RESEARCH REPORT
ANIMAL SCIENCE

Title: Validation of commercial DNA tests for production efficiency, meat quality, and maternal traits in purebred swine - **NPB ID #08-233**

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Industry Summary:

As our knowledge about the genetic factors affecting economically important traits increases, application of such information may add to the efficiency of pig improvement programs through extra response to selection. Commercial DNA tests have recently become available for use by US independent purebred seedstock producers. GeneSeek Inc., a commercial genotyping company in Lincoln, Nebraska has licensed the testing of numerous markers implicated to impact production efficiency of commercial pork production systems. However, the phenotypic effects of many of these markers have been evaluated in commercial crossbred populations with vastly different genetic backgrounds than that of purebred animals within US purebred seedstock herds.

The objective of this study is to examine the potential use of commercially available genetic markers in marker-assisted selection schemes employed by independent purebred seedstock producers, accomplished by the characterization of the allele segregation of commercially available markers in US purebred Duroc, Landrace, and Yorkshire populations as well as verification of the effects of commercially available DNA tests within the US purebred population. Phenotypic data and DNA samples were utilized from three different repositories representing purebred pigs. Growth performance, carcass composition, and meat quality data from the National Barrow Show Sire Progeny Test (N = 522) as well as the National Swine Registry (NSR) Pork Quality Alliance Program (N = 364) were used to evaluate associated DNA tests. Maternal records from purebred Landrace and Yorkshire females (N = 885) from the NSR national performance database were utilized to evaluate maternal DNA tests.

The potential impact of selecting for a genetic marker depends on both the magnitude of its effect and its frequency in the population. Thus, the allele frequencies provide useful information suggesting to the potential application of available DNA markers. The two maternal markers tested within the current study (ESR and EPOR) are fixed for the unfavorable allele within the Duroc breed. Therefore, these DNA tests do not provide a useful avenue for improvement of maternal performance in Durocs.

In the present study, an advantage in number born alive was observed for first-parity Yorkshire females that carry two copies of the favorable form of the ESR marker (GG). Also, a significant advantage in number born alive was also detected within Landrace females at parity 1 for the EPOR marker. Sows that inherit two copies of the C-variant of the EPOR marker were associated with approximately 2 more pigs per litter when compared to sows homozygous for the T allele. The observed litter size differences in first-parity females for each maternal marker provides an opportunity for genetic improvement of litter size within the US purebred Yorkshire and Landrace populations.

Selection for the G-variant of the MC4R marker may prove to be a useful tool for Duroc populations aimed at increased carcass leanness, as a 0.07 in. advantage in leanness was observed for pigs that carry two copies of the favorable MC4R allele in the current study. For an additional DNA marker, HMGA1, a significant difference between HMGA1 genotype classes was observed for backfat within the Duroc breed (difference between homozygote classes = 0.05 in.). The effect of HMGA1 genotype was not significant for backfat in the remaining two breeds. The CCKAR marker was also evaluated for its potential application to improvement of growth performance. The Duroc breed was fixed for the favorable CCKAR allele and was not included in the analysis. However, a significant effect was estimated within the Landrace breed for both measures of growth performance, and may prove to be a useful tool in selection programs.

Two different DNA tests were evaluated for their effects on meat and eating quality traits. The favorable form of the PRKAG3 marker was only abundant enough to effectively evaluate the DNA test within the Duroc breed. In general, PRKAG3 genotype was not found to be a significant source of variation for the meat quality measures evaluated in this study. A second DNA-based meat quality test (CAST) was also evaluated within all three breeds. Two separate markers of the same gene are currently available. Within the current study, both markers were evaluated together as a single test (haplotype). Evaluated as the number of inherited copies of the favorable allele across markers, the CAST haplotype did not significantly contribute to enhanced meat or eating quality within the current study. However, a significant difference among haplotype classes was observed for juiciness score within the Yorkshire breed, where pigs with a greater number of A alleles corresponded to increased juiciness as detected by a sensory taste panel.

As is the case with populations where maker tests are discovered, the findings of validation studies are largely dependent on the specific characteristics of the populations under evaluation, which may lead to differing results from separate populations. Therefore, before marker-assisted selection is initiated, DNA markers found to be associated with an advantage for a trait of interest need to validated within the population where selection will be conducted. In the current study, several DNA tests currently available for testing and use have been confirmed to provide significant opportunity to gain additional selection accuracy and genetic progress. On the other hand, some DNA markers implicated to have effects on economically relevant characters in previous studies were not confirmed within the present study. It is important to note, however, that lack of statistically significant associations should not be interpreted as a marker effect equal to zero. As was observed in the current study, minor allele frequencies may be so small that there is no real opportunity to effectively evaluate the DNA test.

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Scientific Abstract

Use of molecular genetic information in selection programs can increase the capacity of management and selection tools already available. Commercial DNA tests have recently become available for use by US independent purebred seedstock producers. GeneSeek Inc., a commercial genotyping company in Lincoln, Nebraska has licensed the testing of numerous markers implicated to impact production efficiency of commercial pork production systems. The primary objective of this study is to examine the potential use of commercially available genetic markers in marker-assisted selection schemes employed by independent purebred seedstock producers, accomplished by the characterization of the allele segregation of commercially available markers in US purebred Duroc, Landrace, and Yorkshire populations as well as verification of the associations between phenotypes and alleles segregating within genes commercially available for testing.

Phenotypic data and DNA samples were utilized from three different repositories representing purebred pigs (N = 886 for growth, composition, and meat quality analysis; N = 885 for maternal analysis). Analyses were performed separately for each breed and locus for measures of growth, composition, and meat quality; however for the analysis of maternal traits, analysis was also performed separately by parity.

In parity 1 Yorkshire females, an increase in number born alive was observed for females of the GG genotype class. The difference in homozygous classes for the ESR gene in parity 1 Yorkshire females was approximately 0.87 pigs per litter. A significant difference between EPOR genotype classes was detected for first-parity Landrace females, where females homozygous for the C allele had 2.23 more pigs per litter than females carrying two copies of the T allele. The analysis of MC4R revealed no significant differences for measures of growth performance (days to 250 lbs. and average daily gain) among all three breeds evaluated. Analysis of tenth-rib backfat revealed a significant difference between homozygous MC4R genotype classes within the Duroc breed (0.60 in. for GG animals vs. 0.67 in for AA animals). PRKAG3 genotype was not found to be a significant source of variation for the meat quality measures evaluated in this study. The effect of the CAST gene was evaluated as the haplotype of both CAST loci (CAST249 and CAST638) within all three breeds, and the number of inherited copies of the favorable allele did not significantly contribute to enhanced meat or eating quality within the current study. However, a significant difference among haplotype classes was observed for juiciness score within the Yorkshire breed, where pigs with a greater number of A alleles corresponded to increased juiciness as detected by a sensory taste panel. This result was not found for the remaining two breeds, however.

