Asociación Mexicana de Médicos Veterinarios Especialistas en Cerdos
XXXVI Congreso Nacional Querétaro 2001
Julio 25 – 29 de 2001

APPLYING NOVEL TECHNOLOGY TO THE GENETIC IMPROVEMENT OF SWINE

David G. McLaren, Ph.D. and Graham S. Plastow, Ph.D.
PIC (Pig Improvement Company)

Summary

The last decade has seen remarkable progress in the understanding of genomes, including those of farm animals such as the pig. The swine industry has played a significant part in these developments, both by providing direct research support and by its rapid uptake of the practical tools that have been generated. The genetic linkage map now contains over 2000 loci including several hundred genes. This information has enabled researchers to search for loci influencing traits of economic importance using genome scans (or quantitative trait loci, QTL, analysis) and candidate gene approaches. QTL for growth and backfat, meat quality traits and reproduction have been identified. The causative mutations for traits such as porcine stress syndrome (HAL or CRC1), acid meat (RN) and coat colour have all been identified. A mutation in the FUT1 gene confers resistance to a common *E. coli* associated with a post-weaning scours. In addition, results obtained with polymorphisms in candidate genes have also been encouraging (e.g., ESR and PRLR for litter size; heart and adipocyte FABP for meat quality). The swine breeding industry is actively using this information to improve pork production using a combination of quantitative statistical and qualitative molecular techniques collectively referred to as marker assisted selection (MAS). Animal genomics will expand dramatically in the next few years, both in its own right and through the use of comparative methods, which will enable the exploitation of information from other species such as mouse and man including their genome sequences. New approaches such as high throughput gene expression analysis will supplement the DNA approaches used to date. The results obtained from molecular genetics will increasingly be combined with new reproduction techniques to change the way breeders produce and deliver genetic improvement to the global pork chain.

Introduction

Molecular genetics has revolutionised how research geneticists examine genetic differences existing in the pig. In the past few years, efforts have been directed toward the development of genomic maps consisting of anonymous genetic markers and known genes. In addition, comparative genome maps have aided greatly in our search for interesting and potentially useful genes in the pig. We are now at the stage where results from the laboratory are applied to swine breeding programs at an ever increasing rate.

This year marks the tenth anniversary of the first application of DNA marker technology in pig breeding (the Halothane gene test, see Fujii *et al.* 1991). Since then the number of marker tests available for use has increased steadily. In PIC we have successfully utilised markers for litter size, meat quality and more recently the first disease resistance marker in pigs, in order to provide improved and increasingly customized products for our pork chain customers.
The majority of markers used to date have been developed using candidate gene approaches, although in some cases positional information from mapping was crucial in the development of the marker tools. Candidate gene analysis is undertaken when a gene is chosen based on the physiology of the trait and where the candidate gene is assumed to affect trait performance.

More recently we have seen an increase in the number of results generated from QTL linkage analyses. These QTL analyses involve employing a genomic scan where generally F2 or backcross families are used and genotypes are obtained for many (>75) markers evenly spaced across the genome. Several such experiments have recently been completed and are beginning to produce interesting and useful results. However, there are a number of drawbacks to QTL analyses in terms of application. Firstly, QTL mapping has so far involved model populations and very few studies have been conducted using commercial pig populations. It is not known, therefore, how applicable these results are in commercial situations. Secondly, the region of the genome that is identified is relatively large and will contain several hundred or thousand genes making it difficult to identify the gene or genes explaining the QTL.

Identification of the relevant genes remains a significant hurdle in farm animals, although the development of improved comparative maps and other resources such as large fragment libraries are now available and will allow better use of information from "gene-rich" species such as the mouse and human. In the short term projects are underway to determine how such initial QTL findings can be used in commercial populations. For example, the European Community (EC) Biotechnology Project entitled "Transferring QTL technology to the pig breeding industry (PigQTech)", coordinated by Leif Andersson at the Swedish University of Agricultural Sciences, aims to produce a route map to assist animal breeders to exploit genome mapping information. The project involves Quality Genetics (Sweden), a Spanish breeder, Copaga and PIC as well as the Roslin Institute, Centre UdL-IRTA and the University of Barcelona.

Present and future genetic improvements will result from the more detailed genetic maps and our growing understanding of the function and structure of the individual genes and gene families that are responsible for traits of economic importance in the pig. The purpose of this paper is to review the recent application of results of novel molecular genetics technology in pigs and to forecast future developments and their use in pork production.

