to ensure that identity is properly transferred from the live animal to the carcass, under commercial slaughter conditions (eg 380 to 420 head per hour), errors in transfer of identity could occur. Therefore, we routinely compare carcass weight, which is recorded at the packing plant, with live weight, which is recorded at the research centre's feedlot. Drastic deviation of the carcass weight from what is expected based on the animal's live weight would indicate that the carcass was misidentified. When it is suspected that errors in identity have occurred, we collect biological material from the carcasses and compare genotypes of the carcasses and the animals (ie blood) to determine the proper identity of the carcasses.

Genetic contribution to variation in phenotypes

Numerous estimates of the heritability for phenotypes of interest have been published (Gregory *et al*, 1994; Kim *et al*, 1998; Koch *et al*, 1982; O'Connor *et al*, 1997; Wheeler *et al*, 1996b, 2001). Many of these estimates are based on data sets with relatively small numbers of progeny per sire, which increases the standard error. The ranges in heritability estimates for various traits are 0.12 to 0.53 (shear force), 0.22 to 0.50 (sensory tenderness rating), 0.35 to 0.78 (marbling score), 0.25 to 0.84 (12th rib fat thickness), 0.37 to 0.69 (12th rib longissimus area) and 0.41 to 0.71 (percentage retail product). Nevertheless, these estimates provide a starting point for examining the anticipated impact of genetic improvement.

Many have suggested that controlling the genetics of the slaughter cattle population would more or less solve the beef industry's tenderness problem. Genetics makes a significant contribution to the total variation in tenderness, as tenderness varies among and within breeds. However, analyses indicate that genetic and

environmental factors make about equal contributions to variation in tenderness. The heritability (within-breed additive gene effects) of tenderness is estimated to be 30%. Therefore, within a breed, 70% of the variation is explained by environmental and non-additive gene effects. Additional variation is due to between-group management factors (eg preharvest handling, electrical stimulation, chilling rate, etc). Between-breed variation in tenderness is about equal to or less than variation within breeds. Therefore, among cattle of all breeds, approximately 46% of the variation in tenderness is genetic and 54% is environmental. Thus, significant improvement in tenderness can be made by controlling those factors responsible for the environmental effects as well as through selection of breed and genetic selection within a breed.

The rate of genetic improvement is a function of the heritability of the trait, the generation interval and the selection differential. In traditional breeding programmes, selection for traits that can only be measured *post mortem* can be accomplished only through progeny testing. However, progeny testing is time-consuming and increases generation interval. For example, improvement of meat tenderness relying entirely on progeny testing would be a slow process. Data collected at the Meat Animal Research Center (MARC) indicate that extreme culling would have to be imposed to eliminate all tenderness problems through genetics. If we make the following assumptions: use 13 sires, hold inbreeding to less than 1%, 100 head cow herd size, heritability estimates of 0.30 for shear force and 0.42 for marbling, the genetic correlation of 0.25 between shear force and marbling (Koch *et al*, 1982), standard deviation of 1.0 kg for shear force, then it would take 12.0 years and 40.7 years to improve shear force by 1.0 kg by selection for shear force or marbling respectively. If we increase the size of cow herd to 500, the above estimates will be 6.8 and 23.1 years respectively. Obviously, a significant change in the above parameters will affect these estimates. Furthermore, MARC data indicate that the maximum selection differential that could be imposed for tenderness is relatively small. In fact, the distributions of shear force values overlap for the progeny of the toughest and most tender 10% of sires. Moreover, if we culled the toughest 10% of sires we would only decrease the frequency of shear values above 4 kg from 20% to 16%. Thus, extreme culling would have to be imposed to eliminate all tenderness problems through genetics.

One of the reasons it has been difficult to take advantage of genetic sources of variation to improve meat and carcass traits is the genetic antagonisms among these traits. It may be possible to overcome this problem with a genomic approach to genetic selection that allows greater selection pressure on genes that have less antagonistic effects on multiple traits.

Genomics approaches

Detection of quantitative trait loci

Economically important traits are regulated by the combination of environmental variables and genetics. The location of a gene on a chromosome is referred to as a locus, and when a trait is influenced by variation in several genes the contributing loci are termed quantitative trait loci (QTL). It is important to establish the magnitude of the effect that variation at each QTL has on expression of the trait, as selection would have the greatest impact using those regions with greatest effect on the trait of interest. Once these loci are identified and their effects quantified, they can be used in selection schemes.

The basic principle underlying published beef cattle QTL experiments is the production of relatively large, half-sib families, starting with crossbred sires. Typically, 200 to 1,000 offspring of a single sire or small group of sires are used. The number of animals required for accurate mapping of QTL is a function of the magnitude of the effect of substituting the two sire alleles; the smaller the effect, the more animals are required. The number is also affected by the extent to which environmental variables affect the trait; the larger the variation due to the environment, the more animals are required to average out the impact of this factor. The statistical analysis generally examines the contrast between the two alleles of the sire along each chromosome, which is why crossbred sires are employed as they increase the probability of allelic contrast. If pure-bred sires were used in these designs, it would decrease the likelihood that at a given locus the two alleles would have distinguishable effects on phenotype. The guiding concept is that these experimental populations increase the efficiency of detecting genetic variation impacting on production traits; however, one consequence is that identification of QTL for carcass traits is being done in animals with limited genetic background.

