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# Validation of commercial DNA tests for beef quality traits


## Abstract

Gene mapping and discovery programs have resulted in the detection of numerous DNA "˜markers' for various beef cattle production traits. Prior to commercializing genetic markers, it is important to validate their purported effects on the traits of interest in different breeds and environments, and assess them for correlated responses in associated traits. One of the biggest challenges in achieving this objective is the availability of cattle populations with sufficient phenotypic data to assess the association between various traits and newly discovered genetic markers. Results from such validation studies to date have not been widely published and genetic marker tests sometimes may be commercialized prior to the collection of field validation data. In addition, conflicting reports about some commercially available markers, as well as the recognized occurrence of well-proven bulls with a high EPD for a given trait but carrying two copies of the "wrong"? (unfavorable) marker for that trait, have made some producers wary of investing in DNA-based testing. Producers want to know whether DNA-based tests perform in accordance with the claims of the marketing company and are interested in third-party, independent validation of these tests. The objective of this study was to validate three commercially-available genetic tests (GeneSTAR Quality Grade8, GeneSTAR Tenderness8, and Igenity TenderGENE9).



## Keywords

Cattlemen's Day, 2007; Kansas Agricultural Experiment Station contribution; no. 07-179-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 978; Beef; Cattle; DNA markers; Beef quality traits





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# VALIDATION OF COMMERCIAL DNA TESTS FOR BEEF QUALITY TRAITS[1]

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## Introduction

Gene mapping and discovery programs have resulted in the detection of numerous DNA 'markers' for various beef cattle production traits. Prior to commercializing genetic markers, it is important to validate their purported effects on the traits of interest in different breeds and environments, and assess them for correlated responses in associated traits. One of the biggest challenges in achieving this objective is the availability of cattle populations with sufficient phenotypic data to assess the association between various traits and newly discovered genetic markers. Results from such validation studies to date have not been widely published and genetic marker tests sometimes may be commercialized prior to the collection of field validation data. In addition, conflicting reports about some commercially available markers, as well as the recognized occurrence of well-proven bulls with a high EPD for a given trait but carrying two copies of the "wrong" (unfavorable) marker for that trait, have made some producers wary of investing in DNA-based testing. Producers want to know whether DNA-based tests perform in accordance with the claims of the marketing company and are interested in third-party, independent validation of these tests. The objective of this study was to validate three commercially-available genetic tests (GeneSTAR Quality Grade[8], GeneSTAR Tenderness[8], and Igenity *Tender*GENE[9]).

## Experimental Procedures

**Validation Process.** The National Beef Cattle Evaluation Consortium (NBCEC, www.NBCEC.org) conducts independent validations of commercially-available genetic tests for beef cattle production traits. This process is a collaboration of owners of the DNA and phenotypes (e.g., breed associations) and commercial testing companies, facilitated by the NBCEC.

**DNA Testing Companies and Sample Populations.** Phenotypic data and DNA were mostly collected as part of the Carcass Merit Project funded by the Cattlemen's Beef Board and cooperating breed associations. Each

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[1]This research is a condensed version of a research manuscript accepted for publication in the Journal of Animal Science with Alison Van Eenennaam as the first author.

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[8]GeneSTAR Quality Grade and GeneSTAR Tenderness are registered trademarks of Bovigen, LLC.
[9]Igenity *Tender*GENE is a registered trademark of Merial.

commercial testing company selected the breed groups to be used for the validation.

Bovigen, LLC (Harahan, LA), chose to validate its two GeneSTAR 'marker' panels on both Charolais sired calves (n = 400) out of commercial Angus dams and Hereford-sired cattle (n = 285) primarily out of Hereford or Hereford × Red Angus dams. The GeneSTAR Tenderness panel was validated on two populations of Brahman-sired cattle (Brahman dams, n = 674). Approximately half of the Brahmans (n = 318) were Carcass Merit Project cattle from the USDA-ARS SubTropical Agricultural Research Station in Brooksville, FL. The remaining Brahmans were the offspring of 68 Brahman sires bred to Brahman cows at the Louisiana State University Agricultural Center. Merial (Duluth, GA) used the same Charolais-sired and the Carcass Merit Project Brahman-sired cattle populations, plus cattle sired by Red Angus (Red Angus and Red Angus cross dams; n = 310) and Brangus (Brangus and Brangus cross dams; n = 181) for Igenity *Tender*GENE test validation.