Results

Potential of DNA marker assisted selection in the pig industry

Information at the DNA level can help industry breeders and geneticists to fix a specific major gene, such as the normal Halothane gene, a mutation of which is associated with Porcine Stress Syndrome (PSS) and pale soft exudative (PSE) meat. It can also be used to assist in the selection of quantitative traits including those that can be selected by traditional means. Molecular information can increase the accuracy of selection and therefore the selection response. The size of the extra response expected to be obtained through MAS schemes has been considered from a theoretical point of view by many workers. For example, Meuwissen and Goddard (1996) noted that the greatest potential is for traits such as reproduction and meat quality that are difficult to progress using traditional methods. In certain situations they estimated that selection responses can be increased by between 38 to 64% for these traits. Dekkers and van Arendonk (1998) have shown that these results can be further improved by using control theory to optimise response. These results are extremely encouraging. Importantly, these responses can be sustained if new markers are continually identified. For example, new markers can be added to the selection index as old markers begin to reach fixation.

ESR and DNA markers for litter size.

Efficiency of pork production is greatly influenced by female reproductive performance, especially litter size. As pork production becomes more integrated, small to moderate gains in litter size can mean large gains in overall efficiency. Traditionally, pig geneticists have used the methods of crossbreeding and, to a lesser extent, selection to make improvements in litter size. However, as noted above the identification of genes influencing litter size followed by MAS can be employed in conjunction with traditional selection methods to accelerate the rate of improvement, particularly in low heritability and sex-limited traits such as litter size.

Candidate gene analysis for reproduction has shown considerable merit. Results have clearly demonstrated that the estrogen receptor (ESR) is significantly associated with litter size (Rothschild *et al.* 1996; Short *et al.* 1997). This gene was chosen because of the central role estrogen plays in the reproductive cycle of the pig. Estimates of allelic effects vary from 1.15 pig/litter in Meishan synthetics to 0.42 pigs/litter in Large White lines. The ESR marker was incorporated successfully into the PIC selection indices for Large White based dam lines, resulting in an increase in the rate of genetic response in its nucleus herds (Short, Wilson, McLaren and Plastow, unpublished data). Furthermore, the increase in average litter size is observed in crossbred products derived from these lines.

More recently we have reported significant effects on litter size for a number of other markers based on candidate gene analysis. These include prolactin receptor (PRLR) (Vincent *et al.*, 1998) and retinol binding protein 4 (RBP4) (Rothschild *et al.*, 2000). Other effects have been reported for retinoic acid receptor gamma (RARG), RBP4, melatonin receptor 1A (MTNRIA) (Messer *et al.*, 1996; Ollivier *et al.*, 1997, Rothschild and Plastow, unpublished data) and follicle stimulating hormone beta (FSHB) genes (Li *et al.*, 1998). In addition, several QTL studies have revealed promising results on chromosome 8 for litter size (reviewed in Rothschild and Plastow, 1999).

Marker assisted selection and meat quality

As indicated in the introduction, the ability to use DNA markers to assist with selection for improved meat quality is one of the most exciting opportunities for the industry. The first test available, provided by the discovery of the causative mutation for the Halothane gene by David MacLennan and colleagues (Fujii *et al.*, 1991), was a key step in MAS and directly addressed variation in pork quality. It had been known for some time that PSE pork was associated with frequency of the recessive Halothane gene. The test gave breeders the ability to accurately separate all three genotypes (instead of just reactors (nn) from non-reactors (NN and Nn)) allowing more detailed work on the effect of this mutation on meat quality.

A second major gene for meat quality was also identified in the Hampshire breed. This gene, called RN, is associated with lower ultimate pH and increased glycogen content in the meat. The RN gene was first mapped to chromosome 15 (Milan *et al.*, 1996; Mariani *et al.*, 1996a; Reinsch *et al.*, 1997). This allowed DNA tests to be developed using markers linked to the RN gene (e.g., De Vries *et al.*, 1997). Subsequently Milan and colleagues (Milan *et al.* 2000) were able to identify the gene involved and the specific mutation that confers the RN phenotype. This enabled PIC to introduce direct selection for rn+ animals in 1999. The gene involved is a new member of a gene family coding AMP-activated protein kinases (named PRKA). Interestingly, the same gene might explain certain forms of diabetes in humans and the consortium is looking at the opportunities for their research to benefit human health.