Four resource families were developed for the identification of QTL for carcass composition and meat quality traits at the US Meat Animal Research Center. Two half-sib families were developed from a Brahman × Hereford (BH) or a Brahman × Angus (BA) sire (Keele *et al*, 1999; Stone *et al*, 1999). Both sires produced over 500 offspring. Two additional half-sib families were developed from a Piedmontese × Angus (PA) or a Belgian Blue × MARC III (BM) sire (MARC III is a 25% Angus, 25% Hereford, 25% Red Poll, 25% Pinzgauer composite). These families produced 246 and 209 offspring respectively (Casas *et al*, 1998). Although detection of QTL is an ongoing effort at MARC, Table 1 shows the QTL detected to date for carcass composition and meat quality traits, including chromosome number, relative position within each chromosome (according to Kappes *et al*, 1997), LOD drop-off support interval (Ott, 1992), trait of interest, and the family in which the QTL was detected. Results presented are for QTL detected at least at the genome-wide suggestive level (Lander and Kruglyak, 1995), that is, one false positive per scan would be expected (nominal P = 0.002).

Families BM and PA were generated primarily to refine the location of the gene responsible for the double-muscling phenotype (this gene is now known to be myostatin) that is in high frequency in the Piedmontese and Belgian Blue breeds. Double-muscling in cattle was studied to establish its effect on growth, carcass composition and meat quality traits in different genetic backgrounds, to compare the effects of the syndrome inherited from Belgian Blue or Piedmontese, and to

Table 1. Quantitative trait loci detected in the four half-sib resource families.

Chromosome	Relative position cM)[a]	Support interval (cM)[a,b] *Lower*	*Upper*	Trait[c]	Family[d]	Similar[e]
1	50	38	74	FATYD	BA	
1	53	37	72	RPYD	BA	
1	63	41	77	YG	BA	
2	4	2	6	RPYD	BM, PA	1
2	4	2	6	LMA	BM, PA	1
2	4	2	6	YG	BM, PA	1
2	4	2	6	MAR	BM, PA	1
2	4	2	6	FAT	BM, PA	1
2	4	2	6	KPH	BM, PA	1
2	52	38	79	YG	BH	
2	54	21	60	FAT	BH	
2	54	45	70	MAR	BA	
3	28	0	42	MAR	BH	2
3	36	23	46	FAT	BH	
3	56	9	74	MAR	BA	2
3	65	47	85	MAR	BM	2
3	68	64	85	RPYD	BM	3
3	70	55	83	RPYD	BA	3
3	77	69	85	KPH	BA	
4	19	4	34	WBS3	BM	
4	33	24	41	HCW	BM	
5	53	38	66	LMA	BH	
5	62	41	78	FAT	PA	
5	64	53	71	FATYD	BH	
5	67	37	91	WBS14	PA	
5	68	36	112	RPYD	PA	
5	72	54	102	YG	PA	
5	75	62	80	MAR	BH	
6	9	0	26	LMA	BH	
6	52	45	67	LMA	BM	
6	52	44	76	HCW	BM	
7	55	44	71	FAT	BH	
8	9	0	26	MAR	BM	
8	23	0	36	FAT	BM	4
8	30	17	43	FAT	PA	4
9	26	19	34	WBS14	BM	
9	67	63	92	RPYD	BH	
9	71	46	76	MAR	BH	
10	4	0	28	MAR	BH	
10	24	0	30	HCW	BH	
10	59	47	76	MAR	BM	
11	66	27	80	YG	BH	
13	60	43	64	RPYD	BH	
14	14	10	25	FAT	PA	5
14	16	0	22	FAT	BH	5
14	19	0	24	YG	BH	
14	47	30	87	MAR	BH	
15	28	23	32	WBS14	BH	
16	44	25	55	MAR	BA	
16	45	21	69	KPH	BH	6
16	49	32	57	HCW	BA	
16	62	39	73	KPH	BA	6
17	21	0	68	MAR	BM	
17	35	0	63	FATYD	BA	
18	23	11	38	HCW	BH	
18	85	79	85	RPYD	BH	
19	5	0	15	RPYD	BH	
19	18	0	37	YG	BH	
20	66	55	75	WBS3	BH	
20	72	52	75	WBS14	BH	
23	30	21	42	MAR	BH	
26	26	15	41	RPYD	BA	
26	26	16	38	FATYD	BA	
26	26	21	36	YG	BA	
27	29	12	51	MAR	BH	
27	60	49	64	MAR	BM	
29	49	40	62	RPYD	BH	

Table 1 continued

Chromosome	Relative position cM)[a]	Support interval (cM)[a,b] *Lower*	*Upper*	Trait[c]	Family[d]	Similar[e]
29	54	45	58	HCW	BH	
29	54	30	65	WBS14	BH	7
29	54	40	64	WBS3	PA	7
29	54	50	64	WBS14	PA	7

[a] cM = relative position in centimorgans from the beginning of the linkage map (Kappes *et al*, 1997).
[b] 1-LOD drop-off support interval (Ott, 1992).
[c] HCW = hot carcass weight, MAR = marbling, LMA = longissimus area, YG = USDA yield grade, KPH = estimated kidney, heart and pelvic fat, FAT = fat depth, FATYD = fat yield, RPYD = retail product yield, WBS3 = meat tenderness measured as Warner-Bratzler shear force at day 3 *post mortem*, and WBS14 = meat tenderness measured as Warner-Bratzler shear force at day 14 *post mortem*.
[d] BM = sired by a Belgian Blue × MARC III bull; PA = sired by a Piedmontese × Angus bull; BH = sired by a Brahman × Hereford bull; BA = sired by a Brahman × Angus bull.
[e] Similar numbers within column are potentially the same quantitative trait loci detected in at least two families.