Before marker-assisted selection is initiated, markers found in linkage disequilibrium with QTL that have an effect on a trait of interest need to validated within the population where selection will be conducted. In the current study, several DNA test currently available for testing and use have been confirmed to provide significant opportunity to gain additional selection accuracy and genetic gain within US purebred herds. On the other hand, some DNA markers implicated to have effects on economically relevant characters in previous studies were not confirmed within the current study.

Introduction

Due to an increasingly volatile and competitive hog market, smaller-scale purebred seedstock producers within the US have struggled with the issue of how to remain economically viable within the pork industry. Due to recent drastic increases in feed and energy costs, coupled with heightening concerns of consumer acceptance, traits related to lean growth, maternal efficiency, and meat quality have received more relative interest in swine selection schemes.

Use of molecular genetic information in selection programs can increase the capacity of management and selection tools already available. As our knowledge about the genetic factors affecting economically important traits increases, application of such information may add to the efficacy of pig improvement programs through extra responses to selection (Dekkers, 2004). Biological and positional candidate genes affecting several traits of economic importance have been documented due to advancements of linkage and physical maps of the pig genome as well as a growing understanding of the function and structure of the individual genes and gene families that are responsible for swine traits (Rothschild and Plastow, 1999).

Commercial DNA tests have recently become available for use by US independent purebred seedstock producers. GeneSeek Inc., a commercial genotyping company in Lincoln, Nebraska has licensed the testing of numerous markers implicated to impact production efficiency of commercial pork production systems (http://www.geneseek.com, last accessed 10 July 2008). However, the phenotypic effects of many of these markers have been evaluated in commercial crossbred populations with vastly different genetic backgrounds than that of purebred animals within US purebred seedstock herds.

The widespread adoption of marker-assisted selection (MAS) in the purebred industry will likely depend upon the successful integration of marker information into national swine genetic evaluation methodology to enable the development of DNA marker-assisted EPDs. Validation studies can serve to generate information that is essential for the process of incorporating DNA tests into a national genetic evaluation. Such information includes the size of the allelic substitutions in a range of breeds, genetic backgrounds, and production environments. Validation studies may help build confidence in marker technology and assist in providing the necessary information to implement marker data into national genetic evaluation systems (Van Eenennaam et al., 2007).

Purebred producers have a strong interest in the further understanding of whether marker effects are consistent with the findings of the original studies where phenotypic associations were detected. Thus, producers are interested in third-party, independent evaluation of these available marker tests in a purebred genetic background, similar to their own herds. However, due to the general size and scope of US independent purebred seedstock breeders, these producers have limited availability of funds for research and development, specifically as it pertains to the development of large, well-organized, thoroughly phenotyped populations for independent marker validation studies.

There is a need to set priorities by establishing which major genes and genetic markers are the most promising for application and to evaluate the benefits and costs associated with each promising gene or genetic marker before substantial funds are invested in their use by US seedstock breeders. The information developed from this project will inevitably aid in this effort and thus will be used to facilitate the genetic progress of purebred breeders through the implementation of marker-assisted EPDs within the national swine genetic evaluation. Additionally, many US independent seedstock producers serve as the Great Grandparent (GGP) and nucleus herds for numerous multiplier herds, and thus affect a significant proportion of market hogs marketed domestically and internationally. Added genetic gain captured through marker-assisted technology will ultimately increase the exposure and sustainability of US purebreds in international and domestic markets.

Objectives

The primary objective of this study is to examine the potential use of commercially available genetic markers in marker-assisted selection schemes employed by independent purebred seedstock producers. This objective will be accomplished through two underlying steps: 1) characterization of the allele

segregation of commercially available markers in US purebred Duroc, Landrace, and Yorkshire populations; and 2) verification of the association between phenotypes and alleles segregating within genes as claimed by the commercial genotyping company. The information developed from the accomplishment of the above objectives will be used to facilitate the genetic progress of purebred breeders through the implementation of marker-assisted EPDs within the national swine genetic evaluation system.

Materials and Methods

DNA Tests

The candidate gene approach has been successful in identifying major genes that affect traits of economical importance. To date, several positional candidate genes, based on comparative analysis of identified QTL, along with biological candidate genes chosen due to their influence on the underlying physiology of the trait have been found (Rothschild and Plastow, 1999). The following candidate genes detected through the above approaches that have been implicated to be associated with growth, meat quality, and maternal traits, and are currently available for commercial testing. GeneSeek Inc., a commercial genotyping company in Lincoln, Nebraska has licensed the testing of these markers (http://www.geneseek.com, last accessed 10 July 2008).

Protein kinase AMP-activated γ_3-subunit (PRKAG3). Ciobanu et al. (2001) recently identified novel genetic variants of the PRKAG3 gene within a commercial swine population. Over 1800 animals were genotyped for three separate missense substitutions, and association analysis revealed the presence of new economically important alleles of the PRKAG3 gene affecting the glycogen content in the muscle and resulting meat quality characteristics. Specifically, significant differences between homozygote genotype classes for the I199V locus were detected for numerous meat quality characteristics.

Calpastatin (CAST). Several association studies have evaluated variations in sequence of the CAST gene with meat tenderness in cattle and pigs (Lonergan et al., 1995; Kocwin-Podsiadla et al., 2003). CAST has been well documented as a specific inhibitor of μ- and m-calpain proteases, and it has been shown that postmortem activity of calpastatin is related to meat tenderness. Based on its location and documented function, CAST was considered as a potential candidate gene to possibly describe variation in meat quality characteristics. Ciobanu et al. (2004) evaluated the presence and association of new alleles/haplotypes within the CAST gene with several meat quality traits. This study detected significant associations between two different CAST alleles (249 and 638) and tenderness values.

Melanocortin-4 receptor (MC4R). Various mutations within the MC4R gene have been shown to be associated with obesity in humans. Adan et al. (2006) reported that humans with differing genotypes for MC4R coincide to differences in eating behavior. Kim et al. (2000) reported similar effects of feeding behavior that have been previously found in humans and mice in an evaluation involving pigs. This study reports that a missense mutation (G/A) within the seventh transmembrane domain at codon 298 on porcine chromosome 1 is associated with growth and fatness. Overall, pigs in this study with the 11 genotype had approximately 8% less backfat and gained 37 g/day less than pigs homozygous for allele 2.

High Mobility Group AT-hook Protein 1 (HMGA1). Kim et al. (2004) found that the HMGA1 polymorphism approached fixation in populations that have undergone intense selection for lean percentage. In commercial nucleus lines, a significant association between HMGA1 genotype and backfat measures was detected in lines where allelic segregation was sufficient for adequate power to test associations. In commercial Landrace and Large White populations, favorable allele frequencies were 67% and 51%, respectively, while the favorable allele frequency in a commercial Duroc population was higher (81%).

Cholecystokinin Type A Receptor (CCKAR). Houston et al. (2006) evaluated the effects of a novel polymorphism of the CCKAR gene in commercial nucleus populations, where significant associations between measures of feed intake, average daily gain, and days to market were estimated. Within commercial lines, analysis revealed a dominant mode of inheritance, indicating that progeny of homozygous parents reveal effects of enhanced growth and appetite irrespective of the genotype of the other parent.

