



TECHNICAL SUMMARY

March 2009

GeneSTAR MVPs — Molecular Value Predictions for beef feed efficiency, marbling¹ and tenderness

Key Points

- GeneSTAR® is a DNA-marker test for important production traits in all breeds of beef cattle.
- GeneSTAR MVPs™ employ a panel of 56 DNA markers to produce GeneSTAR Molecular Value Predictions (MVPs).
- GeneSTAR MVP reliability values average 30, 26 and 49 percent for feed efficiency, marbling and tenderness, respectively.
- GeneSTAR MVPs have been internally and externally validated in a variety of beef cattle populations globally.

Introduction

GeneSTAR is a DNA test for quality and production traits in all breeds of beef cattle. It tests DNA markers known to be associated with feed efficiency, marbling and tenderness. GeneSTAR was initially released in 2000 as a single-marker test for an important gene for marbling. Advances in animal genomics have allowed GeneSTAR to expand to include additional markers for marbling and the traits listed above. The rate at which new markers/traits are incorporated into the GeneSTAR product range is ever increasing, aided over the past few years by an exponential growth in our knowledge of the bovine genome.

Until now, four DNA markers each for feed efficiency, marbling and tenderness have been utilised in the GeneSTAR platform. Previous GeneSTAR results have been presented on a scale of 0 to 8 stars for each trait (each marker having a result of zero, one or two

stars with the overall trait result calculated by adding the results for each individual marker together). Under this system, the higher the number of stars, the better the animal was likely to perform for that trait.

As the true value of gene marker tests lies in panels of markers, Pfizer Animal Genetics aims to incorporate additional markers into these panels as they are discovered through a range of ongoing gene mapping and sequencing projects. These marker panels become tools for predicting the true breeding value of an animal and better enable customers to utilise individual animal precision management practices and fully exploit genome-enabled genetic improvement programmes. This comprehensive approach is the cornerstone of Pfizer Animal Genetics' long-term goal of providing full-service genomic solutions to the global cattle industry.



GeneSTAR MVPs

GeneSTAR Molecular Value Predictions (MVPs) as described in this summary have been in development since 2006 based on exclusively licensed technology augmented with publicly available markers. The platform utilises a panel of 56 DNA markers to calculate MVPs for three core management traits.

These traits include:

- Feed efficiency as defined by net (or residual) feed intake (NFI or RFI)
- Marbling (measured as AUS-MEAT Marble Score)
- Tenderness (measured as Warner-Bratzler shear force at 14-day postmortem aging)

The commercial development of GeneSTAR MVPs has involved an extensive and rigorous four-step process:

- 1) Initial discovery of DNA markers that have statistically important effects on performance in economically relevant traits of interest.
- 2) Development of statistical methodology for the inclusion of individual markers into “genome-wide” panels for prediction of MVPs and associated prediction reliabilities.
- 3) Evaluation of the relationship between MVPs and phenotypic performance in a range of populations within the Pfizer Animal Genetics research programme.
- 4) Independent validation of the effects of the marker panel predictions of MVPs.

Inclusion of Markers in GeneSTAR MVPs

The first gene marker distributed under GeneSTAR was the marbling marker (MI). This product was offered commercially in 2000. Between 2002 and 2006 two additional marbling and four tenderness markers were added. The addition of each marker was a major undertaking with an extensive amount of external and internal discovery and validation work required. As the technology and the field of animal genetics advanced, one marbling and four feed efficiency markers were incorporated in 2006. This came as a result of the first bovine genome-wide analysis using a publicly available 10,000 single nucleotide polymorphism (SNP) array.

GeneSTAR MVPs now incorporate 56 DNA markers for use in multiple-trait prediction of feed efficiency, marbling and tenderness. The previously offered 12 markers are included in the new panel along with an additional 44 markers from more recent discovery and validation efforts. These additional 44 markers have been identified from a range of sources including a recent Pfizer Animal Genetics whole genome scan, public domain information and a series of collaborative research projects. More detailed information on the discovery effort involved in the production of both the original 12 markers and the recently released additional 44 markers can be found at www.pfizeranimalgenetics.com.au.