**Genetic Tests.** Genotyping for the GeneSTAR Quality Grade and GeneSTAR Tenderness marker panels (Bovigen, LLC) and the *Tender*GENE marker panel (Merial) was done by the respective companies. The 2 tenderness panels share two common μ-calpain and similar but different calpastatin markers. The μ-calpain enzyme system is primarily responsible for tenderization that occurs during aging. Calpastatin is an inhibitor of the calpain enzyme system, so high levels of calpastatin are undesirable.

**Phenotypes.** Traits analyzed were longissimus lumborum (loineye) Warner-Bratzler shear force (WBSF) and subjectively recorded marbling score. Muscle sections were harvested 24 to 48 hours postmortem from numerous processing plants, with nearly all using relatively high-voltage electrical stimulation. Steaks (1 inch thick) were vacuum packaged and aged at 1° to 2°C until 14 days postmortem. Steaks were cooked to an internal temperature of 71°C at 163°C. After reaching the endpoint temperature, steaks were cooled at 1° to 2°C for 24 hours, and eight 1/2-inch-diameter cores were removed parallel to the muscle fibers and sheared with a WBSF V-blade attached to an Instron Universal Testing Machine. A numeric score was used to record marbling, with 400 corresponding to Slight$^{00}$, 500 corresponding to Small$^{00}$, and 600 corresponding to Modest$^{00}$, etc. Quality grade was analyzed as percentage qualifying as USDA Choice or Prime based entirely on marbling score ≥ 500 (all carcasses were A maturity).

**Statistical Analyses.** The basic model was y = CG + marker effect + sire + e, where CG denotes a fixed contemporary group and sire was a random effect. For GeneSTAR Tenderness and Igenity *Tender*GENE marker panels, there were two linked markers, so the regression was on the expected number of copies of each of the four haplotypes (one of which was rare). Genotype frequencies were estimated and analyses carried out with SAS Proc HAPLOTYPE and Proc Mixed, respectively (SAS 9.1.3, SAS Inst. Inc., Cary, NC).

## Results

**Allele Frequencies.** The sample genotypes and allele frequencies for each of the markers included in the commercial tests in this validation study are shown in Table 1. Some alleles were extremely rare (< 0.5%) in certain populations. Specific haplotype frequencies are reported in Table 2. Haplotypes are combinations of genes affecting different traits.

**GeneSTAR Quality Grade.** One of the Quality Grade (QG) alleles was almost fixed in the Hereford-sired sample population, so the analysis included only the 387 Charolais-sired × Angus cattle (Table 1). The GeneSTAR QG test was not associated with marbling score; however, an increase in the percentage of Choice plus Prime approached

significance (P≤ 0.06; Table 3). The association of the test with quality grade was primarily attributable to the effect of the favorable allele of the QG marker. Bovigen, LLC categorizes the different genotypes into categories of 0, 1, or 2 "stars". In this sample population, the average effect of each "star" (0, 1, or 2) of the GeneSTAR QG test was associated with a 6.2% increase in the percentage of Choice plus Prime.

**GeneSTAR Tenderness.** Improved tenderness was associated with substituting a T allele at Calpastatin-T1 and a C allele at both μ-calpain loci. The GeneSTAR Tenderness analysis included 1302 cattle (372 Charolais × Angus, 260 Hereford, and 670 Brahman). The association of calpastatin-T1 and the μ-calpain haplotypes with WBSF were each highly significant (P<0.01), as was the combination of these markers (P<0.0001). Each calpastatin T was associated with a decrease of 0.33 lb. in WBSF, and substituting the Calpain T2-T3 C-C haplotype for the Calpain T2-T3 G-T haplotype was associated with a decrease of 0.75 lb in WBSF (Table 4).