Estrogen Receptor (ESR). Mutations in the estrogen receptor gene can produce considerable phenotypic changes in the mammalian reproductive system. Initial studies of the ESR gene, utilizing the Chinese Meishan breed discovered variation at the ESR locus in swine (Rothschild et al., 1996). The advantageous allele has a positive additive effect on total number born and number born alive in swine. The effect of this allele has been shown to range from 1.25 pigs per litter in Meishan crosses to 0.4 pigs per litter in Large White (Rothschild et al., 1996; Short et al., 1997).

Erythopoietin Receptor (EPOR). EPOR has been shown to be associated with uterine capacity and litter size in swine. Sows homozygous for the favorable allele have been shown to have increased uterine capacity, which corresponds to an increase in live births (Vallet et al., 2005). In two different swine populations at USDA-MARC, an extra pig per litter was observed when comparing boars that carry two copies of the favorable allele (T) of the EPOR marker with boars homozygous for the C allele.

Sample Populations

Phenotypic data and DNA samples were utilized from three different repositories representing purebred pigs (Table 1). The first of which (dataset A) consists of an extensive phenotypic database that has been established through the National Barrow Show® Sire Progeny Test. Through this project, extensive growth, carcass composition, meat quality, and eating quality data has been annually recorded on purebred animals from different herds (Goodwin et al., 2001). Corresponding loin tissue samples were collected on pigs from 2004 and after. The second dataset (dataset B) comprised of meat quality phenotypes and corresponding loin tissue samples collected within the NSR Pork Quality Alliance Program (Schwab et al., 2007), which consists of monthly producer cut-tests. The third dataset (dataset C) is comprised of maternal records on purebred Landrace and Yorkshire females from the national performance database at NSR. Corresponding blood samples were collected on Whatman™ blotter cards currently utilized by NSR for various other DNA testing procedures. Datasets A and B were used for the validation of genetic markers implicated to have an effect on growth, composition, and meat quality traits, while dataset C will be used for the validation of maternal genetic markers. All tissue samples and blotter cards were sent to GeneSeek Inc. for genotyping of markers that correspond to the appropriate dataset as summarized in Table 1.

Table 1. Summary of datasets used for DNA marker validation.

Dataset[1]	Animals	Herds	Sires	Dams
A	522	13	71	248
B	364	9	82	186
C	885	15	317	625

[1]Dataset A = National Barrow Show Sire Progeny Test, 2004-2009;
Dataset B = National Swine Registry Pork Quality Alliance Program;
Dataset C = maternal records from NSR national performance database.

Meat and Eating Quality Measurement

Standard carcass collection procedures (NPPC, 2000), were followed to obtain carcass measurements. Carcass pH was measured 24 h post-mortem on the 10th rib face of the longissimus muscle using a pH star probe (SFK Ltd, Hvidovre, Denmark). Objective color measurements of Minolta L* were obtained on the 10th face of the loin using a Minolta CR-310 (Minolta Camera Co., Ltd., Osaka, Japan) with a 50-mm-diameter aperture, D65 illuminant, and calibrated to the white calibration plate. Within the NBS Progeny Test (Dataset A), a section of bone-in loin containing the 10th to 12th ribs was excised from the carcass and transported to the Iowa State University Meat Laboratory. A slice from the 10th rib face was then removed and utilized for percent lipid content analysis (Bligh and Dyer, 1959). The 11th and 12th rib sections were cut into 1 in. chops and set freshly cut side up for 10 min to allow the sample to bloom. Subjective measures of color (1 = pale pinkish gray to white; 6 = dark purplish red) and marbling (1 = 1.0% IMF; 10 = 10.0% IMF) were evaluated on the 11th rib face according to NPPC (2000) by personnel trained in meat quality evaluation. Water holding capacity was measured on the 11th rib face using the filter paper method described by Kauffman et al. (1986) and is reported in milligrams of water absorbed by the filter paper (lower values are more desirable).

A trained sensory panel with 3 members evaluated cooked loin quality attributes (Huff-Lonergan et al., 2002) on the 11th and 12th rib sections 7 days post mortem. Both rib sections were cooked to 71º C in an electric broiler (Amana model ARE 640, Amana, IA), with sample temperature monitored by Chromega/Alomega thermocouples attached to an Omega digital thermometer (DSS-650, Omega Engineering, Inc., Stamford, CT). Weights prior to and immediately after cooking were used to calculate percent cooking loss. Three equally-sized cubes were removed from the center of the 11th rib sample and evaluated by the trained sensory panel for juiciness (1 = dry, 10 = juicy), tenderness (1 = tough; 10 = tender), and flavor (1 = little pork flavor, bland; 10 = extremely flavorful, abundant pork flavor) using an end-anchored, 10-point scoring system (AMSA, 1995). Individual booths with red overhead lighting were provided for each panelist. Sample evaluations were averaged across panelists for analysis. The 12th rib section was evaluated for tenderness using an Instron Universal Testing Machine (model 1122; Instron Corp., Canton, MA) fitted with a circular, 5-pointed star probe (9 mm diameter with 6 mm between points) (Oltrogge-Hammernick and Prusa, 1987).

Statistical analysis

Differences between genotypes for phenotypic measures of meat quality, maternal, composition, and growth traits were assessed with the use of the PROC MIXED procedure in SAS (SAS Inst., Cary, NC). Analyses were performed separately for each breed and locus for measures of growth, composition, and meat quality; however for the analysis of maternal traits, analysis was also performed separately by parity. The basic model was y = marker genotype + sex + contemporary group + sire + dam. Marker genotype and sex were fitted as fixed effects, while contemporary group, sire, and dam were fitted as random. For the analysis of backfat and days to 250 lbs., a linear and quadratic covariate of off-test weight was also included. For the analysis of average daily gain, linear and quadratic covariates of on-test weight were used in place of off-test weight. Number born alive was pre-adjusted for effects of age at first farrow and parity prior to analysis using current adjustment factors used within the current STAGES™ national genetic evaluation (Stewart et al., 1991). Contemporary group within the analysis of growth and composition measures was defined by off-test date, while contemporary group was defined as slaughter date for meat quality measures. The contemporary group effect for analysis of number born alive was defined as the combination of herd, year, and season of the parity record. The fixed marker effect was defined as the genotype class (i.e. 11, 12, or 22) for all DNA markers except for both CAST loci. For the evaluation of the CAST gene, the effects of both loci were assumed equal and additive, so the

marker genotype fitted in the above model represents the number of favorable alleles across both markers (i.e. 0, 1, 2, 3, or 4).

Results

Allele Frequencies

The genotype and allele frequencies for each SNP evaluated in the commercial tests involved in this validation study are shown in Tables 2, 3, and 4. The frequencies in these tables include all animals currently tested within each of the purebred Duroc, Landrace, and Yorkshire populations and represent the currently estimated allelic segregation for each loci. The potential impact of selecting for a genetic marker depends on both the magnitude of its effect and its frequency in the population. Thus, the allele frequencies detailed above provide some useful information suggesting to what the marker frequency may be within each breed population.