This result represented a tremendous effort by this group of laboratories to move from the chromosomal position to the gene itself (see Introduction). Although still a large amount of work, it clearly demonstrates that the tool kit is now in place to move from a genetic map location to the gene itself in the pig. The group also investigated other breeds, but were only able to trace the acid meat gene to the Hampshire breed. The results represent another important addition to the pig breeder's tool kit in terms of improving meat quality where the Hampshire breed is used.

Intramuscular fat has been identified as an important trait in relation to consumer preference, both in visual appearance and eating quality. Evidence for a major gene affecting intramuscular fat (IMF) was obtained using segregation analysis (Janss *et al.*, 1997), and this group went on to try to identify the gene using QTL mapping. A number of different QTL have been reported (reviewed in de Vries *et al.*, 1998, 2000). The latest analysis from this group reported QTL for this trait on chromosomes 6 and X (de Koning *et al.*, 2000). Interestingly, they suggest that both of these QTL are inherited in a non-Mendelian manner, suggesting that they are imprinted (see below for IGF2).

Among candidate genes investigated for muscle quality are two fatty acid binding protein genes (heart, H-FABP and adipocyte FABP (FABP4)) which may be associated with intramuscular fat (Gerbens *et al.*, 1997, 1998). A comparison of the homozygous haploid classes for H-FABP found that they differed by about 15% of the mean value. Interestingly, the difference in IMF content is only partially explained by backfat content. This should permit selection for increased IMF based on HFABP genotype as long as increased backfat is

countered by ongoing phenotypic backfat selection. More recently, a larger effect on IMF was described with the related gene adipocyte FABP (FABP4, Gerbens *et al.*, 1998). In this case the effect appears to be independent of backfat and so offers great promise for the manipulation of this trait by MAS.

Other markers which have been generated for meat quality based on the candidate gene approach include myogenin (increased fibre number, which may impact overall pork quality, Soumillion *et al.*, 1997) and calpastatin (Ernst *et al.*, 1997). Coat color, although not directly associated with meat quality, is of considerable relevance to the packing industry. White pigs are preferred at slaughter and the cost of a "colored carcass" at some locations may be over US$1/pig. Andersson and colleagues (Johansson-Moller *et al.*, 1996) have now identified the KIT gene as responsible for white coat color and a DNA test is patented and being used in MAS programs (Andersson and Plastow, personal communication). The MC1R (MSHR) gene has been shown to control red and black colour in the pig (Mariani *et al.*, 1996b). These tests for coat color offer the opportunity to develop simple traceability systems for pork products, which may be particularly useful as niche products are developed from traditional breeds.

Finally, the discovery of imprinted gene effects in farm animals is an interesting development as such effects are not amenable to manipulation by traditional selection schemes. The first examples include genes that influence aspects of meat quality, such as muscling and tenderness in sheep (the callipyge gene, Cockett *et al.*, 1998). The clearest example in pigs is the effect of the IGF2 region of chromosome 2 on muscling in pigs of different backgrounds (Jeon *et al.*, 1999, Nezer *et al.*, 1999). The ability to predict the genotype at such loci is a clear benefit of MAS.

Animal health

One of the most exciting areas for the application of genomic technologies is animal health, where variation in the susceptibility to some diseases is known to have a genetic component. The potential is illustrated by the identification of a variant of the FUT1 gene that, when present in the homozygous state, confers resistance to *E. coli F18*. This is an important cause of post weaning scours and edema disease and can result in mortality of up to 40% in naïve herds. The development of a simple DNA marker test for this mutation means that resistant pigs can be bred and selected for use in systems where this bacterium is, or may become, a problem.

Research is now underway to identify genes that are involved in the protection or susceptibility of the host during infection by pathogens. The ability to be able to study the expression of many genes simultaneously using microarrays (1,000s of genes can be spotted onto small glass slides) offers exciting possibilities for the study of the interaction between the pathogen and the host and ultimately the development of new tools to select for reduced susceptibility. PIC, together with Intervet and CISA in Spain, have recently initiated an EC funded project (PathoChip) to analyse gene expression both in the pathogen and the host during infection of the pig by *Haemophilus parasuis*.