determine if the same locus was responsible for the phenotype in both breeds. The locus causing this condition was mapped to the centromeric end of chromosome 2 (Casas *et al*, 1998) in both PA and BM populations, indicating that the same locus was involved in both breeds. Subsequently, it was determined that the syndrome was caused by mutations in the myostatin gene. A specific point mutation in myostatin was identified in the PA population and a small deletion in the BM animals, both of which were predicted to interfere with function of the gene (Smith *et al*, 1997; Kambadur *et al*, 1997). Loss of myostatin function causes dramatic effects on many traits in these populations, which are similar in nature for both Piedmontese and Belgian Blue alleles. QTL analysis of the rest of the genome in these populations established epistatic effects with other chromosomal regions for several traits (Casas *et al*, 2000; Casas *et al*, 2001), demonstrating that the status of myostatin expression could affect the influence of variation in other loci.

The BH and BA families were developed primarily to detect QTL for tenderness, with secondary objectives of detection of QTL for carcass composition and other meat quality traits. QTL on chromosome 2 were identified for carcass traits in both BH and BA families, which do not segregate double-muscle alleles of myostatin, suggesting the possibility of other less drastic alleles of this gene. However, these QTL fell in a region of chromosome 2 significantly distal from the centromeric location of myostatin, such that we could rule out the possibility that they reflected alternative alleles of this major gene. In both BH and BA families, QTL for fat deposition traits were detected in a similar location on chromosome 2 (Table 1). It is important that the QTL were detected in both families, because identification of QTL with a similar impact on a given trait, at the same chromosomal position in multiple families, is important supporting evidence that the QTL is valid and the variation reasonably common. However, it was not possible to determine whether similar variation existed in the BM and PA families, because the magnitude of the myostatin effects interfered with the ability to detect smaller effects on the same chromosome in these populations.

A QTL for marbling was detected on chromosome 3 in families BH, BA and BM, supporting the idea that variation in a gene on this chromosome affects fat deposition in various cattle breeds. Similarly, a QTL for retail product yield was detected on this chromosome in both the BA and BM families. In the BA and BM families the QTL for marbling and retail product yield reside in a similar chromosomal region, but the QTL for marbling in the BH family was centromeric. However, the support intervals for marbling in all three families overlapped, suggesting they could all reflect allelic variation at the same QTL. Overlap of QTL influencing expression of marbling and retail product yield in the BA and BM families indicates that these traits may be affected by alleles of the same gene, or group of genes.

Our data indicate that chromosome 5 contains loci important for expression of a number of relevant production traits. In families BH and PA, QTL were detected for longissimus area, marbling, fat depth, retail product yield, USDA yield grade and fat yield. In addition, chromosome 5 harbours one of the loci that interact with myostatin status in the PA family, specifically a QTL for Warner-Bratzler shear force measured at 14 d *post mortem*. These chromosome 5 QTL reside in a region neighbouring the location of the insulin-like growth factor I gene (*IGF1*). At present it is not possible to ascertain whether the observed variation is due to the pleiotropic effect of one gene on multiple traits, or different genes closely linked with independent effect on all traits, because the confidence intervals for the traits substantially overlap. However, the fact that the peaks of probability for each trait were not at identical positions along the chromosome tends to support the latter.

The presence of a QTL for longissimus muscle cross-sectional area was observed in families BH and BM on chromosome 6. The one on the BH family was in the centromeric region of the chromosome, while the one in the BM family was distal. The support intervals do not significantly overlap, suggesting that two distinct QTL for the same trait lie on this chromosome and are segregating in these populations.

An important feature of QTL expression is the possibility for variation at two or more loci to interact in

determining phenotype. That is, the effect of an allele at a QTL may depend on the particular allele present at one or more other loci. An example of this, in an obvious phenotype, is the interaction of alleles at the msh receptor and agouti loci, where coat colour in cattle is primarily determined by the allele of the msh receptor but can be 'overridden' by certain alleles at the agouti locus (reviewed in Voisey and van Daal, 2002). Similar epistatic interactions between loci have been postulated to exist for QTL (Falconer, 1989), and current technology allows their detection. Microsatellite markers have been successfully used to detect regions interacting with myostatin on chromosome 4 for Warner-Bratzler shear force measured at 3 d *post mortem*, on chromosome 5 for Warner-Bratzler shear force measured at 14 d *post mortem*, on chromosome 8 for fat depth and on chromosome 14 for fat depth. Evidence suggests that, in the BM family, there is a direct effect of the chromosome 8 QTL on fat depth, whereas in the PA family there is evidence of an interaction with myostatin. Specifically, in the PA family the effects of the two sire alleles of this QTL depend on whether there are one or two functional copies of the myostatin gene present in the animal. It is not clear why the QTL did not interact with myostatin status in the BM family despite doing so in the PA family. It is possible that functionally different alleles on the chromosome 8 locus could be involved, although it is also theoretically possible that the result represents the first apparent distinction between the Piedmontese point mutation of myostatin and the Belgian Blue deletion mutation. In any event, the discovery of QTL interacting with myostatin represents the first report of such interactions in livestock.