Table 2. Genotypic and allele frequencies of purebred Duroc, Landrace, and Yorkshire animals currently tested for ESR and EPOR.

	No. of Animals			Genotypic Frequency (%)			Allele Frequency (%)	
***ESR*[a]**	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	**A**	**G**
Duroc	95	1	0	98.96	1.04	0.00	99.48	0.52
Landrace	617	46	1	92.92	6.93	0.15	96.39	3.61
Yorkshire	308	370	118	36.69	46.48	14.82	61.93	38.07
***EPOR*[b]**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	**C**	**T**
Duroc	97	0	0	100.00	0.00	0.00	100.00	0.00
Landrace	403	230	37	60.15	34.33	5.52	77.31	22.69
Yorkshire	726	75	0	90.64	9.36	0.00	95.32	4.68

[a]Estrogen Receptor (ESR) gene; G has been designated as the favorable allele in previous studies (Rothschild et al., 1996; Short et al., 1997) and implicated to be associated with increased litter size.
[b]Erythopoietin Receptor (EPOR) gene; Sows homozygous for the favorable allele (T) have been shown to have increased uterine capacity, which corresponds to an increase in live births (Vallet et al., 2005).

Allelic segregation differs significantly by breed for each of the two maternal markers (Table 2). The Duroc breed is fixed for the undesirable allele for ESR, while the Yorkshire breed has the highest frequency of the desirable allele (38.07%). Similar results were observed for the EPOR locus. Durocs appear to be fixed for the undesirable form of the gene and Yorkshires carry this same allele in very low frequency (4.68%). However, the highest frequency of the favorable allele (22.69%) is in the Landrace breed.

MC4R has been implicated to have an underlying effect on appetite, thus each variant of the gene has been shown to have different effects that can be considered favorable. Pigs that are homozygous for the fast growth allele (AA) have been shown to reach market weight 3 days sooner when compared to pigs that are homozygous for the lean allele. If producers chose to select for the MC4R lean allele (G), it has been implicated that pigs will have 8% less backfat and eat significantly less feed (improving feed efficiency). This gene is segregating within all three breeds evaluated (Table 3). The G-variant, implicated to be associated with additional feed efficiency has a higher frequency among all three breeds, while the Landrace breed has the highest frequency of nearly 70%. The growth form of the gene (A) is most frequently found in the Yorkshire breed, while the Duroc breed has nearly equal segregation of each allele.

Similar allele frequencies have been characterized across all three breeds for CCKAR, as the favorable allele (G) appears to be greater than 80%. In comparison to the Yorkshire and Landrace breeds who have minor allele frequencies slightly greater than zero, the Duroc breed appears fixed for this gene. The favorable allele for HMGA1, as implicated in previous studies, has a higher frequency in the Duroc and Yorkshire breeds of 44.57% and 28.52%, respectively. However, the Landrace breed has the favorable allele in much lower frequency (12.62%) when compared to the remaining two breeds (Table 3).

Table 3. Genotypic and allele frequencies of purebred Duroc, Landrace, and Yorkshire animals currently tested for MC4R, CCKAR, and HMGA1.

	No. of Animals			**Genotypic Frequency (%)**			**Allele Frequency (%)**	
***MC4R*[a]**	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	**A**	**G**
Duroc	182	327	241	24.27	43.60	32.13	46.07	53.93
Landrace	151	138	30	47.34	43.26	9.40	68.97	31.03
Yorkshire	23	149	268	5.23	33.86	60.91	22.16	77.84
***CCKAR*[b]**	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	**A**	**G**
Duroc	0	0	751	0.00	0.00	100.00	0.00	100.00
Landrace	4	63	253	1.25	19.69	79.06	11.09	83.91
Yorkshire	4	88	346	0.91	20.09	79.00	10.96	89.04
***HMGA1*[c]**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	**C**	**T**
Duroc	238	351	157	31.90	47.05	21.05	55.43	44.57
Landrace	237	73	3	75.72	23.32	0.96	87.38	12.62
Yorkshire	236	157	47	53.64	35.68	10.68	71.48	28.52

[a]Melanocortin-4 Receptor (MC4R) gene; The A allele has been shown to be associated with fast growth, while the G variant has been shown to be associated with lean and efficient growth (Kim et al., 2000).
[b]Cholecystokinin Type A Receptor (CCKAR) gene; Animals that carry at least one copy of the G-variant have been shown to have higher feed intake and daily gain (Houston et. al., 2006).
[c]High Mobility Group AT-hook Protein 1 (HMGA1) gene; The T allele has been shown to be associated with added leanness (Kim et. al., 2004).

All meat quality markers (PRKAG3, CAST249 and CAST638) are segregating within all three breeds (Table 4). With the exception of CAST249, the Duroc breed appears to contain the favorable form of the gene in higher frequency. The Landrace breed has a considerably lower frequency of the favorable allele for PRKAG3 (2.72%), while each of the remaining two breeds contain the A-variant at much higher frequencies. Depending on the size of the effect within each breed, discovery and use of sires that carry at least one copy of the favorable allele for PRKAG3 may lead to substantial improvement in meat quality characteristics. Each Calpastatin locus has similar allele frequencies among each breed, where in the case of the CAST638 marker, the frequency of the favorable allele exceeds 60% for all three breeds.

Table 4. Genotypic and allele frequencies of purebred Duroc, Landrace, and Yorkshire animals tested for PRKAG3, CAST249, and CAST638.

	No. of Animals			Genotypic Frequency (%)			Allele Frequency (%)	
PRKAG3[a]	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	**A**	**G**
Duroc	63	285	402	8.40	38.00	53.60	27.40	72.60
Landrace	1	16	314	0.30	4.83	94.86	2.72	97.28
Yorkshire	11	111	337	2.40	24.18	73.42	14.49	85.51
CAST249[b]	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**	**A**	**G**
Duroc	199	359	192	26.53	47.87	25.60	50.47	49.53
Landrace	49	125	153	14.98	38.23	46.79	34.10	65.90
Yorkshire	128	243	89	27.83	52.83	19.35	54.26	45.76
CAST638[b]	**AA**	**AC**	**CC**	**AA**	**AC**	**CC**	**A**	**C**
Duroc	505	221	25	67.24	29.43	3.33	81.96	18.04
Landrace	178	122	27	54.43	37.31	8.26	73.09	26.91
Yorkshire	170	228	63	36.88	49.46	13.67	61.61	38.39

[a]Protein Kinase, AMP Activated, γ_3-subunit (PRKAG3); The A-variant of the marker has been illustrated in research populations to be associated with muscle glycogen content, pH, and color (Ciobanu et al., 2001).
[b]Calpastatin (CAST). Calpastatin is responsible for inhibiting proteases that affect meat tenderness after harvest. Two separate genetic variants have been identified within the CAST gene (CAST249 and CAST638). The A allele is considered favorable for each locus (Ciobanu et al., 2004).