All 56 markers contained in GeneSTAR MVPs are SNPs—DNA sequence variations that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered at a particular location in the genome. All SNPs used in the GeneSTAR MVPs are di-allelic, meaning they have two possible alleles which can be combined to form three possible genotypes (i.e., if we assume the two alleles of a SNP are Q and q , then the three possible genotypes for that marker are QQ , Qq and qq).

The markers included in this new panel have all been found to have statistically significant effects on one or more traits of interest.

GeneSTAR MVP Methodology

The reporting and interpretation system for the GeneSTAR platform has undergone a significant expansion in order to accommodate the additional information explained by GeneSTAR MVPs. In the era of increasingly large marker panels, the aim is to predict the true breeding value (and resulting phenotype) of an individual as opposed to a simple genotype result. The previous process simply counted the number of favourable alleles—zero, one or two—for a particular marker, which was then translated into a number of stars. With the realisation that not all markers carry similar effects and that individual markers may contribute to more than one trait simultaneously, a simple star rating may fail to best represent the true genetic value of an individual. GeneSTAR MVPs employ a much larger number of markers as shown in Table 1.

The approach taken to improve both the reliability and utility of GeneSTAR test results has been to develop a Molecular Value Prediction (MVP) of an animal's true breeding value. The accuracy of the MVP is maximised by fitting marker additive effects as a regression on the number of favourable alleles. Under this approach, all individual marker effects are assumed to be additive, but not necessarily equal. The resulting MVP is then produced by adding the individual marker effects of the genotypes observed for an animal across all marker loci in the panel, according to the model shown in Table 2.

In order to ensure that the MVP is not only accurate but also conservative in the face of complex predictive equations dealing with genotypic information, a "shrinking" methodology (Kinghorn B. 2008) was developed for Pfizer Animal Genetics. This approach allows for the "shrinking" of individual marker effects based on the standard error of the marker genotype effect before contributing to the overall MVP. The shrinking approach approximates the results

that would be expected from a whole genome analysis using thousands of markers; however, as only 56 markers are currently used, the potential variance accounted for is less than would be possible from a full-scale whole genome analysis.

As with all predictions of genetic value, assessment of reliability and accuracy is important. The primary standard for assessing the accuracy and predictive power of the MVP for a trait is the reliability value. Reliability is based on the correlation (R) between the MVP and the animal's genetic breeding value if all information were known. Because MVPs are predictions of genetic value (i.e., breeding value) for the trait, the maximum value for R is the square root of the heritability of the trait in the population. The reliability value describes this ratio and is expressed as a percentage of the maximum accuracy attainable. The reliability is a highly useful indication of how much additional information may be added in the future as greater numbers of markers are added to the panels used to calculate the MVP.

Table 1 – From STARS to MVPs.

| | Number of Markers | Results Expressed | Reliability Value | Validated |
|-------------------|-------------------|-------------------------------|-----------------------|-----------|
| Original GeneSTAR | 12 ^a | STARS ^b | NA ^c | ✓ |
| New GeneSTAR MVP | 56 | Trait Prediction ^d | 26 – 49% ^e | ✓✓ |

^aFour markers each for feed efficiency, marbling and tenderness

^bNumber of favourable alleles

^cNot applicable

^dEstimate of molecular breeding value

^e% Reliability value ranges across traits

Table 2 – Molecular breeding values at a single-marker locus.

| Genotype | Number of Favourable Alleles | Molecular Breeding Values as Deviation from Population Mean | Error Variance |
|----------|------------------------------|---|---------------------|
| QQ | 2 | $(2 - 2p)\alpha$ | $(2 - 2p)^2V\alpha$ |
| Qq | 1 | $(1 - 2p)\alpha$ | $(1 - 2p)^2V\alpha$ |
| qq | 0 | $(0 - 2p)\alpha$ | $(0 - 2p)^2V\alpha$ |

Molecular breeding value as the deviation from population mean and its error variance for genotypes with zero, one or two copies of the favourable allele of a marker (α is the substitution effect of the marker, p is the frequency of the favourable allele, V is the square of the standard error of substitution effect estimate).

