Pfizer *Animal Health*
Animal Genetics

TECHNICAL SUMMARY

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GeneSTAR MVPs — Molecular Value Predictions for beef feed efficiency[1] marbling[2] 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 39 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 and marbling and three markers for tenderness have been utilized 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 utilize individual animal precision management practices and fully exploit genome-enabled genetic improvement programs. This comprehensive approach is the cornerstone of Pfizer Animal Genetics' long-term goal of providing full service genomic solutions to the global cattle industry.

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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 utilizes 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 USDA degree of marbling)

- 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 accuracies.

3) Evaluation of the relationship between MVPs and phenotypic performance in a range of populations within the Pfizer Animal Genetics research program.

4) Third-party 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 (M1). This product was offered commercially in 2000. Since 2004 three additional marbling and three tenderness markers have been 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, 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 11 markers are included in the new panel along with an additional 45 markers from more recent discovery and validation efforts. These additional 45 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 11 markers and the recently released additional 45 markers can be found at www.pfizeranimalgenetics.com.

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 favorable alleles—zero, one or two—for a particular marker, which was then translated into a number of stars. With the realization 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 maximized by fitting marker additive effects as a regression on the number of favorable 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	11[a]	STARS[b]	NA[c]	✓
New GeneSTAR MVP	56	Trait Prediction[d]	26 – 39 %[e]	✓✓

[a]Four markers each for feed efficiency and marbling and three for tenderness
[b]Number of favorable alleles
[c]Not applicable
[d]Estimate of molecular breeding value
[e]% Reliability value ranges across traits

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

Genotype	Number of Favorable Alleles	Molecular Breeding Values as Deviation from Population Mean	Error Variance
QQ	2	$(2 - 2p)a$	$(2 - 2p)^2 Va$
Qq	1	$(1 - 2p)a$	$(1 - 2p)^2 Va$
qq	0	$(0 - 2p)a$	$(0 - 2p)^2 Va$

Molecular breeding value as the deviation from population mean and its error variance for genotypes with zero, one or two copies of the favorable allele of a marker (a is the substitution effect of the marker, p is the frequency of the favorable 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 (NFI/RFI); marbling is measured as marbling score (MS) and quality grade (USDA QG); 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 = 2,866) with all other populations (N = 4,455) 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 (summarized 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 39 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

[a]Marbling 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).
[b]Composite containing *Bos Indicus* and *Bos Taurus* breeds.
[c]This population includes Angus, Brahman, Charolais, Hereford, Limousin, Maine-Anjou, Red Angus, Shorthorn and South Devon breeds.
[d]Number of observations varied by trait within the range given.
[e]Marbling score assessment of degree of intramuscular fatness under the USDA grading system (4.0 = Small 0 degree of marbling).
[f]Marbling measured as % IMF.

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

Trait	Range in N	Correlation (R)[d]	% Reliability[e]	% Genetic Variation[f]	P-value[g]
			Fitting 56-marker panel[a,b,c]		
Feed Efficiency					
Overall	1,749	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	6,127	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	2,030	0.30	39.0	24.6	
Range	206–996	0.17–0.35	30.5–53.1	9.3–28.2	0.02–0.01

[a]Prediction of MVPs based on the 56-marker panel.
[b]Overall value for R, % reliability, and % genetic variation is calculated as the weighted average over all populations in which the trait was evaluated.
[c]Range in values across populations is shown in the second row.
[d]The maximum value possible for R is the square root of the heritability of the trait.
[e]Ratio of R to the square root of the heritability of the trait.
[f]Percentage of additive genetic variation accounted for by variation in the MVPs.
[g]Range in P-values across the populations evaluated is given to match the range in N.

Results of Third-Party Independent Validation Analyses

The final step in the development of GeneSTAR MVPs was third-party 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.

Realizing 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 39 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
B (NBCEC)	European Maternal Line Composite	Steers 150–180	785	MS	4.08	0.55
			785	IMF	2.99	0.68
			723	% Choice	0.57	0.49
C (NBCEC)	Bos Indicus-influenced Composites[b]	Steers: 70 d Feed Intake	392	MS	4.28	0.97
			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
D (CRC)	Multi-breed[c]	Various[d]	1228	MS	1.00	0.88
			1345	IMF	4.86	1.99
			1377	NFI	0.03	1.11
			1244	WBSF	4.73	1.24

[a]MS = AUS-MEAT Marble Score (0–9) for population D and 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.
[b]Equal percentage of Simbrah, Red Brangus and Braford maternal line composites.
[c]Breeds included Angus, Brahman, Belmont Red, Hereford, Murray Grey, Santa Gertrudis and Shorthorn.
[d]Cattle were either grass finished or grain finished to either domestic specifications (90 days on feed), Korean (150 days) or Japanese (300 days).
[e]SD = Standard deviation
[f]Warner-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

[a]Warner-Bratzler shear force at one-day aging.

Overall Meta-analysis of Results

In order to summarize 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 datasets, 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	-0.73	0.01	-0.93
% 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. 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*-values[3]

	NFI	Marb	IMF	Tend
North American Populations				
Bos Taurus	0.023	0.175	0.008	<0.001
Bos Indicus-influenced	0.449	0.096	0.001	0.051
North American Combined	0.085	0.096	<0.001	<0.001
Australian Populations				
Bos Taurus	0.007	0.376	0.296	0.126
Bos Indicus-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				
Bos Taurus	<0.001	0.244	0.007	<0.001
Bos Indicus-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.

Breed: Angus			Molecular Value Prediction		
			FE	**Marb**	**Tend**
	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 39 percent for the three traits, respectively. These MVPs have been further validated in third-party 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 summarizing the research and development studies underlying the GeneSTAR MVP system are on file as Pfizer Animal Genetics Technical Reports 57, 58 and 59.)

1 Validated in *Bos Taurus* populations only, additional information available on file for *Bos Indicus*. Feed Efficiency is measured as Net Feed Intake.
2 In NBCEC validation, the marbling MVP is significantly associated with Ultrasound as % IMF at 60 days prior to harvest.
3 Full meta-analysis data on file.

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