Maternal DNA Tests

Large differences in allelic segregation was observed for each of the Landrace and Yorkshire breeds for each of the maternal DNA tests. The minor allele frequency in Landrace for ESR is only 3.61%, so analysis only included the Yorkshire breed. In contrast, the minor allele frequency in Yorkshire for EPOR was only 4.68%. As a result, the analysis for marker effects for EPOR only included the Landrace breed. In parity 1 Yorkshire females, an increase in number born alive was observed for females of the GG genotype class (Table 5). The difference in homozygous classes for the ESR gene in parity 1 Yorkshire females was approximately 0.87 pigs per litter. A significant difference between EPOR genotype classes was detected for first-parity Landrace females, where females homozygous for the C allele had 2.23 more pigs per litter than females carrying two copies of the T allele. Genotype effects were observed in first-parity females for both loci only. Analysis of marker effects was performed for later parities (genotype means for parities 3-5 not shown); however, no significant differences between genotype classes were detected.

Table 5. Effect of two different genetic markers on litter size in purebred Yorkshire and Landrace sows[1].

	ESR Genotype			
Yorkshire	**AA**	**AG**	**GG**	**Pr > F**
Parity 1	11.57 ± 0.24 (146)	11.87 ± 0.21 (196)	12.44 ± 0.37 (55)	0.158
Parity 2	11.58 ± 0.26 (125)	11.95 ± 0.23 (156)	12.03 ± 0.38 (59)	0.499
	EPOR Genotype			
Landrace	**CC**	**CT**	**TT**	**Pr > F**
Parity 1	12.10 ± 0.21 (184)	11.39 ± 0.26 (118)	9.87 ± 0.52 (23)	0.005
Parity 2	12.18 ± 0.22 (152)	12.00 ± 0.28 (97)	11.21 ± 0.66 (18)	0.503

[1]Number of observations for each genotype class are in parentheses

Growth and Composition DNA Tests

Least squares means for growth and composition traits for each genotype class for MC4R are presented in Table 6 for Duroc, Landrace, and Yorkshire pigs. No significant differences were detected for measures of growth performance (days to 250 lbs. and average daily gain) among all three breeds evaluated. Analysis of tenth-rib backfat revealed a significant difference between homozygous genotype classes within the Duroc breed (0.60 in. for GG animals vs. 0.67 in for AA animals). A trend for increased leanness in GG animals was observed for the Landrace breed; however, no significant difference was detected within the Yorkshire breed.

Table 7 shows the effect of HMGA1 genotype on backfat in Duroc, Landrace, Yorkshire pigs. A significant difference among genotypes was observed for the Duroc breed, while the difference between genotype classes approached significance in the Yorkshire breed (P = 0.06). However, only 2 observations were observed for the TT genotype in the Landrace breed, and a significant genotype effect was not detected.

The Duroc breed is fixed for the desirable allele of CCKAR, and was not included in analysis. Additionally, a low minor allele frequency was observed for each of the Landrace and Yorkshire breeds (11.09% and 10.96%, respectively). Despite the skewed allelic segregation in each breed, a significant genotype effect was detected for Landrace animals for both measures of growth performance.

Table 6. Effect of MC4R genotype on growth and composition traits in purebred Duroc, Landrace, and Yorkshire swine[1].

Trait / Breed	Genotype			
Days to 250 lbs.	**AA**	**AG**	**GG**	**Pr > F**
Duroc	161.20 ± 1.38 (334)	162.91 ± 1.30 (374)	162.49 ± 1.43 (206)	0.162
Landrace	164.20 ± 2.37 (28)	167.77 ± 1.40 (130)	168.27 ± 1.41 (141)	0.235
Yorkshire	170.51 ± 1.27 (237)	171.11 ± 1.43 (147)	170.22 ± 2.98 (18)	0.887
Average daily gain	**AA**	**AG**	**GG**	**Pr > F**
Duroc	1.85 ± 0.06 (59)	1.82 ± 0.06 (110)	1.82 ± 0.06 (38)	0.351
Landrace	1.82 ± 0.08 (7)	1.82 ± 0.07 (49)	1.84 ± 0.06 (47)	0.830
Yorkshire	1.82 ± 0.08 (92)	1.83 ± 0.08 (49)	1.77 ± 0.09 (7)	0.371
Tenth-rib backfat	**AA**	**AG**	**GG**	**Pr > F**
Duroc	0.67 ± 0.02 (215)	0.65 ± 0.02 (282)	0.60 ± 0.02 (150)	<0.001
Landrace	0.83 ± 0.04 (27)	0.78 ± 0.03 (120)	0.75 ± 0.03 (128)	0.138
Yorkshire	0.74 ± 0.02 (227)	0.74 ± 0.02 (131)	0.80 ± 0.04 (18)	0.307

[1]Number of observations for each genotype class are in parentheses

Table 7. Effect of HMGA1 genotype on tenth-rib backfat in purebred Duroc, Landrace, and Yorkshire swine[1].

	Genotype			
Breed	**CC**	**CT**	**TT**	**Pr > F**
Duroc	0.67 ± 0.02 (221)	0.64 ± 0.02 (320)	0.62 ± 0.02 (153)	<0.001
Landrace	0.77 ± 0.02 (212)	0.81 ± 0.03 (65)	0.94 ± 0.13 (2)	0.227
Yorkshire	0.79 ± 0.02 (204)	0.75 ± 0.02 (138)	0.73 ± 0.03 (41)	0.062

[1]Number of observations for each genotype class are in parentheses

Table 8. Effect of CCKAR genotype on growth traits in purebred Landrace and Yorkshire swine[1].

Trait / Breed	Genotype			
Average daily gain	**AA**	**AG**	**GG**	**Pr > F**
Landrace	1.57 ± 0.10 (2)	1.76 ± 0.06 (27)	1.84 ± 0.05 (82)	0.008
Yorkshire	NA	1.79 ± 0.07 (42)	1.81 ± 0.07 (113)	0.722
Days to 250 lbs.	**AA**	**AG**	**GG**	**Pr > F**
Landrace	185.87 ± 5.94 (3)	169.03 ± 1.86 (55)	167.02 ± 1.19 (242)	0.008
Yorkshire	163.59 ± 7.92 (4)	169.43 ± 1.70 (75)	171.24 ± 1.22 (322)	0.356

[1]Number of observations for each genotype class are in parentheses

Meat and Eating Quality DNA Tests

The marker effect of PRKAG3 was only evaluated within the Duroc breed due to the lack of sufficient segregation in Landrace and Yorkshire. Presented in Table 9 are the least squares means by PRKAG3 genotype within the Duroc breed. In general, PRKAG3 genotype was not found to be a significant source of variation for the meat quality measures evaluated in this study. The only exception, where a significant difference between genotypes was detected, occurred for the analysis of subjective marbling score where pigs carrying two copies of the A allele had a greater amount of marbling than animals homozygous for the G allele. However, this result was not confirmed for intramuscular fat percentage.