Discussion

The success obtained with ESR demonstrates the value of MAS for polygenic traits such as litter size. Although the proportion of variation explained by the ESR genotype is relatively small, the added accuracy of selection in large schemes enables the value to be demonstrated and captured by the producer. Variation in meat quality is explained by differences in numerous genes, some of which will have a relatively small effect. However, identifying such genes can be extremely useful in facilitating genetic improvement in quality traits that are typically lowly heritable and impossible to measure in the live pig.

Exploitation of major genes for meat quality

Improving meat quality is not just about changing levels of traits like tenderness or marbling, but also about increasing uniformity. The existence of major genes has provided excellent opportunities for improving meat quality, since it allows large steps to be made in the desired direction (e.g., improving technological yield of ham process by selecting against RN gene). The utilisation of markers for major genes also helps reduce variation, since relevant genes can be selected to be fixed in products. Another aspect is the use of such genes in differentiation for specific markets. For example, in certain types of dry cured ham a high IMF is required, whereas other products like cooked ham require a low amount of IMF. Genes explaining variation in IMF content could be used here. For the future, it is expected that processors and retailers will specify a whole series of genes that have to be present or absent for each product that they process or sell.

Developing tailored products

Characterisation of meat quality enables the breeder to identify the genetic effects on specific components of meat quality. This knowledge can be used to tailor different products for the processing industry. The RN gene represents one example; once a DNA test was developed by PIC in 1996 it became possible to differentiate between crossbred animals carrying the dominant RN allele that could be sent for fresh pork production (as this genotype is associated with better fresh pork eating quality) and RN-free animals to be sent for the production of hams (thereby avoiding the yield losses associated with RN).

Guaranteeing the value chain

Once specific attributes of genotypes have been identified and selection and differentiation implemented to increase value and reduce variation then it becomes necessary to identify these products in order to preserve the added value. This can easily be achieved using relatively simple passport systems, although new technology, including electronic identification, will likely play a part in these systems. DNA markers, however, represent a powerful tool to audit simple passport systems, especially after slaughter when the carcass is split into its constituent parts. An animal-specific DNA fingerprint can be obtained from fresh or processed meat and linked back to samples taken at birth or transport stages. Furthermore, PIC has utilised its knowledge of the pig genome to develop breed specific markers that simplify this process,

making efficient audit systems a reality for schemes reliant upon breed specifications, for example those including Duroc for eating quality or European Wild Boar for particular flavor.

DNA markers also represent ideal ways of characterising and developing the different genetic resources that are available in different markets for the development of pork products. PIC is participating in an EC project coordinated by Louis Ollivier (INRA, Jouy-en-Josas) which aims to characterise genetic variation in European pigs to facilitate the maintenance and exploitation of biodiversity. The project is using both microsatellites and amplified fragment length polymorphisms (AFLPs) to estimate genetic distance between populations. Recently, however, we have evaluated an alternative approach based on the use of candidate gene markers associated with variation in economically important traits (Ciobanu *et al.*, 1999). Such an approach offers genetic information about the origin of the breeds and illustrates the potential utility of new genetic markers for accelerating genetic progress not only in modern established commercial lines, but also in the selection of adapted local breeds for commercial meat production systems.

Conclusions

Molecular genetics is already gaining significant uptake in the swine industry (see Table 1). However, there is much still to be done in terms of identifying the genes involved in explaining variation in traits such as reproduction and meat quality. Significant progress will be made by utilizing candidate genes and searching for population-wide linkage disequilibrium, using tools such as AFLP, an extremely powerful methodology as it can generate large numbers of markers very rapidly in populations of interest. AFLP can be used with bulk segregant analysis (BSA) in a form of map-less QTL analysis. For example, markers were identified close to the dominant white locus (Plastow *et al.*, 1998). This approach is applicable for other traits, an example of its application would be the generation of simple markers for QTL identified using microsatellite based QTL mapping. Such markers would then enable use without the need for family information, as was the case originally for coat color and RN. The first results obtained in this respect support the applicability of the AFLP BSA approach for polygenic traits.

Table 1. Molecular Genetic Tests Used By The Swine industry.

Parentage tests	non exclusive use
HAL	meat quality - non exclusive use
ESR	litter size - exclusive use (PIC)
PRLR	litter size - exclusive use (PIC)
KIT	white color - exclusive use (PIC)
MC1R	red/black color - exclusive use (PIC)
MC4R	growth and fatness – exclusive use (PIC)
FUT1	edema disease E. coli F18–exclusive use (PIC/ITH Switzerland)
RN	meat quality - exclusive and non exclusive tests

AFABP, HFABP	intramuscular fat - non exclusive
IGF2(?)	carcass composition – exclusive use (Seghers/Quality Genetics)
Trade secret tests	several traits

Future developments in pig genomics research and quantitative genetics, combined with additional technologies such as gene expression analysis, will likely accelerate the discovery of additional genes and their use in marker assisted selection.