Relationship of GeneSTAR MVPs to Phenotypic Performance

GeneSTAR Molecular Value Predictions have been evaluated in research populations in Australia (AU) and the United States (U.S.) selected to provide a representative range of commercial breeds and situations. Table 3 describes the populations.

Feed efficiency is measured as net feed intake or residual feed intake (NFI/RFI); marbling (MS) is measured as AUS-MEAT Marble Score or USDA Marbling Score, and tenderness is measured as Warner-Bratzler shear force (WBSF) and sensory panel tenderness score (ST).

GeneSTAR MVPs were developed using populations A through E (N = 2866) with all other populations (N = 4455) used to evaluate and further refine the calibration of the prediction equations.

Summary results of the relationship of GeneSTAR MVPs with phenotypic performance are presented in Table 4

(on the next page). The criteria for this relationship are the statistical significance of the prediction on the trait of interest (where $P < 0.05$ is the minimum threshold of significance) and the correlation (R) between the MVP and adjusted phenotype. While individual populations can vary in level of heritability for traits, average levels for the traits of interest result in maximal values of R of 0.50, 0.52 and 0.55 for feed efficiency, marbling and tenderness, respectively.

Predictions from the new 56-marker panel were compared in all populations with predictions from the previously available GeneSTAR markers. The average improvement in R observed ranged from 45 percent for marbling to 250 percent for feed efficiency.

The GeneSTAR MVPs are significantly improved across all populations studied as compared to the previous marker panels available. While results varied across populations studied internally (summarised in Table 4), the overall trend for the MVP reliability values was on the order of 30 percent for feed efficiency, 26 percent for marbling and 49 percent for tenderness.

Table 3 – Description of evaluation populations, management regimens as days on feed (DOF) and average trait performance.

| Population | Breed Composition | Regimens (DOF) | N | Phenotype Examined ^a |
|------------|--------------------------|------------------------------|----------|---------------------------------|
| A (AU) | Wagyu | 300–450 | 605 | MS |
| B (AU) | Angus | 250 | 920 | MS |
| C (AU) | Angus | 150–180 | 235–332 | MS ^d , NFI |
| D (AU) | Composite ^b | <150 | 755 | MS |
| E (AU) | Shorthorn – Progeny Test | 100–180 | 114–254 | MS, NFI |
| F (AU) | British-X | <180 | 508 | MS |
| G (US) | Angus-X | 150 | 872 | MS ^e |
| H (AU) | Angus x Hereford | <180 | 387 | MS |
| I (US) | Multi-breed ^c | 150–180 | 828–1037 | MS ^e , WBSF, ST |
| J (AU) | Angus | 150–180 | 348 | MS ^f |
| K (US) | Angus | Bulls: 70 d Feed Intake | 605 | NFI |
| L (US) | Brangus | Heifers: 70 d Feed Intake | 468 | NFI |
| M (US) | Santa Gertrudis | Steers: 70 d Feed Intake | 206–230 | MS ^e , WBSF, NFI |

^aMarbling is AUS-MEAT Marble Score (0 – 9) or USDA Marbling Score (4.0 = Small 0 degree of marbling); Net Feed Intake is the difference between an animal's actual and expected feed intake based on its body weight and growth rate; W-B Shear Force (kg); Sensory Panel Tenderness (1 – 8).

^bComposite containing *Bos Indicus* and *Bos Taurus* breeds.

^cThis population includes Angus, Brahman, Charolais, Hereford, Limousin, Maine-Anjou, Red Angus, Shorthorn and South Devon breeds.

^dNumber of observations varied by trait within the range given.

^eMarbling score assessment of degree of intramuscular fatness under the USDA grading system (4.0 = Small 0 degree of marbling).

^fMarbling measured as % IMF.