The effect of the CAST gene was evaluated as the haplotype of both CAST loci (CAST249 and CAST638) within all three breeds. Least squares means for each haplotype, designated as the number of favorable alleles (0, 1, 2, 3, 4) across both markers, are presented in Table 10. The individual effects of each CAST marker were also evaluated for each meat and eating quality measure, however, no significant associations were detected (data not shown). As illustrated in Table 10, the number of inherited copies of the favorable allele did not significantly contribute to enhanced meat or eating quality within the current study. However, a significant difference among haplotype classes was observed for juiciness score within the Yorkshire breed, where pigs with a greater number of A alleles corresponded to increased juiciness as detected by a sensory taste panel. This result was not found for the remaining two breeds, however.

Table 9. Effect of PRKAG3 genotype on meat quality traits in purebred Duroc swine[1].

	Genotype			
Trait	**AA**	**AG**	**GG**	**Pr > F**
24 hr. pH	5.75 ± 0.06 (44)	5.74 ± 0.06 (198)	5.73 ± 0.06 (280)	0.622
Color Score	2.90 ± 0.23 (37)	2.77 ± 0.21 (180)	2.70 ± 0.21 (282)	0.192
Marbling Score	2.22 ± 0.39 (44)	2.10 ± 0.36 (190)	1.86 ± 0.35 (271)	0.014
Intramuscular Fat %	2.64 ± 0.21 (31)	2.79 ± 0.11 (87)	2.76 ± 0.09 (139)	0.739
Cooking Loss %	17.46 ± 0.66 (31)	18.04 ± 0.42 (85)	18.33 ± 0.35 (137)	0.326
Instron	5.04 ± 0.17 (31)	5.34 ± 0.10 (87)	5.27 ± 0.08 (139)	0.204
Minolta L*	51.33 ± 1.11 (13)	51.79 ± 0.88 (110)	51.96 ± 0.87 (143)	0.709

[1]Number of observations for each genotype class are in parentheses

Table 10. Effect of CAST haplotype on meat and eating quality traits in purebred Duroc, Landrace, and Yorkshire swine.

Trait/Breed	Number of A alleles*				
24 hr. pH	**0**	**1**	**2**	**4**	**Pr > F**
Duroc	5.79 ± 0.06	5.73 ± 0.06	5.73 ± 0.06	5.73 ± 0.06	0.259
Landrace	5.60 ± 0.04	5.57 ± 0.04	5.58 ± 0.03	5.59 ± 0.04	0.720
Yorkshire	5.58 ± 0.04	5.59 ± 0.05	5.61 ± 0.04	5.61 ± 0.04	0.279
Cooking Loss %	**0**	**1**	**2**	**4**	**Pr > F**
Duroc	15.76 ± 1.21	17.21 ± 0.73	17.97 ± 0.44	17.99 ± 0.49	0.229
Landrace	18.98 ± 1.66	17.69 ± 0.83	19.37 ± 0.56	18.40 ± 0.91	0.285
Yorkshire	20.06 ± 0.85	19.15 ± 1.06	18.14 ± 0.49	18.73 ± 0.56	0.133
Instron	**0**	**1**	**2**	**4**	**Pr > F**
Duroc	5.12 ± 0.28	5.09 ± 0.17	5.10 ± 0.10	5.14 ± 0.11	0.990
Landrace	5.97 ± 0.34	5.43 ± 0.19	5.29 ± 0.14	5.34 ± 0.21	0.218
Yorkshire	5.39 ± 0.24	5.66 ± 0.29	5.48 ± 0.13	5.38 ± 0.15	0.768
Tenderness Score	**0**	**1**	**2**	**4**	**Pr > F**
Duroc	6.97 ± 0.44	7.01 ± 0.26	6.85 ± 0.14	6.66 ± 0.17	0.595
Landrace	6.09 ± 0.55	6.71 ± 0.29	6.41 ± 0.21	6.50 ± 0.31	0.653
Yorkshire	6.33 ± 0.39	6.37 ± 0.48	6.69 ± 0.23	6.71 ± 0.27	0.732
Juiciness Score	**0**	**1**	**2**	**4**	**Pr > F**
Duroc	7.14 ± 0.43	6.82 ± 0.26	6.59 ± 0.16	6.52 ± 0.18	0.415
Landrace	6.16 ± 0.58	6.45 ± 0.32	6.15 ± 0.24	6.26 ± 0.35	0.809
Yorkshire	5.47 ± 0.36	5.85 ± 0.44	6.36 ± 0.21	6.53 ± 0.25	0.041
Flavor Score	**0**	**1**	**2**	**4**	**Pr > F**
Duroc	2.29 ± 0.28	2.40 ± 0.18	2.46 ± 0.13	2.52 ± 0.14	0.774
Landrace	2.12 ± 0.18	2.06 ± 0.10	1.97 ± 0.08	2.09 ± 0.11	0.569
Yorkshire	1.98 ± 0.15	1.80 ± 0.18	1.97 ± 0.09	2.02 ± 0.10	0.660

*No observations were present for 3 copies of the A allele; haplotype combination of CAST249 and CAST638, represented as the number of A alleles across both markers.

Discussion

The frequency of the alleles for each gene observed within this study will play a large role on the potential impact of selection for pigs with the desired genotype. In most cases, when the favorable allele frequency is closest to 0.5, the greatest opportunity for genetic progress in the short term exists. However, when the frequency of the favorable allele approaches fixation (>80%), the incremental value of fixing the gene in the population is not as large, while when the frequency of the favorable allele is 0, there is no opportunity for genetic improvement through genotypic selection. In general, marker allele frequencies indicate that a majority of the genes are segregating. In most cases, the breed populations are not fixed for a certain allele and that there is potential for trait improvement where significant associations are detected between markers and phenotypes.

The two maternal markers tested within the current study are fixed for the unfavorable allele within the Duroc breed. Therefore, these DNA tests do not provide a useful avenue for improvement of maternal

performance in Durocs. Due to the long-term selection objectives employed within the US Duroc population through time, significant emphasis on post-weaning characters may have indirectly altered allele frequencies toward fixation. Similarly, each of the two maternal markers evaluated here correspond to largely different allelic segregation for the Landrace and Yorkshire breeds. Specifically, the ESR marker has a very low minor allele frequency in Landrace, whereas the minor allele frequency for the EPOR marker is very low for Yorkshire. Each situation, in combination with the effect of each marker that is sufficiently segregating, present different potential opportunities for genetic improvement of litter size.