Two QTL for marbling were detected on chromosome 10 in independent families. A QTL for marbling was identified in the centromeric region of chromosome 10 in the BH family. A QTL for the same trait was detected in a distal region in the BM family. Given that the QTL are well separated, it appears unlikely that the same gene, or group of genes, is involved in the expression of the same trait in families with two different genetic backgrounds. This situation is similar to the one observed for longissimus area on chromosome 6.

A QTL for fat depth was detected in two families on chromosome 14. A direct effect was observed at the centromeric region of chromosome 14 in the BH family. In the PA family, the same chromosomal region influenced fat depth; however, the region was interacting with myostatin. An interaction was not expected in the BH family because that family was not segregating a loss-of-function mutation in myostatin.

Additional QTL were identified on three other bovine chromosomes. A QTL for estimated kidney, pelvic and heart fat, an important carcass composition trait in cattle, was detected in families BH and BA on chromosome 16. Support intervals overlap, allowing the possibility that they are the same QTL, but further studies are required to establish whether this is a single QTL observed in independent families. There was evidence supporting the presence of two QTL for marbling on chromosome 27. A centromeric QTL was identified in the BH family, and a distal QTL was observed in the BM family. The position of the support intervals suggests the presence of independent QTL, although further studies are required to establish this conclusion. A suggestive QTL for Warner-Bratzler shear force was detected on the telomeric end of chromosome 29 in the PA and BH families, and has been the focus of further study to identify genetic markers as outlined in the following section.

Identification of genetic markers for QTL

Traits of economic importance are regulated by interaction of the animal's genetics and the environment in which it is raised (feed availability or quality, weather, handling), as mentioned above. Variation in the DNA sequence of genes that influence biochemical or developmental pathways relevant to a trait are a principal cause of differences among animals raised in a particular environment, and are responsible for many of the phenotypic differences between breeds. This is because the variation can effect the expression, activity or localization of the protein produced by the gene. The goal of the bovine genomics programme at MARC is to locate and identify DNA sequence variation *predictive* of genetic merit for specific traits.

The first step of the gene identification process is the determination of chromosomal regions that harbour variation affecting traits, as described in the preceding section. Subsequently, the task is to identify the most appropriate genetic markers enabling the functional variation to be monitored in commercial stock. This involves the creation of a dense set of markers targeted to the region containing the QTL, which in turn depends on the cattle genetic map. Although it may be possible to use the same markers that were used to identify the QTL to monitor genotype, the narrower the interval between markers and the closer the markers are to the causative DNA sequence variation, the more efficient and robust the application of the technology. The ultimate marker describes the actual causative variation, such as the point mutation in myostatin in Piedmontese animals, as this allows direct testing of individual animals without the need for pedigree or ancestral phenotypic data.

Functional genomics represents an alternative approach to the identification of genes potentially affecting production traits. This approach compares gene expression at the RNA or protein level in phenotypically distinct tissue samples to identify candidate genes that might affect production traits. For example, one might compare proteins present in samples of tough and tender meat, to identify particular genes whose level of expression can be correlated with this trait. While these approaches may effectively identify factors that affect tissue status, they do not necessarily reveal the underlying genetic variation leading to the phenotype, and thus are not directly applicable to the improvement of livestock through breeding. However, the results of such studies in conjunction with QTL studies could identify genes that are both functional and positional candidates if the map position of the affected genes is known or can be predicted.

The genetic map of cattle that we have used to identify QTL was primarily constructed with markers of a type called microsatellites (Kappes *et al*, 1997), which are highly effective in tracking inheritance and were used in the studies described in the preceding section. This type of marker was very useful for the detection and

localization of QTL to chromosomal segments, but provided little information about potential genes that might be responsible for the observed effects. However, the development of higher quality comparative maps, which align the cattle genetic map with those of other species (Band *et al*, 2000; Stone *et al*, 2002), and the concurrent completion of the human genome sequence, have recently allowed a much better picture of genes in relation to QTL to emerge. This is due to the fact that all mammals have essentially the same set of genes performing, in the majority of cases, identical functions. Furthermore, the DNA sequence of genes is relatively conserved between mammals, for example, humans and cattle, so that a direct comparison of a cattle gene sequence with the database of the human gene sequence will frequently identify the gene, its likely position in the genome, and some idea of its function. Moreover, due to the evolutionary history of mammals, their genes are generally grouped in the same way on their chromosomes, allowing us to use human map information to predict what genes will lie in the QTL interval. Our ongoing programme at MARC of collecting bovine cDNA sequence, in combination with the online resources available from the human effort and the developing human:bovine comparative map, now makes it possible to identify and characterize the genes that lie in QTL intervals much more efficiently than before.

There have been a number of successes reported in the identification of specific DNA sequence variation impacting on production traits in livestock. All of them relied on comparative analysis with other mammalian genomes, underscoring the importance of this approach. It is significant that all of them have involved variation that has a major effect on a trait or traits. This is because when the allele substitution effect for one version of a gene with a variant version of a DNA sequence is large, it is much easier to narrow down the genetic interval in which the locus resides. It also means that identification of the variation will probably be easier, because it involves a significant change in some aspect of the gene responsible. The example cited in the above section was the myostatin gene, for which mutations have been described that cause double-muscling in cattle. In Piedmontese cattle, a single base change in the DNA sequence of the gene is sufficient to inactivate the myostatin protein and cause the double-muscle syndrome (Kambadur *et al*, 1997). This mutation has been definitively shown to be the cause, as disruption of myostatin activity has a very similar effect on phenotype in mice, and it predicts an amino acid substitution for a residue known to be critical for protein activity (Lee and McPherron, 1999). It is therefore possible to construct a DNA test for double-muscling in Piedmontese cattle that will have 100% accuracy in predicting the presence of this allele, without any prior knowledge of the genotypes or phenotypes of related animals.