**Igenity TenderGENE.** Improved tenderness was associated (P<0.001) with substituting a C allele at calpastatin and a C allele at both μ-calpain loci. The association of calpastatin and the μ-calpain alleles with WBSF were each highly significant (P<0.001), and the combination of all three even more so (P<0.0001). Table 5 shows the improvement in WBSF for each of the possible haplotypes contrasted to the least tender genotype (calpastatin GG, μ-calpain TT, μ-calpain GG) calculated from a combined analysis of 1209 cattle (181 Brangus, 400 Charolais × Angus crosses, 310 Red Angus and 318 Brahman). In this sample population, each calpastatin C was associated with a decrease of 0.42 lb in WBSF and substituting C-C for G-T at CAPN1 was associated with a decrease of 0.73 lb of WBSF. Combined genotypic effects for GeneSTAR Tenderness and Igenity *Tender*GENE are presented in Table 6. There was a 2.2 lb difference in WBSF between the most and least tender genotypes in both panels.

## Discussion

Our study did not show a significant association of the GeneSTAR Quality Grade marker with marbling score, but there was a definite trend (P = 0.06) toward increased quality grade associated with substituting the favorable allele in Charolais × Angus crossbred cattle that had been fed for less than 250 days. However, the binary trait of percentage of Choice plus Prime represents a considerable loss of information compared to the continuous trait of marbling score. The association of quality grade with the results from the GeneSTAR Quality Grade test in the absence of a significant association with marbling score was probably the result of a high proportion of animals on the borderline of the USDA Select/Choice grade. The absolute improvement in quality grade associated with any marker will always be dependent upon marbling endpoint, which emphasizes the importance of environment and management on results derived from validation studies.

The genotype effects of the two tenderness panels, GeneSTAR Tenderness and Igenity *Tender*GENE, were very similar to each other (Table 6), suggesting that the two calpastatin alleles are marking the same tenderness-associated region of the genome. The magnitude of the WBSF reduction associated with the most favorable genotypes compared to the least favorable genotypes is distinctly greater than the difference in tenderness that has been recorded between Select and low Choice quality grades, as well as being greater than the tenderness difference between Select and "premium" Choice (upper two-thirds of Choice) beef. From the perspective of genetic improvement, it is interesting to observe that the frequency of the μ-calpain G-T haplotype is relatively high (Table 2). This suggests that the beef industry may have the opportunity to

improve tenderness by increasing the frequency of the μ-calpain C-C haplotype.

Failure to achieve statistical significance should never be interpreted as evidence that an effect is zero. In this case, the major allele frequency in one or more validation populations may be so high that there is no real opportunity to evaluate the effect of the test. This should not be considered a negative result, but rather a 'no result' (e.g. the GeneSTAR QG test in the Hereford population in this study). Given these considerations, it is perhaps not surprising that few marker validation studies in cattle have been published. However, validation of the effects of genetic markers in independent populations is likely to be vital to the success of genetic testing technology, as producers are likely to be reluctant to invest in unproven markers.

Validation studies can also serve to generate information that is essential for the process of incorporating DNA tests into cattle evaluation. Although there is a tendency to label DNA tests as being associated with one particular trait, markers with a large effect on any one trait are also likely to have correlated effects on other traits because most genes influence a variety of traits. The widespread adoption of marker-assisted selection in the industry will likely depend upon the successful integration of marker information into cattle evaluation schemes to enable eventual development of "DNA marker-assisted EPDs."

## Implications

Tenderness could be markedly improved by selecting for the favorable calpastatin and μ-calpain genotypes included in GeneSTAR Tenderness and Igenity *Tender*GENE marker panels. Using the GeneSTAR Quality Grade marker panel could result in an increased percentage of USDA Choice plus Prime carcasses.