Table 4 – Associations between GeneSTAR MVPs and trait phenotypes from internal populations.

| Trait | Range in N | Correlation (R) ^d | Fitting 56-marker panel ^{a,b,c} | | |
|------------------------|------------|------------------------------|--|----------------------------------|----------------------|
| | | | % Reliability ^e | % Genetic Variation ^f | P-value ^g |
| Feed Efficiency | | | | | |
| Overall | 1749 | 0.13 | 29.8 | 12.1 | |
| Range | 114–605 | 0.02–0.28 | 3.4–63.1 | 0.1–39.8 | 0.87–0.001 |
| Marbling | | | | | |
| Overall | 6127 | 0.12 | 25.5 | 9.2 | |
| Range | 206–1,037 | 0.02–0.28 | 3.1–61.5 | 0.1–37.8 | 0.84–0.01 |
| Tenderness | | | | | |
| Overall | 2030 | 0.30 | 49.1 | 24.6 | |
| Range | 206–996 | 0.17–0.35 | 30.5–53.1 | 9.3–28.2 | 0.02–0.01 |

^aPrediction of MVPs based on the 56-marker panel.

^bOverall value for R, % reliability, and % genetic variation is calculated as the weighted average over all populations in which the trait was evaluated.

^cRange in values across populations is shown in the second row.

^dThe maximum value possible for R is the square root of the heritability of the trait.

^eRatio of R to the square root of the heritability of the trait.

^fPercentage of additive genetic variation accounted for by variation in the MVPs.

^gRange in P-values across the populations evaluated is given to match the range in N.

Results of Independent Validation Analyses

The final step in the development of GeneSTAR MVPs was independent validation studies to confirm commercial relevance.

One group that played an important role in this process was the U.S. National Beef Cattle Evaluation Consortium (NBCEC, <http://www.ansci.cornell.edu/nbcec/nbcec.html>). The purpose of NBCEC validation is to independently verify associations between genetic tests and traits claimed by a commercial genotyping company using phenotypes and DNA from different cattle populations. In the NBCEC system, experimental populations with well-defined phenotypic records are used in the validation process. In this case, Pfizer Animal Genetics provided MVPs of the cattle to the NBCEC for analysis of the relationship to trait

performance in each population. The results of this validation approach are a confirmation of the claims made for each trait in the marker test. Until recently, the NBCEC service was the only publicly available model for DNA-marker test validation.

Realising important differences in the production and marketing environments between Australia, New Zealand and North America, a similar validation process was carried out in Australia. These validations were commissioned by Pfizer Animal Genetics with the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC, <http://www.beefcrc.com.au/aus-beef-dna-results>), who commissioned the Animal Genetics and Breeding Unit (AGBU) of the University of New England (Armidale, NSW) to conduct the analysis following the same guidelines used in the NBCEC model.

Based on Pfizer Animal Genetics cumulative internal data, the average reliability values were on the order of 30 percent for feed efficiency, 26 percent for marbling and 49 percent for tenderness.

Table 5 provides a summary of the populations used in external validation while Table 6 presents the results from the individual analyses of these populations.

Table 5 – Description of validation populations, management regimens as days on feed (DOF) and average trait performance.

| Population | Breed Composition | Regimen (DOF) | N | Phenotype Examined ^a | Phenotypic Mean | Performance SD ^e |
|--------------|--|--------------------------|------|---------------------------------|-----------------|-----------------------------|
| A (NBCEC) | European Maternal Line Composite | Bulls 100–130 | 462 | WBSF | 4.14 | 0.07 |
| | | | 671 | NFI | -0.08 | 0.84 |
| | | | 785 | MS | 4.08 | 0.55 |
| B (NBCEC) | European Maternal Line Composite | Steers 150–180 | 785 | IMF | 2.99 | 0.68 |
| | | | 723 | % Choice | 0.57 | 0.49 |
| | | | 392 | MS | 4.28 | 0.97 |
| C (NBCEC) | <i>Bos Indicus</i> -influenced Composites ^b | Steers: 70 d Feed Intake | 394 | IMF | 3.63 | 0.56 |
| | | | 392 | % Choice | 0.57 | 0.50 |
| | | | 390 | WBSF ^f | 3.61 | 0.98 |
| | | | 395 | NFI | 0.03 | 0.93 |
| | | | 1228 | MS | 1.00 | 0.88 |
| D (CRC) | Multi-breed ^c | Various ^d | 1345 | IMF | 4.86 | 1.99 |
| | | | 1377 | NFI | 0.03 | 1.11 |
| | | | 1244 | WBSF | 4.73 | 1.24 |

