ANNUAL PROGRESS REPORT
NATIONAL RESEARCH SUPPORT PROJECT – NRSP008
Year Ending 2004
Preliminary Information-Not for Publication

Submitted by Max F. Rothschild and Christopher K. Tuggle
Iowa State University
January 15, 2005

I. Project: NRSP-8: Swine Genome Committee

II. Cooperating Agencies and Principal Investigators

A. Agencies and Departments Cooperating: Iowa Agriculture Experiment Station and Animal Science Department, Iowa State University

B. Leaders of the Project: Max Rothschild (leader) and Christopher Tuggle (co-leader)

C. Cooperating Investigators: Jack Dekkers, Elizabeth Huff-Lonergan, Steven Lonergan, Lloyd Anderson, Zhiliang Hu, Chad Stahl, Ken Stalder, Iowa State University; Daniel Pomp, University of Nebraska; S.M.D. Bearson, USDA-ARS-NADC; J. Lunney, USDA-ARD-BARC; R. Geisert, Oklahoma State University; D. Milan, INRA-Toulouse, France; and many PiGMaP collaborators.

III. Objectives

Objective 1: *Enhance and integrate genetic and physical maps of agriculturally important animals for cross species comparisons and sequence annotation.*

Objective 2: *Facilitate integration of genomic, transcriptional, proteomic and metabolomic approaches toward better understanding of biological mechanisms underlying economically important traits.*

Objective 3: *Facilitate and implement bioinformatic tools to extract, analyze, store and disseminate information.*

IV. General Project Plan

A. Mapping of Type I Comparative Loci (Objective 1).

A significant interest of the group continues to be characterization and mapping of candidate genes for improvement of the linkage map as well as investigate type I loci for their role in quantitative genetics of economically important traits in the pig. We are using several general approaches. These include mapping cDNAs that have been sequenced in the pig or in other closely related species. These will be selected on the basis of expected physiological role and on the basis of their position on the human map. Using DNA from a panel of somatic cell hybrids provided by Martine Yerle and Joel Gellin (Toulouse) or the radiation hybrid panel supplied by the University of Minnesota, we will physically map Type I loci. We will

amplify genes using species-specific PCR and DNA from each hybrid cell line, and data obtained will be sent to Toulouse for analysis. Genes that have been genetically mapped will be of high priority, as physical mapping of linked genes will tie together the genetic and physical maps. We have used and will continue to use the radiation hybrid panel and FISH techniques for physical mapping.

B. Developing and Mapping Expressed Sequence Tagged Sites (ESTs) (Objective 1)

Using the IMpRH RH mapping panel and newly developed porcine sequence, we are mapping genes with sequence similarity to human loci. This approach can rapidly and efficiently improve pig:human comparative maps.

C. Reference Pedigrees and Linkage Mapping (Objective 1).

A large Berkshire x Yorkshire family was developed. A total of 2 Berkshire grandsires and 9 Yorkshire grand dams were used to produce 6 sires and 28 dams in the F_1 generation. Phenotypes and DNA from 525 animals are being used. A total of nearly 180 genetic markers have been added to the map and this population is quite useful for gene mapping.

D. Candidate Genes for Reproduction, Growth, Health, Sow Longevity and Quality Meat Traits (Objective 2)

Several interesting genes that may affect a variety of traits in the pig are being chosen. Polymorphisms are first identified and the genes are then mapped. Association analyses have produced useful results for several genes.

E. QTL Identification (Objective 2)

A large QTL genome scan for meat quality traits using a Berkshire x Yorkshire family was developed. A total of 2 Berkshire grandsires and 9 Yorkshire grand dams were used to produce 6 sires and 28 dams in the F_1 generation. Phenotypes and DNA from 525 animals are being used. A total of nearly 180 genetic markers have been added to the map. Imprinted QTL are being investigated also.

F. Transcript Profiling of Pig Embryo, Endometrium and other tissues (Objective 2)

Use both custom arrays as well as a new microarray made of 13,000 oligonucleotides for transcriptional profiling of several pig tissues.

G. Identification of genes responding to *Salmonella* infection (Objective 2)
An experiment was initiated to use molecular techniques to identify some of the genes which increase or decrease expression levels in the early response to *Salmonella choleraesuis* or *Salmonella typhimurium* infection.

H. Database development (Objective 3)

Database development is continuing through the help of the US Pig Genome Coordination program.

V. Work Progress

A. Mapping of Type I Comparative Loci (Objective 1).

Several candidate genes have been mapped this year using linkage and physical mapping. These are being placed in the regions of known QTL. Further identification and mapping of several other genes is under way.

B. Developing and Mapping Additional Expressed Sequence Tagged Sites (ESTs) (Objective 1)

We have completed the physical mapping of 443 loci and generated a high resolution RH and comparative map for sections of pig chromosomes 1 and 7 (SSC1, SSC7). This work was completed in collaboration with the Midwest Consortium and O. Demeure and D. Milan (INRA-Toulouse). High confidence in the final mapping location was achieved for 309 loci, with most of these genes mapping to SSC1, 4, 7, 8 and X. Our porcine/human comparative mapping results reveal possible new homologies for SSC1, 3, 5, 6, 12 and 14 and refine synteny breakpoints for chromosome 7. This work has been submitted for publication ("Large-scale EST mapping refines the comparative maps for SSC1 and SSC7 with the human genome " Demeure et al., 2005, expected).