**Table 1.**

| Marker | Favorable allele | Population description | Genotype (%) 0 | 1 | 2 | No. animals | Frequency unfavorable | Frequency favorable |
|---|---|---|---|---|---|---|---|---|
| GeneSTAR Calpastatin T1 | T | Charolais × Angus | 1 | 11 | 88 | 409 | 0.06 | 0.94 |
| | | Hereford | 16 | 50 | 34 | 322 | 0.41 | 0.59 |
| | | Brahman | 11 | 46 | 43 | 674 | 0.34 | 0.66 |
| Igenity TenderGENE Calpastatin UoG | C | Charolais × Angus | 5 | 33 | 62 | 412 | 0.21 | 0.79 |
| | | Brangus | 5 | 32 | 63 | 203 | 0.21 | 0.79 |
| | | Red Angus | 8 | 36 | 56 | 305 | 0.26 | 0.74 |
| | | Brahman | 33 | 47 | 20 | 344 | 0.57 | 0.43 |
| GeneSTAR µ-calpain T2 | C | Charolais × Angus | 27 | 54 | 19 | 435 | 0.54 | 0.46 |
| | | Brangus | 20 | 51 | 29 | 219 | 0.45 | 0.55 |
| | | Red Angus | 26 | 54 | 21 | 307 | 0.53 | 0.47 |
| | | Hereford | 71 | 25 | 4 | 305 | 0.84 | 0.16 |
| | | Brahman | 88 | 11 | 1 | 674 | 0.94 | 0.06 |
| GeneSTAR µ-calpain T3 | C | Charolais × Angus | 58 | 37 | 4 | 435 | 0.77 | 0.23 |
| | | Brangus | 67 | 31 | 2 | 217 | 0.82 | 0.18 |
| | | Red Angus | 59 | 36 | 5 | 307 | 0.77 | 0.23 |
| | | Brahman | 96 | 4 | 0 | 674 | 0.98 | 0.02 |
| | | Hereford | 56 | 40 | 4 | 309 | 0.76 | 0.24 |
| GeneSTAR Quality Grade Thyroglobulin | T | Charolais × Angus | 62 | 34 | 5 | 409 | 0.78 | 0.22 |
| | | Hereford | 81 | 18 | 1 | 324 | 0.90 | 0.10 |
| GeneSTAR Quality Grade | | Charolais × Angus | 63 | 33 | 4 | 420 | 0.79 | 0.21 |
| | | Hereford | 97 | 3 | 0 | 311 | 0.99 | 0.01 |

**Table 2. μ-Calpain Allele Frequencies**

| μ-Calpain | G-T | C-T | G-C | C-C | N |
|---|---|---|---|---|---|
| Charolais × Angus | 0.51 | 0.03 | 0.26 | 0.20 | 400 |
| Brangus | 0.45 | 0 | 0.37 | 0.17 | 181 |
| Red Angus | 0.51 | 0.01 | 0.25 | 0.23 | 310 |
| Brahman | 0.92 | 0 | 0.07 | 0.02 | 318 |
| Hereford | 0.12 | 0.04 | 0.64 | 0.20 | 260 |

**Table 3. Effects of GeneSTAR Quality Grade Panel Results on Marbling Score and % of Animals Grading Choice and Prime Phenotypes from 387 Charolais-sired × Angus Cattle**

| Trait | Marker | Estimate, effect | SE | *P* |
|---|---|---|---|---|
| Marbling Score | GeneSTAR Quality Grade[2] | 5.7 | 4.2 | 0.18 |
| % Choice and Prime | GeneSTAR Quality Grade[2] | 6.2 | 3.2 | 0.06 |

[1]Average effects of Quality Grade favorable alleles.

**Table 4. Effects of GeneSTAR Tenderness Panel Results on Warner-Bratzler Shear Force (lb) Phenotypes from 372 Charolais-sired ×Angus, 260 Hereford, and 670 Brahman Cattle**

| No. Head | Marker | Allele/ Haplotype | Sample Frequency | Estimated Effect (lb) | SE |
|---|---|---|---|---|---|
| 1302 | Calpastatin T1 | T | 0.72 | -0.33 | 0.23 |
| | | C | 0.28 | 0.00 | |
| 1302 | μ-calpain T2-T3 | C-C | 0.11 | -0.75 | 0.37 |
| | | C-T[1] | 0.02 | -0.35 | |
| | | G-C | 0.23 | -0.40 | 0.31 |
| | | G-T | 0.64 | 0.00 | |

[1]The low number of animals with the C-T haplotype in this study made it difficult to accurately estimate their effects.