^aMS = AUS-MEAT Marbling Score (0 – 9) for population D or USDA Marbling Score (4.0 = Small 0 degree of marbling) for populations B and C; IMF = % intramuscular fat estimated by ultrasound scanning for populations B and C, and carcass intramuscular fat estimated chemically for population D; % Choice is the percentage of animals grading USDA Choice (CH) or higher; WBSF = Warner-Bratzler shear force at 14-d aging (kg); NFI = net feed intake.

^bEqual percentage of Simbrah, Red Brangus and Braford maternal line composites.

^cBreeds included Angus, Brahman, Belmont Red, Hereford, Murray Grey, Santa Gertrudis and Shorthorn.

^dCattle were either grass finished or grain finished to either domestic specifications (90 days on feed), Korean (150 days) or Japanese (300 days).

^eSD = Standard deviation

^fWarner-Bratzler shear force at one-day aging.

Table 6 – Associations between panel effects and phenotypes in validation populations.

| MVP Trait | Validation Phenotype | Population | N | Fitting 56-marker panel | | |
|-----------------|----------------------|----------------|------|-------------------------|-----------------|---------|
| | | | | Regression Coefficient | Correlation (R) | P-value |
| Feed Efficiency | NFI | A | 671 | 0.400 | 0.340 | 0.020 |
| | | C | 395 | -0.020 | 0.010 | >0.500 |
| | | D | 1377 | 0.340 | 0.080 | 0.001 |
| Marbling | MS | B | 785 | 0.120 | 0.040 | 0.146 |
| | | C | 392 | 0.370 | 0.070 | 0.096 |
| | | D | 1228 | -0.010 | 0.001 | >0.500 |
| Marbling | IMF | B | 785 | 0.310 | 0.090 | 0.011 |
| | | C | 394 | 0.660 | 0.130 | 0.001 |
| | | D | 1345 | 0.210 | 0.020 | 0.430 |
| Marbling | % Choice | B | 723 | -0.030 | 0.010 | >0.500 |
| | | C | 392 | 0.276 | 0.110 | 0.028 |
| Tenderness | WBSF | A | 462 | 0.670 | 0.310 | 0.021 |
| | | C ^a | 390 | 0.360 | 0.210 | 0.007 |
| | | D | 1244 | 0.197 | 0.060 | 0.001 |

^aWarner-Bratzler shear force at one-day aging.

Overall Meta-analysis of Results

In order to summarise the collective results from studies in the development and validation of the MVPs, a meta-analysis was designed and conducted. The meta-analysis effectively combined the statistical results into outcome groups by geographic region (Australia and North America) and cattle subpopulations (*Bos Taurus* and *Bos Indicus*-influenced). The results of this meta-analysis are shown in Table 7 for North America, Australia and the combination of the North American and Australian data sets, respectively.

This analysis revealed:

- Validation for tenderness MVPs across all populations and markets
- Validation in both North America and Australia for the influence of the feed efficiency MVP in *Bos Taurus* populations
- Validation of feed efficiency MVPs for *Bos Indicus*-influenced populations in Australia with further substantiation of this association in internal North American data
- For marbling MVPs a significant association was substantiated in NBCEC validations with % IMF estimated by ultrasound scanning 60 days prior to harvest. Furthermore, a significant association was documented in North American *Bos Indicus*-influenced populations with % Choice.

Reporting Format

The GeneSTAR MVP platform produces more information on an individual animal than any previous iteration of the GeneSTAR test. The outcome of this test is no longer reported according to the original star system, but as a GeneSTAR MVP. An example of the new GeneSTAR MVP reporting format is shown below.