- DNA technology provides excellent opportunities to improve reproduction and meat quality in selection schemes within lines.
- Selection on major genes will not only increase average levels of quality but also decrease variability (i.e., increase uniformity). Additionally, major genes can be exploited for differentiation for specific markets.
- These methods can be used to identify genotypes best suited for specific added value products.
- DNA markers provide a system to support quality assurance schemes to identity preserve the added value created by selection schemes, custom product development and integrated, aligned or coordinated pork production systems.

Acknowledgements

The authors gratefully acknowledge the many colleagues around the world who have contributed so successfully to the development of marker assisted selection in pigs. The AFLP work is conducted in collaboration with Keygene n.v and was supported by the EC (contract no. BIO4-CT95-0081). The PigQTech (contract no. BIO4-CT97-2243), Pig Biodiversity (contact no. BIO4-CT98-0188) and PathoChip (Contract QLK2-CT-2000-00726) projects are also supported by the EC.

References

Andersson, L., Milan, D., Jeon, J-T., Looft, C., Amerger, V., Robic, A., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Reinsch, N., Gellin, J., Kalm, E., Le Roy, P., and Chardon, P. (2000) Positional cloning of a major gene (RN) controlling glycogen content in pig skeletal muscle. International Plant and Animal Genome VIII Abstract S15.

Ciobanu, D.C., Nagy, A., Wales, R., Day, A.E., and Plastow, G.S (1999) Genetic Variation of Two Local Romanian Pig Breeds Assessed Using DNA Markers, 50th Annual Meeting of the EAAP, Zurich (and in preparation).

Cockett, N.E., Berghams, S., Beckers, M-C., Shay, T.L., Jackson, S.P., Snowder, G.D., and Georges, M. (1998) Proc 6th World Congress on Genetics Applied to Livestock Production 26: 525-528.

Dekkers, J.C.M. and van Arendonk, J.A.M. (1998). Optimizing selection for quantitative traits with information on an identified locus in outbred population. Genetical Research 71:257-275.

De Koning, D-J., Rattink, A.P., Harlizius, B., van Arendonk, J.A.M., Brascamp, P., and Groenen, M.A.M. (2000) Evidence for non-Mendelian control of body composition traits in Meishan crossbreds. International Plant and Animal Genome VIII Abstract W210.

De Vries, A.G., Faucitano, L., Sosnicki, A., and Plastow, G.S. (2000) The use of gene technology for optimal development of pork meat quality. Food Chemistry, in press.

De Vries, A.G., Sosnicki, A., Garnier, J.P., and Plastow, G.S. (1998) The role of major genes and DNA technology in selection for meat quality in pigs. Meat Science 49: Suppl 1, S245-S255.

De Vries, A.G., Timm, H.H., Wilson, E.R., Evans, G., Keller, V., and Plastow, G.S. (1997) An effective DNA-marker test for the 'acid meat' problem. Book of Abstracts 48th Ann. Meet. EAAP, Vienna, 3:340.

Ernst, C.W., Robic, A., Yerle, M., Wang, L., and Rothschild, M.F. (1998) Mapping of calpastatin and three microsatellites to porcine chromosome 2q2.1-q2.4. Animal Genetics 29: 212-215.

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V.K., Weiler, J.E., O'Brien, P.J., MacLennan, D.H. (1991). Science, 253, 448.

Gerbens, F., Rettenberger, G., Lenstra, J.A., Veerkamp, J.H. and Tepas, M.F.W. (1997) Characterization, chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene. Mammalian Genome. 8:328-332.

Gerbens F., Jansen A., Van Erp A.J.M., Harders F., Meuwissen T.H.E., Rettenberger G., Veerkamp J.H., Te Pas M.F.W. (1998) The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs. Mammalian Genome 9: 1022-1026.

Janss, L.L.G., Van Arendonk, J.A.M. and E.W. Brascamp, E.W. (1997) Segregation analyes for presence of major genes affecting growth, backfat and litter size in Dutch Meishan crossbreds. J. Anim. Sci. 75:2864-2876.