**Table 5. Effects of Igenity *Tender*GENE panel results on Warner-Bratzler Shear Force (lb) Phenotypes from 181 Brangus, 400 Charolais-sired × Angus Cross, 310 Red Angus and 318 Brahman Cattle**

| No. Head | Marker | Allele/ Haplotype | Sample Frequency | Estimated Effect (lb) | SE |
|---|---|---|---|---|---|
| 1209 | CalpastatinUoG | C | 0.72 | -0.42 | 0.11 |
| | | G | 0.28 | 0.00 | |
| 1209 | μ-calpain | C-C | 0.16 | -0.73 | 0.15 |
| | | C-T[1] | 0.01 | 0.00 | 0.50 |
| | | G-C | 0.22 | -0.40 | 0.13 |
| | | G-T | 0.61 | 0.00 | |

[1]The low number of animals with the C-T haplotype in this study made it difficult to accurately estimate its effect.

**Table 6. Combined Three-marker Genotype Effects, and Frequencies for the Two Tenderness Panels GeneSTAR Tenderness and Igenity *Tender*GENE[1]**

| Genotype GeneSTAR's T1 or Igenity's Calpastatin UGa | T2 | T3 | GeneSTAR Tenderness | | IgenityTenderGENE | |
|---|---|---|---|---|---|---|
| | | | Estimate (lb) | % | Estimate (lb) | % |
| 2 or CC | 2 = CC | 2 = CC | -2.2 | 0.8 | 2.2 | 1.5 |
| | | 1 = CT[1] | -.1.8 | 0.7 | -1.1 | 0.7 |
| | | 0 = TT[1] | -1.3 | 0.0 | -0.2 | 0.0 |
| | 1 = CG | 2 = CC | -.1.8 | 5.5 | -2.0 | 5.0 |
| | | 1 = CT | -1.3 | 6.1 | -1.5 | 10.2 |
| | | 0 = TT[1] | -1.1 | 1.0 | -0.4 | 0.7 |
| | 0 = GG | 2 = CC | -1.5 | 4.2 | -1.5 | 2.7 |
| | | 1 = CT | -1.1 | 11.0 | -1.3 | 15.0 |
| | | 0 = TT | -0.7 | 24.7 | -0.9 | 17.5 |
| 1 or CG | 2 = CC | 2 = CC | -1.8 | 0.4 | -1.8 | 0.7 |
| | | 1 = CT[1] | -1.5 | 0.2 | -0.7 | 0.1 |
| | | 0 = TT[1] | -1.1 | 0.0 | -0.4 | 0.0 |
| | 1 = CG | 2 = CC | -1.5 | 2.9 | -1.5 | 3.5 |
| | | 1 = CT | -1.1 | 1.9 | -1.1 | 6.1 |
| | | 0 = TT[1] | -0.7 | 0.5 | 0.0 | 0.3 |
| | 0 = GG | 2 = CC | -1.1 | 4.8 | -1.3 | 1.9 |
| | | 1 = CT | -0.7 | 4.1 | -0.9 | 7.5 |
| | | 0 = TT | -0.4 | 21.9 | -0.4 | 16.9 |
| 0 or GG | 2 = CC | 2 = CC | -1.5 | 0.0 | -1.5 | 0.2 |
| | | 1 = CT[1] | -1.1 | 0.1 | -0.2 | 0.1 |
| | | 0 = TT[1] | -0.7 | 0.0 | -0.9 | 0.0 |
| | 1 = CG | 2 = CC | -1.1 | 0.7 | -1.1 | 0.6 |
| | | 1 = CT | -0.7 | 0.5 | -0.7 | 0.7 |
| | | 0 = TT[1] | -0.4 | 0.5 | -0.4 | 0.0 |
| | 0 = GG | 2 = CC | -0.4 | 1.3 | -0.9 | 0.4 |
| | | 1 = CT | -0.4 | 1.2 | -0.4 | 2.5 |
| | | 0 = TT | 0.0 | 5.2 | 0 | 5.2 |

[1]Estimated from 1302 (372 Charolais-sired × Angus, 260 Hereford, and 670 Brahman), and 1209 (181 Brangus, 400 Charolais-sired × Angus Cross, 310 Red Angus and 318 Brahman) Cattle, Respectively

[2]These rows include genotypes involving the rare *CAPN1* 316/4751 C-T haplotype. The low number of animals with the C-T haplotype in this study made it difficult to accurately estimate its effect.