It is likely that most DNA sequence variations with a large effect could be identified using currently available tools, given a sufficient effort to investigate them in livestock. However, much variation of interest does not result from the action of single, large-effect sequence differences, since these are generally amenable to classical selection and do not require genomic technology. For example, the QTL affecting meat tenderness identified on chromosome 29 in the PA family (Table 1) has a moderate (0.4 standard deviations) but significant effect on the trait. One consequence of the moderate effect is that the precision of mapping the QTL is much lower, as evidenced by the large (24 cM) support interval, than the precision for major genes such as myostatin knock-out mutations (which may be narrowed to regions as small as 2 or 4 cM). Variation with moderate effect is also more difficult to ascribe to observed DNA sequence differences, as it is unlikely to have as obvious an alteration as the abrogation of activity seen for myostatin in double-muscling (and the effect is more likely to be hidden by non-genetic effects, so that the phenotypic effect may be highly variable in different experiments or populations). This complicates the task of creating genetic markers with predictive merit; however, these loci of moderate effect are precisely those for which genetic markers would be ideal to guide selection.

The chromosome 29 QTL has been the subject of substantial effort to generate genetic markers that might be used to improve consistency of meat tenderness, and serves to illustrate the process of generating useful genetic markers. Using the positional information (support interval between 40 and 64 cM), a process of comparative mapping to the human genome was used to identify several genes whose known function suggested they were candidates for variation causing the observed allele contrast. One of these candidate genes, having the abbreviated symbol *CAPN1*, encodes the large subunit protein of micromolar calcium-activated neutral protease (mu-calpain; Smith *et al*, 2000). The map position of *CAPN1* places it under the peak of highest probability in the support interval for the QTL. Furthermore, mu-calpain has been shown to be the primary enzyme responsible for *post mortem* proteolysis in beef (Koohmaraie, 1996), making *CAPN1* both a positional and functional candidate gene.

The moderate effect and consequent uncertainty of the WBS QTL required a modified approach to creating a DNA test, proceeding in several stages. First, the complete sequence of the *CAPN1* gene was determined for a single allele, providing the basis for searching for variation among animals (Smith *et al*, 2000). The gene is relatively large, covering more than 60,000 base pairs, with a coding region interrupted by 21 introns. The next stage was to determine the sequence of the entire coding portion of the gene in a set of animals representing 15 beef breeds popular in the USA, to search for variation in the sequence between animals and breeds (described in Page *et al*, 2002). This procedure identified 167 nucleotide differences in at least one member of the panel of 96 animals chosen for sequencing. Two of these differences predicted changes to the mu-calpain protein, including substitution of alanine for glycine at position 316, and isoleucine for valine at position 530. We focused on these two changes as possible markers for determining functional variation with respect to the WBS QTL. It should be noted that the original population used to discover the QTL was the PA half-sib family. The QTL is detected as a difference between the two alleles of the sire, in this case the Piedmontese allele *versus* the Angus allele. We therefore sequenced both alleles of the PA sire

to determine which of the 167 variable nucleotides were heterozygous, and identified 35 positions that included those predicting amino acid changes. Further work established that the Angus allele of the PA sire contained alanine and valine, the Piedmontese allele glycine and isoleucine.

Tests for these two variations were genotyped across the animals used in the QTL study, establishing that the Piedmontese allele was associated with the reduction in tenderness identified by the QTL analysis. Unfortunately, this type of association would be expected for any variation that exists in the QTL interval, and does not represent proof that these would be pertinent markers for use outside the experimental population. It is necessary to evaluate markers outside the original herd to validate the utility of DNA markers. In the specific case of *CAPN1*, a second experimental herd in New Zealand had also revealed a QTL for meat tenderness on chromosome 29, providing an independent test of the hypothesis. We genotyped the crossbred (Limousin × Jersey) sire for this population and determined it was also heterozygous for alanine/valine and glycine/isoleucine alleles, with the Limousin glycine/isoleucine allele being associated with reduced meat tenderness. This result provided critical support for the concept that the specific variation being tested was *predictive* of merit for tenderness, while providing only circumstantial evidence that the observed amino acid changes are the *cause* of the observed phenotypic variation. The situation is further obscured by the presence in cattle of alleles with alanine/isoleucine (a rare allele) and glycine/valine (a common allele), which were not present in the sires for testing in the QTL population.

Studies with the *CAPN1* markers in a more diverse set of germplasm are necessary to evaluate their association with meat tenderness and utility for selection purposes. Such studies are under way and preliminary results indicate that they can be successfully used to predict the presence of undesirable alleles with respect to meat tenderness (Page, unpublished data). The point to be made here is that identification of specific DNA sequence variation causing phenotypic variation is a complex and daunting task for QTL of moderate effect. It is likely that formal proof of causation for these particular amino acid substitutions will never be accomplished, even if they are in fact the causative variants. It is not obvious to what degree the activity of the enzyme would have to be altered, for example, to explain the observed variation in tenderness; nor do assays exist that are sensitive and specific enough to detect these putative changes. Nevertheless, the task of producing DNA markers such as those in *CAPN1* sufficient to be predictive of phenotype is becoming feasible, and should be sufficient to make the application of genomic research possible in livestock.