In previous studies, the ESR gene marker has been found to be associated with litter size in pigs, where sows that are homozygous for the favorable allele have been shown to have 0.8 more total pigs per litter when compared to females homozygous for the unfavorable allele (Rothschild et al., 1996). In the present study, a similar difference between homozygous genotype classes was observed for first-parity Yorkshire females. However, the same marker effect was not found for later parities. EPOR has been shown to be associated with uterine capacity and litter size in swine. Sows homozygous for the favorable allele have been shown to have increased uterine capacity, which corresponds to an increase in live births (Vallet et al., 2005). A significant advantage in number born alive was also detected within Landrace females at parity 1 within the current study. Sows that inherit two copies of the C-variant of the EPOR marker were associated with approximately 2 more pigs per litter when compared to sows homozygous for the T allele. Similar to the results for Yorkshire parity-1 females at the ESR locus, the effect of the EPOR marker was only observed for first-parity performance. This finding may be caused by several factors. Due to the numerous physiological differences that exist among young sows (i.e. ovulation rate, age at puberty, acclimation stress, etc.), this result is not surprising. Additionally, the maternal performance in later parities may be associated with a higher level of selection bias that may alter the effects of each marker at later parities. Females that are retained in the herd after parity 1 generally must have demonstrated sufficient maternal performance, which may create a bias for maternal characters used to evaluate the marker effects in this study. However, the observed litter size differences in first-parity females for each maternal marker provides an opportunity for genetic improvement of litter size within the US purebred Yorkshire and Landrace populations.

Various mutations within the MC4R (Melanocortin-4 Receptor) gene have been shown to be associated with obesity in humans. Kim et al. (2000) reported similar effects of feeding behavior that have been previously found in humans and mice in an evaluation involving pigs for a missense mutation (G/A) on porcine chromosome 1. Pigs that are homozygous for the fast growth allele (AA) have been shown to reach market weight 3 days sooner when compared to pigs that are homozygous for the lean allele. In the current study, no differences were detected for measures of growth performance in any of the three breeds evaluated. For the analysis of backfat, however, a significant genotype effect was found within the Duroc breed, where pigs that carry two copies of the G allele have 0.07 in less backfat than pigs homozygous for the A allele. This effect was not observed within Landrace and Yorkshire in the current study, which may be due to the low number of observations within homozygous marker classes (AA for Landrace = 27; GG for Yorkshire = 18). Selection for the G-variant of the MC4R marker may prove to be a useful tool for Duroc populations aimed at increased carcass leanness.

The HMGA1 marker is segregating within all three breeds evaluated in the current study; however, large differences in allelic segregation were observed across breeds. Allele frequencies for the favorable allele (T) were 44.57%, 12.62%, and 28.52% for the Duroc, Landrace, and Yorkshire breeds, respectively. A significant difference between HMGA1 genotype classes was observed for backfat within the Duroc breed (difference between homozygote classes = 0.05 in.) where the minor allele frequency was the highest. The effect of HMGA1 genotype was not significant for backfat in the remaining two breeds. However, the

HMGA1 marker was not segregating to the same extent in the Landrace and Yorkshire breeds when compared to Duroc, which may have limited the power to detect an association within these lines. Kim et al. (2004) found that the HMGA1 polymorphism approached fixation in populations that have undergone intense selection for lean percentage. In commercial nucleus lines, a significant association between HMGA1 genotype and backfat measures was detected in lines where allelic segregation was sufficient for adequate power to test associations. In commercial Landrace and Large White populations, favorable allele frequencies were 67% and 51%, respectively, while the favorable allele frequency in a commercial Duroc population was higher (81%). These frequencies differ from those observed in the current study, which may explain the difference in estimated genotype effects.

Houston et al. (2006) evaluated the effects of a novel polymorphism of the CCKAR gene in commercial nucleus populations, where significant associations between measures of feed intake, average daily gain, and days to market were estimated. Within commercial lines, analysis revealed a dominant mode of inheritance, indicating that progeny of homozygous parents reveal effects of enhanced growth and appetite irrespective of the genotype of the other parent. In the present study, however, associations between average daily gain, and days to 250 lbs. were only estimated in Landrace and Yorkshire populations. The Duroc breed was fixed for the favorable allele and was not included in the analysis. Additionally, varying results were observed for each of the Landrace and Yorkshires pigs in the current study. A significant effect was estimated within the Landrace breed for both measures of growth performance. However, no difference was detected between genotype classes within the Yorkshire breed. It is important to note, however, that a small number of pigs carrying two copies of the A allele was observed, and limits the power associated with the association analysis (especially in the case of dominant gene action).

Allelic segregation differed substantially between the three breeds in the current study for the PRKAG3 marker. The Duroc population was the only breed where segregation of the marker allowed a sufficient number of observations within each genotype class was available to test the association between the PRKAG3 marker and measures of meat quality. In general, PRKAG3 genotype was not found to be a significant source of variation for the meat quality measures evaluated in this study. The only exception, where a significant difference between genotypes was detected, occurred for the analysis of subjective marbling score where pigs carrying two copies of the A allele had a greater amount of marbling than animals homozygous for the G allele. However, this result was not confirmed for intramuscular fat percentage. Ciobanu et al. (2001) identified novel genetic variants of the PRKAG3 gene within a commercial swine population. Over 1800 animals were genotyped for three separate missense substitutions, and association analysis revealed the presence of new economically important alleles of the PRKAG3 gene affecting the glycogen content in the muscle and resulting meat quality characteristics. Specifically, significant differences between homozygote genotype classes for the I199V locus were detected for numerous meat quality characteristics. For measures of meat quality, where a small amount of phenotypic variation typically exists, a large number of animals is needed to test associations. If in fact the PRKAG3 marker is associated with relatively small differences in meat quality, the number of available observations within the current study likely limits the ability to detect such differences.

Several association studies have evaluated variations in sequence of the CAST gene with meat tenderness in cattle and pigs (Lonergan et al., 1995; Kocwin-Podsiadla et al., 2003). CAST has been well documented as a specific inhibitor of μ- and m-calpain proteases, and it has been shown that postmortem activity of calpastatin is related to meat tenderness. Based on its location and documented function, CAST was considered as a potential candidate gene to possibly describe variation in meat quality characteristics. Ciobanu et al. (2004) evaluated the presence and association of new alleles/haplotypes within the CAST gene with several meat quality traits. This study detected significant associations between two different

CAST alleles (249 and 638) and tenderness values. In the current study, the effect of the CAST gene was evaluated as the haplotype of both CAST loci (CAST249 and CAST638) within all three breeds, as the minimum minor allele frequency was approximately 30% in all cases. The individual effects of each CAST marker were also evaluated for each meat and eating quality measure, however, no significant associations were detected. The number of inherited copies of the favorable allele did not significantly contribute to enhanced meat or eating quality within the current study. However, a significant difference among haplotype classes was observed for juiciness score within the Yorkshire breed, where pigs with a greater number of A alleles corresponded to increased juiciness as detected by a sensory taste panel. This result was not found for the remaining two breeds, however.

Due to the complexity and polygenic nature of traits such as growth, composition, and meat quality, one additional possibility for the discrepancy of results between previous studies/populations when compared to the results presented here may be due to background gene effects. There is an abundance of evidence indicating that epistasis has a significant effect on observable marker effects across populations. Mayo and Franklin (1998) implicated that epistasis among loci with large effects on a quantitative trait are common in studies involving various traits in plants.