Molecular Value Prediction

| | FE | Marb | Tend |
|--------|-------|------|-------|
| MVP | -0.33 | 0.01 | -0.42 |
| % Rank | 26% | 49% | 7% |

The GeneSTAR MVP is expressed in units of the trait, deviated on a +/- scale from zero where the zero point is defined as the average of all animals examined to date. To allow benchmarking of animals within contemporary breed subpopulations, the overall breed average and individual animal percentile ranks are also reported. Additionally, average and range of reliability values are reported for each trait in the report summary (Figure 1).

Table 7 – Meta-analysis summary of associations between MVPs and phenotypes recorded as P-values²

| | NFI | Marb | IMF | Tend |
|---|--------|-------|--------|--------|
| North American Populations | | | | |
| <i>Bos Taurus</i> | 0.023 | 0.175 | 0.008 | <0.001 |
| <i>Bos Indicus</i> -influenced | 0.449 | 0.096 | 0.001 | 0.051 |
| North American Combined | 0.085 | 0.096 | <0.001 | <0.001 |
| Australian Populations | | | | |
| <i>Bos Taurus</i> | 0.007 | 0.376 | 0.296 | 0.126 |
| <i>Bos Indicus</i> -influenced | 0.008 | 0.309 | 0.124 | <0.001 |
| Australian Combined | <0.001 | 0.442 | 0.101 | <0.001 |
| Combined North American and Australian Populations | | | | |
| <i>Bos Taurus</i> | <0.001 | 0.244 | 0.007 | <0.001 |
| <i>Bos Indicus</i> -influenced | 0.053 | 0.125 | 0.001 | <0.001 |
| External Combined | <0.001 | 0.111 | <0.001 | <0.001 |

Figure 1 – Example of the summary page in the new GeneSTAR MVP reporting format.

| | | Molecular Value Prediction | | | |
|------------------------------|--|----------------------------|-------|-------|-------|
| | | FE | Marb | Tend | |
| Breed: | Breed MVP Statistics: | Average | 0.48 | 0.17 | -0.17 |
| | | Min | -0.84 | -0.30 | -0.92 |
| | | Max | 1.29 | 0.52 | 0.57 |
| | Herd MVP Statistics: | Average | 0.53 | 0.19 | -0.20 |
| | | Min | -0.38 | -0.25 | -0.83 |
| | | Max | 1.21 | 0.52 | 0.57 |
| | Herd Distribution of Animals Relative to Breed Quartiles: | Top 25% (76–100 quartile) | 5% | 52% | 29% |
| | | 51–75% (quartile) | 21% | 27% | 25% |
| | | 26–50% (quartile) | 25% | 18% | 25% |
| Bottom 25% (1–25 quartile) | | 48% | 4% | 21% | |
| Herd Reliability Statistics: | Average | 33.8% | 21.8% | 38.9% | |
| | Min | 26.0% | 15.8% | 25.9% | |
| | Max | 35.9% | 22.9% | 41.4% | |

Conclusions and Implications for Use

The GeneSTAR MVP tool produces Molecular Value Predictions based on a panel of 56 DNA markers for feed efficiency, marbling and tenderness. These MVPs have average reliability values of 30, 26 and 49 percent for the three traits, respectively. These MVPs have been further validated in independent research in both Australian and North American cattle populations representing a wide range of breeds and production systems.

GeneSTAR MVPs are directly applicable to genetic improvement in these economically relevant traits for both seedstock and commercial cow-calf production systems. They are also applicable as tools for use in predicting phenotypic performance of cattle in commercial production systems, making them valuable tools for facilitating precision management to enhance profitability across the beef value chain.

(Complete technical reports summarising the research and development studies underlying the GeneSTAR MVP system are on file as Pfizer Animal Genetics Technical Reports 57, 58 and 59.)

1 The marbling MVP is significantly associated with Ultrasound as % IMF at 60 days prior to harvest.

2 Full meta-analysis data on file.

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