Implementation

A major hurdle for genome technology, which up to this point has not been sufficiently addressed, is implementation in an industry setting. One reason for this is that the optimal strategy for implementation depends on the state of knowledge about the trait and the QTL, as well as on the particular germplasm, production system, and the resources of the producer who is considering use of the technology. In this section we will consider production situations that could benefit from the results of genome technology, and propose methods for implementation. In the following section we will then consider some of the current constraints on pursuing these objectives.

Flanking markers v direct tests

The only information available for the great majority of QTL in beef cattle is the approximate chromosomal interval in which the locus lies (Table 1). The ability to apply this information in a target population is thus limited to use of previously identified genetic markers that bound the support interval. For example, if the desired result is to decrease the amount of carcass fat in the target population, one might use genetic markers flanking carcass fat QTL to make selection decisions in the population. However, the limited information available in this scenario means selection may be ineffective without significant investment from the producer. The first step must be to determine whether significant variation exists at the QTL in the population. It is possible that the animals to be used are fixed for one or another functional alleles, which will not be revealed by the markers unless they are run on pedigrees within the population. This effort will also determine the phase of the marker genotypes with respect to the desirable QTL alleles, but adds expense to implementation of marker-assisted selection. Furthermore, a segment of the population will experience recombination between the markers, which causes their genotype at the QTL to be unknown. Large production systems in which substantial numbers of phenotyped progeny per sire are available for examination are necessary to implement this strategy efficiently.

Genetic markers that lie very near to the gene affected by the causative DNA sequence variation represent a more efficient strategy for implementation. Recombination between the causative variation and the marker will be greatly reduced, so fewer animals will reside in the 'unknown' category. If the marker genotype is sufficiently predictive, then the genotype of individual animals will be adequate and extensive testing of pedigrees within the herd will not be required. Single nucleotide polymorphism (SNP) markers are particularly well suited for this type of application, as their alleles are extremely stable through many generations, and it is rare that the same SNP will occur independently in the cattle species. The examples of SNP-based tests cited above are the myostatin gene, in which the causative variation is known, and mu-calpain, in which the cause is less clear and we are relying on linkage disequilibrium with the causative variation. Moreover, identification of SNP with predictive power for a QTL shifts the burden of investment from the producer, who can be supplied with a genetic test with reasonably well defined success rates and characteristics, to the research laboratory, where the initial discovery and testing of markers will be carried out. It is likely that the most successful introduction of genetic marker technology in beef cattle production on a commercial scale will be SNP-based direct DNA tests for these reasons. However, the number of direct DNA tests available for production traits is much smaller than the

number of QTL identified for which only flanking microsatellite marker data are known, so that for most traits the only currently available approach is one using flanking markers. Our research programme in this area aims to provide additional SNP markers for some of these other QTL.

Approaches to utilizing results of genetic testing

If genetic testing technology is truly 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 genetic tests will be related to several traits. Furthermore, for the foreseeable future, genetic tests will only account for some of the genetics of any trait; breeders will still need expected progeny differences (EPDs). We envision a system in which phenotypes and genetic tests on the individual and its relatives are combined, through national cattle evaluation (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 genetic testing.

It may be tempting to try to convert genetic test results into adjustment factors that could be added to EPDs to adjust for the additional information. However, genetic test results affect low-accuracy EPDs much more than they do high-accuracy EPDs. Furthermore, genetic test results affect the evaluation of progeny of heterozygous parents more than they do the progeny of homozygous parents. Therefore, any 'adjustment factors' that might be developed for genetic tests would not be generally applicable. Results of genetic tests should be included in the same analysis as the phenotypes through NCE.

If genetic tests are to be included in NCE, then some criteria must be established to determine which genetic tests are to be included in the evaluations. A number of factors should be considered, including the effect of each test genotype on each trait of interest and the frequencies of the test genotypes in the breeds of interest. It is important that the tests be evaluated for as many of the economically relevant traits as is practical. Few genes influence only one trait, just as few traits are determined by only one gene. It is unfortunate that there is a tendency to label genetic tests with one specific trait.

An independent institution using standard resource populations with phenotypes for the desired traits in cooperation with the testing company could provide the information required to decide whether or not to use a genetic test. The National Beef Cattle Evaluation Consortium (NBCEC) is currently developing a system for independent evaluation of DNA tests. Under this approach, the NBCEC provides DNA to the genetic testing company, which runs the test on the DNA and sends the test results back to the NBCEC. The NBCEC then analyses the data and reports the results publicly in a standardized format.

Independent evaluation of commercialized genetic tests should provide breeders with the information they need in order to decide which DNA tests to use in their breeding programmes, as well as providing information that breed associations could use to decide which tests to include in NCE. Furthermore, it should enable responsible genetic testing companies to market tests more effectively and with greater confidence. The process will also generate information (such as the effect of the test) that is needed in order for genetic testing data to be included in NCE.