Schwab et al. (2008) evaluated various genetic markers previously reported to have an effect on adipogenesis within two lines of purebred Duroc pigs. Variations in gene effects between lines found in this study further indicate that possible epistatic effects within different background genomes exist among different swine populations. Existence of the *MC4R* and *FABP3* mutations evaluated in this study may be useful markers in MAS schemes aimed at intramuscular fat improvement, provided that alleles within these genes are segregating and the presence of an association is detected within the population of interest.

The implications of epistasis for both detecting and using QTL in breeding programs are important when evaluating the economic benefit of incorporating molecular information. Therefore, before marker-assisted selection is initiated, markers found in linkage disequilibrium with QTL that have an effect on a trait of interest need to validated within the population where selection will be conducted. In the current study, several DNA tests currently available for testing and use have been confirmed to provide significant opportunity to gain additional selection accuracy and genetic gain. On the other hand, some DNA markers implicated to have effects on economically relevant characters in previous studies were not confirmed within the current study. It is important to note, however, that lack of statistically significant associations should not be interpreted as a marker effect equal to zero. As was observed in the current study, minor allele frequencies may be so small that there is no real opportunity to effectively evaluate the DNA test. As indicated in the above discussion, the findings of validation studies are largely dependent on the specific characteristics of the populations under evaluation. Though this is problematic for industry-wide extrapolation of results, independent validation of commercial DNA tests provides some assurance to purebred breeders that DNA tests perform according to previous findings (often from populations comprised of different genetic background, selection pressure, management, etc.), and assist in the development of needed data to integrate DNA-based technology into national genetic evaluation systems.

Literature Cited

Adan, R. A., B. Tiesjema, J. J. Hillebrand, S. E. la Fleur, M. J. Kas, and M. de Krom. 2006. The MC4R receptor and control of appetite. Br. J. Pharmacol. 149:815-827.

AMSA. 1995. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat. Am. Meat Sci. Assoc., Chicago, IL.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 3:911-917

Ciobanu, D., J. Bastaansen, M. Malek, J. Helm, J. Woollard, G. Plastow, and M. Rothschild. 2001. Evidence for new alleles in the Protein Kinase Adenosine Monophosphate-Activated γ_3-Subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics. 159:1151-1162.

Ciobanu, D. C., J. W. M. Bastiaansen, S. M. Lonergan, H. Thomsen, J. C. M. Dekkers, G. S. Plastow, and M. F. Rothschild. 2004. New alleles in calpastatin gen are associated with meat quality in pigs. J. Anim. Sci. 82:2829-2839.

Dekkers, J. C. M. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons. J. Anim. Sci. 82(E. Suppl.):E313-328.

Goodwin, R. 2001. Genetic parameters of pork quality traits. Proc. of the National Swine Improvement Federation Annual Meeting. St. Louis, MO. December 7, 2001.

Huff-Lonergan, E., T. J. Baas, M. Malek, J. C. Dekkers, K. Prusa, and M. F. Rothschild. 2002. Correlations among selected pork quality traits. J. Anim. Sci. 80:617-627.

Houston, R. D., C. S. Haley, A. L. Archibald, N. D. Cameron, G. S. Plastow, and R. A. Rance. 2006. A Polymorphism in the 5'-Untranslated Region of the Porcine Cholecystokinin Type A Receptor Gene Affects Feed Intake and Growth. Genetics. 174: 1555-1563.

Kauffman, R. G., G. Eikelenboom, P. G. van der Wal, B. Engel, and M. Zaar. 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Sci. 18:307-322.

Kim, K. S., N. Larsen, T. Short, G. Plastow, and M. F. Rothschild. 2000. A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mamm. Genome 11:131-135.

Kim, K. S., J. M. Reecy, W. H. Hsu, L. L. Anderson, M. F. Rothschild. 2004. Functional and phylogenetic analyses of a melanocortin-4 receptor mutation in domestic pigs. Dom. Anim. Endocrinol. 26:75-86.

Kocwin-Podsiadla, M., J. Kuryl, E. Krzecio, A. Zybert, and W. Przybylsky. 2003. The interaction between calpastatin and RYR1 genes for some pork quality traits. Meat Sci. 65:731-735.

Lonergan, S. M., C. W. Ernst, M. D. Bishop, C. R. Calkins, and M. Koohmaraie. 1995. Relationship of restriction fragment length polymorphisms (RFLP) at the bovine calpastatin locus to calpastatin activity and meat tenderness. J. Anim. Sci. 73:3608-3612.

Mayo, O., and I. R. Franklin. 1998. The place of QTL in the bias of quantitative genetics. I. General considerations. Proc. of the 6th World Congress on Genetics Applied to Livestock Production. 26: 77-80.

NPPC. 2000. Pork Composition and Quality Assessment Procedures. E.P. Berg, ed. National Pork Producers Council. Des Moines, IA.

Oltrogge-Hammernick, M., and K. J. Prusa. 1987. Research note: Sensory analysis and Instron measurements of variable-power microwave-heated baking hen breasts. Poult. Sci. 66:1548-1551.

Rothschild, M. F., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Mileham, and G. Plastow. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc. Natl. Adad. Sci. USA 93:201-205.

Rothschild, M. F., and G. S. Plastow. 1999. Advances in pig genomics and industry applications. AgBiotechNet 1:1-7.

Schwab, C. R., B. E. Mote, Z. Du, R. Amoako, T. J. Baas, and M. F. Rothschild. 2008. An evaluation of four candidate genes for use in selection programs aimed at increased intramuscular fat in Duroc swine. J. Anim. Breed. Genet. (Accepted).

Schwab, C. R., R. Bates, D. Anderson. 2007. NSR Muscle Quality Program: Current Structure. Proc. of the National Swine Improvement Federation Annual Meeting. Kansas City, MO. December 7, 2007.

Short, T. H., M. F. Rothschild, O. I. Southwood, D. G. McLaren, A. de Vries, H. van der Steen, G. R. Eckardt, C. K. Tuggle, J. Helm, D. A. Vaske, A. J. Mileham, and G. S. Plastow. 1997. Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. J. Anim. Sci. 75:3138-3142.

Stewart, T. S., D. L. Lofgren, D. L. Harris, M. E. Einstein, and A. P. Schinckel. 1991. Genetic improvement programs in livestock: swine testing and genetic evaluation system (stages). J. Anim. Sci. 69: 3882-3890.

Vallet, J. L., B. A. Freking, K. A. Leymaster, and R. K. Chrisenson. 2005. Allelic variation in the erythropoietin receptor gene is associated with uterine capacity and litter size in swine. Animal Genetics. 36:97-103.

Van Eenennaam, A. L., J. Li, R. M. Thallman, R. L. Quass, M. E. Dikeman, C. A. Gill, D. E. Franke, and M. G. Thomas. 2007. Validation of commercial DNA tests for quantitative beef quality traits. J. Anim. Sci. 85:891-900.