Jeon, J-T., Carlborg, O., Tornsten, A., Giuffra, E., Amarger, V., Chardon, P., Andersson-Eklund, L., Andersson, K., Hansson, I., Lunstrom, K., and Andersson, L. (1999) A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nature Genetics 21: 157-158.

Johansson Moller, M., Chaudhary, R., Hellmén, E., Höyheim, B., Chowdhary, B. and Andersson, L. (1996.) Pigs with the dominant white coat color phenotype carry a duplicate of the KIT gene encoding the mast / stem cell growth receptor. Mammalian Genome 7: 822-830.

Li, N., Zhao, Y.F., Xiao, L., Zhang, F.J., Chen, Y.Z., Dai, R.J., Zhang, J.S., Shen, S.Q., Chen, Y.F. and Wu C.X. 1998. Candidate gene approach for identification of genetic loci controlling litter size in swine. Proc 6th World Congress on Genetics Applied to Livestock Production 26: 183-186.

Mariani, P., Lundström, K., Gustafsson, U., Enfält, A.C., Juneja, R.K. and Andersson, L. (1996a) A major locus (RN) affecting muscle glycogen content is located on pig chromosome 15. Mammal. Genome 7:52-54.

Mariani, P., Moller, M.J., Hoyheim, B., Marklund, L., Davies, W., Ellegren, H. and Andersson, L. (1996b) The extension coat color locus and loci for blood group O and tyrosine aminotransferase are on pig chromosome 6. Journal of Heredity 87:272-276.

Messer, L., Wang, L., Legault, C. and Rothschild, M.F. (1996) Mapping and investigation of candidate genes for litter size in French Large White pigs. Anim. Genet. 27 (Suppl. 2):114.

Meuwissen, T.H.E. and Goddard, M.E. (1996). The use of marker haplotypes in animal breeding schemes. Genet. Sel. Evol., 28, 161-176.

Milan, D., Woloszyn, N., Giteau, M., Navas, A., Yerle, M., Rogel-Gaillard, C., Chardon, P., Gellin, J., Elsen, J.M., and LeRoy P. (1996) Toward the identification of RN gene involved in meat quality in pigs. Anim. Genet. 27 (Suppl. 2):114.

Milan, D., J. T. Jeon, C. Looft, V. Amarger, A. Robic et al., 2000 A mutation in *PRKAG3* associated with excess glycogen content in pig skeletal muscle. Science 288: 1248-1251.

Nezer, C., Moreau, L., Brouwers, B., Coppieters, W., Detilleux, J., Hanset, R., Karim, L., Kvasz, A., Leroy, P., and Georges, M. (1999) An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nature Genetics 21: 155-156.

Ollivier, L., Messer, L.A., Rothschild, M.F. and Legault, C. (1997) The use of selection experiments for detecting quantitative trait loci. Genet. Research 69:227-232.

Plastow, G.S., Kuiper, M., Wales, R., Archibald, A.L., Haley, C.S. and Siggens, K.W. (1998). AFLP for mapping and QTL detection in commercial pigs. Proc 6th World Congress on Genetics Applied to Livestock Production 26, 209-212.

Reinsch, N., Looft, Chr., Rudat, I. and Kalm, E. (1996) The Kiel RN experiment: final porcine chromosome 15 mapping results. J. Anim. Breed. Genet. 114:133-142.

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

Rothschild, M.F., Messer, L., Day, A., Wales, R., Short, T., Southwood, O., and Plastow, G. (2000) Investigation of the retinol-binding protein 4 (RBP4) gene as a candidate gene for increased litter size in pigs. Mammalian Genome 11: 75-77.

Rothschild, M.F., and Plastow, G. S. (1999) Advances in pig genomics and industry applications. AgBiotechNet, 10: 1-8.

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

Soumillion, A., Erkens, J.H., Lenstra, J.A., Rettenberger, G. and Te Pas, M.F.W. (1997). Genetic variation in the porcine myogenin gene locus. Mammalian Genome, 8: 564.

Vincent, A.L., Short, T.H., Eckardt, G.R., McLaren, D.G., Southwood, O.I., Plastow, G.S., Tuggle, C.K., and Rothschild, M.F. The prolactin gene receptor is associated with increased litter size in pigs. (1998) Proc 6th World Congress on Genetics Applied to Livestock Production 27:15-18.