Selective reporting of genetic testing data is likely to have a much greater effect on NCE than selective reporting of phenotypes. It could cause serious bias in allele frequency estimates. If a breeder reported only favourable genetic test results, then the untested animals in that herd would be predicted to have a much higher frequency of favourable genotypes than the actual frequency, providing a strong incentive to breeders to report only favourable results. This could cause serious overestimation of their QTL effect. Therefore, it would help greatly if the breed associations required that data submitted to NCE be uncensored. However, this would require the cooperation of the genetic testing companies and it might decrease the submission of test data to NCE. It would be useful to have a statistical method of correcting for selective reporting, but such a method is not obvious.

Constraints

Genetic testing is most appealing for traits that are expensive or difficult to measure. Breeders wish to use DNA testing as an alternative to the collection of phenotypes, but some phenotypes will always be needed to keep breeding programmes on target. Although DNA testing could substantially increase the amount of information provided by each phenotype, there are constraints associated with this technology. The most serious current constraint is the lack of informative DNA tests, which there is a substantial worldwide effort to overcome. However, as more tests become available, it is important to consider other constraints inherent in the development of genetic markers.

Constraints on interpretation of research results

Costs associated with the production of animals and collection of phenotypic data commonly result in the use of experimental populations with limited numbers of animals having both phenotype and genotype information. A consequence of this is increased uncertainty regarding the association of marker genotype and status relative to the causative variation, and relatively little knowledge concerning the general applicability of the marker(s) among cattle breeds. Typically, a reasonably planned and executed marker study develops markers that could turn out to be specific to particular crossbreed situations. This is a serious constraint to both the development of DNA tests and their inclusion in NCE. We are attempting to address this at MARC by sampling substantial numbers of progeny of highly influential industry sires and collecting DNA and detailed phenotypic data in offspring of these sires on as many economically relevant traits as possible. This will permit the testing of markers for generality and applicability in industry germplasm.

QTL of very large effect are the easiest to identify, but may disrupt normal physiology too much to be useful.

For example, the inactive form of myostatin has very desirable effects on meat composition and tenderness, but animals that are homozygous for this allele are not suitable for the extensive beef production environments that are prevalent in much of the world.

Another constraint in interpretation of research results is the presence of undetected functional alleles. The proposed myostatin test serves to illustrate this point. The test described may be 100% accurate at measuring the presence of the Piedmontese mutation causing double-muscling, but cannot accurately predict the presence or absence of the double-muscling-causing mutation in samples of unknown or uncertain breed background. This is because there are at least four other mutations present in modern cattle breeds that inactivate the myostatin protein, which would not be identified by the proposed test. In the Belgian Blue breed, for example, an 11-base pair deletion at a different point in the gene is the causative mutation. Presumably the occurrence of multiple 'phenocopy' alleles occurred as a result of widespread selective pressure for increased muscling or decreased fat, which would efficiently capture myostatin-inactivating variations due to the significant impact on phenotype in the heterozygous state. The important point is that similar multiple variants are possible for other genes, and more likely to occur in genes affecting production traits, given that similar selective pressures have been applied over long periods of time by producers with genetically distinct and isolated animal populations. It is likely to be common that a newly developed genetic test will not identify all possible functional alleles of the QTL locus due to the limited genetic background in the research population. Identification of these functional alleles requires that phenotypes continue to be collected and associated with genetic test results. Undetected alleles may result in decreased accuracy of the tests, depending on the frequency of the undetected alleles. They may also cause underestimation of the effect and degree of dominance of the gene in populations with undetected alleles. The inaccuracies may be infrequent, but could be quite large, and are likely to go undetected for a long time. When they are recognized, the consequences could be considerable.

Undetected alleles could also occur in tests that are based on linkage disequilibrium with functional polymorphism, such as the mu-calpain example cited above, rather than tests for known functional variation. In this case, one or more SNP tend to be associated with effects on a trait of interest. In the case of multiple SNP, some haplotypes are associated with favourable effects and others with unfavourable effects. The association is useful, but there are likely to be some animals in which the favourable test result is actually linked to an unfavourable functional allele, or vice versa. In such cases, adding another SNP may allow a haplotype to be subdivided into two haplotypes, one of which is associated with the favourable and one with the unfavourable functional allele. Thus, the accuracy of the test is improved. The difficulty is in knowing where (in which families) to look for deterioration of the association. It seems likely that this will be based primarily on families developed for QTL detection using linkage analysis. Whatever the cause of undetected alleles, there will be a point of diminishing return at which it would not be productive to look for additional markers; however, it may be a challenge to determine when this point has been reached.

Constraints on implementation

Tests based on association with multiple SNP present special challenges in terms of delivery of the test results to the end-user. For a test based on a single SNP, the result can be presented simply as zero, one, or two copies of the favourable allele. However, with two SNP, there are nine possible test results, but some will occur at frequencies too low to estimate their effects. With intermediate haplotype frequencies and high disequilibrium between the SNP, we expect to have six test results that occur at substantial frequency, but it is not necessarily clear how the results are expected to rank, much less what the estimated effect of each result is unless we have a very large population in which to evaluate the test. With more SNP the problem becomes yet more challenging. In the end, the problem becomes one of associating SNP haplotypes with functional alleles, but this association may not be obvious, nor is the pair of haplotypes that makes up a multilocus genotype always unambiguous. Therefore, some work remains to be done in developing statistical methods for analysing such data. Furthermore, association tests are notoriously subject to spurious false positive results. A primary cause of these is population stratification that is not accounted for in the statistical model. As an extreme example, consider an association test for muscling in a mixture of Angus and Limousin cattle. Any gene with different frequencies in Angus and Limousin would show an association with muscling, since Limousin animals have significantly higher muscling than Angus. Specifically, an SNP with two alleles (1 and 2) for which allele 1 is present at 10% frequency in Angus but 60% frequency in Limousin, will appear to be associated with muscling because the higher-muscled Limousin animals will tend to have allele 1 and the lower-muscled Angus will tend to have allele 2. In this case, population stratification has led to a false association for this marker, since it is only required that the marker should have biased allele frequencies in the two breeds, and not necessarily that the marker is close to causative variation in either breed. Consequently, associations should be verified by linkage analysis within reasonably large families. Nonetheless, many association studies are conducted in cattle with deliberately unknown genetic background. This makes it impossible properly to account for population stratification in the analysis, particularly when conducting a single-marker study. When using commercial populations for verification of experimental results, paternal half-sibs are a common source of unknown population stratification in feedlot cattle. Stratification is revealed when analysing larger numbers of markers spread across the genome, since a larger number of markers will appear to be associated than is likely due to true disequilibrium with causative variation. At MARC we are striving to rule out stratification in our multibreed industry sire population by running sufficient markers to identify sources of population stratification.

Another challenge in implementing genetic tests is that

we need to know the effect of the test on each trait, but the effect is unlikely to be the same in all breeds, environments and management systems. For example, in Breed A, animals with the +/+ genotype might average 0.40 marbling units higher than animals with the −/− genotype, but in Breed B, the difference between +/+ and −/− might be only 0.25 marbling units. Furthermore, if the same set of animals of Breed A had been fed 30 days longer, their difference between genotypes might have been 0.50 marbling units. The point is that, although the size of the effect of a genetic test is important information, it is not constant across all situations. Differences in effect between breeds could occur either as a result of interaction with other loci (as in the examples of interaction with myostatin described earlier) or because of differences in the extent of linkage disequilibrium between the test polymorphisms and the functional polymorphisms between breeds.

Realistic expectations

Genetic testing should allow us to obtain evaluations earlier in the life cycle, which should increase the accuracy of selection, especially for traits that are expensive to measure or that can only be measured *post mortem*. It should also provide more benefit from each phenotype that is measured and greater opportunity to select for traits with antagonistic genetic relationships (eg fat thickness and marbling). However, the use of markers will not be very efficient until a sufficient number of tests for a trait, accounting for as much of the genetic variation as possible for each target population, becomes available. Previous studies conducted to create these tests have been arduous and expensive, requiring years of research. Creation of markers (which is the prerequisite to develop genetic tests) was difficult when the crude genetic maps of cattle were first developed in the mid-90s, and accounted for a substantial portion of the time required. Fortunately, the completion of the human genome and the impending project to sequence the bovine genome will greatly accelerate the development of genetic markers in beef cattle. It is reasonable to predict that genetic tests corresponding to a significant fraction of known QTL (Table 1) will be available in the next three years, and that additional QTL will be discovered to continue this development beyond that time frame. It remains to be seen how applicable the QTL discovered, for example, in our Pied × Angus population, may be to a Hereford rancher. Our results to this point make us optimistic that most variations in production traits will not be particularly breed-specific, but it is definitely possible that certain beneficial alleles will only be found with appreciable frequency in particular breeds.

With appropriate genetic tests in hand, the big challenge is to migrate to a price structure in which breeders can afford to test a large number of animals for many loci, while testing companies are able to recover development costs and earn a profit. The real costs of the laboratory procedures required are decreasing rapidly and are expected to continue to decrease. It is realistic to expect that the basic cost of executing an SNP genotype for a bovine DNA sample will soon fall into the 1–10 cents per genotype range (it currently falls into the US$1.00–5.00 range). However, many of these procedures require licensing fees that are priced according to the market for human DNA testing services. It is not clear whether the patent holders will price licences separately for the agricultural market, although there is clearly greater revenue potential from selling many tests at a modest price than from selling a few at a high price per sample. However, the livestock testing market is currently so limited that it is difficult to get the attention of owners of DNA technology.

There are also costs of intellectual property regarding the associations between economically important traits and genes or specific polymorphisms. When these associations are discovered by the testing companies, the research cost is substantial and must be spread over a large number of animals. However, the initial market for such tests is usually small so the research cost per animal is high and this inhibits widespread use of the test. Therefore, it seems more appropriate for the discovery and characterization of associations between polymorphisms and traits to be done by the public sector with the private sector focusing on transferring the technology to the cattle industry in a cost-effective manner.

There is serious interest in the feedlot industry in using genetic testing to sort incoming cattle of unknown backgrounds into different management groups according to genetic potential for various traits. This approach would require that the genetic testing account for a substantial proportion of the genetic variance for the target traits, which is a very ambitious goal. However, if this goal were achieved it could provide sufficient testing volume to drive the costs of testing down to the point where widespread genetic testing in breeding cattle would be feasible.

The uses of genetic testing as a breeding tool in seedstock herds or as a management tool in feedlots are sometimes viewed as alternative or even competing uses for the technology. However, they are actually complementary uses of the technology and given the low profit margins that exist in beef production, using the same product in both markets would be the best way to recover the development costs and provide a product to the industry profitably. It remains to be seen how successful such products will be.

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Notes

[1] Names are necessary to report factually on available data; however the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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