C. Generation of Reference Pedigrees (Objective 2)

The Berkshire x Yorkshire family continues to be used to map genes. DNA can be shared with interested parties. We have added many new genes on the map. F7 animals are being produced.

D. Candidate Genes for Economic Traits (Objective 2)

Several candidate genes have been mapped this year using linkage and physical mapping. These are being placed in the regions of known QTL. These genes continue to be investigated for their role in important economic traits in the pig. This includes additional validation research using commercial pig lines.

i) Candidate genes for meat quality

New research has concentrated on genes affecting meat quality especially as it relates to dry processed products. The results from study investigating the impact of two genes on country-cured ham were published (Stalder et al., 2004). The study objective was to evaluate the effects of mutations in two genes, PRKAG3 and CAST, on fresh and dry-cured processing characteristics. Processing and curing followed normal commercial procedures. A 100 g sample of the *semimembranosus* was excised and frozen for later DNA harvest. Genotypes for CAST, and PRKAG3 were determined after DNA extraction from the frozen samples. The PRKAG3 marker had no affect on dry-cured ham processing characteristics. The CAST gene

marker was a significant source ($P<0.05$) of variation for cured ham moisture content and tended to be a significant source ($P<0.10$) for yield, ham weight loss, salt content and Minolta color change. The data demonstrate that the CAST 11 genotype is associated with greater processing yields. Alternatively, selection or sorting for the CAST 22 genotype should produce cured hams that exhibit more efficient moisture loss and, as a result, require less processing time and contain greater salt content.

Cathepsins are lysosomal enzymes involved in the proteolysis process observed during the dry curing process of hams. The effect of three cathepsin genes (cathepsins B, F and Z) on several processing characteristics of fresh and dry-cured hams was investigated. Genotypes for all genes were obtained using PCR-RFLP procedures developed in our group (cathepsins B and Z) or published in the literature (cathepsin F). Association analysis revealed significant effects of all genes on several traits. Cathepsin Z (CTSZ) genotypes significantly affected several fresh and dry-cured ham traits. The CTSZ genotype 22 was found to be associated not only with higher fresh and cured ham weight but also with higher yield. Similar results were detected for cathepsin F (CTSF). In fact, CSTF variants had a significant effect on fresh and dry-cured ham weight, average marbling and intramuscular fat. The fresh ham traits analyzed were not influenced by the cathepsin B (CTSB) variants. However, this locus had a significant effect on several dry-cured ham traits, including cured weight, yield, weight loss and moisture content. This study indicates that the variants at these three cathepsin genes are associated with increased quality characteristics of fresh and dry-cured hams. Therefore, these genes can be used by the swine industry as tools to select pigs with characteristics more suitable for dry-cured ham production.

ii) Candidate genes for sow longevity

Preliminary research is targeting genes that are associated with sow longevity. These genes may not only have a positive effect for number of litters a sow produces, but some may also have a positive impact on litter size. Sow longevity is of great economic importance to the bottom line of swine production. High sow mortality has contributed to concerns over animal welfare and negatively impacted staff morale. High sow culling or mortality rates are forcing sow dropout rates higher, before many females reach their most productive parities and before investment cost of females can be recovered. Realizing that genetic mechanisms have a major role to play in controlling sow longevity, ISU has teamed with PIC to focus on identification of these key genes. Scientists have identified pathways and genes in model organism, such as mice and flies, which have shown longer lifespans. Information from those model organisms is being examined by the scientists to determine if the same pathways or genes are involved in sow longevity. Two sow groups have been used as research models in preliminary studies. The first group consists of about 1,000 commercial sows, with almost half having less than five parities and the rest having more than eight parities. The second population consists of more than 200 sires, where complete lifetime production records were recorded on their daughters (minimum of 10 daughters/sire). Genotypes from each gene were analyzed for association with sow longevity in both populations. Three of ten candidate genes investigated were identified as possessing significant effects on sow longevity. The effects of these genes ranged from having a beneficial effect of 0.2 to over 2.0 more litters depending on the farm and population of animals analyzed. One of these genes also had a beneficial effect on litter size, suggesting that if selection for this particular gene is made, then the producer can benefit from sows having more litters and also have more live pigs per litter. For a farrow-to-finish producer, an increase in average parities/sow of just a tenth of a parity (ie. going from 3.4 to 3.5 average parities/sow) calculates into a benefit of $0.23 for every market hog sold.

Likewise, a farrow-to-wean producer can realize a return of $0.13 for every pig sold for the same increase of 0.1 average parities. Further research on different and larger pig populations will be necessary before these genes can be used for selection in swine production. But these early results hold promise that advances in sow longevity will be here in the near future.

E. QTL Identification (Objective 2)

A total of 525 animals have been slaughtered and 40 traits have been measured including many meat quality measures. We have now placed about 20 new genes on the map (total 180) markers and genes on the map and genotyped all animals. We are investigating 3 particular QTLs. In addition to the previously reported QTL we are now testing for imprinting and several such imprinted QTL have been identified. To follow-up F7 animals are being bred to produce F8 animals for a possible second set of phenotyped individuals. Analysis to find the underlying genes associated with the QTL has revealed genes causing two of the QTL (chromosome 2 and 15). Others are under review.

F. Transcript Profiling of Pig Embryo, Endometrium and Other Tissues (Objective 2)

i) In a study using a 1,000 gene embryo EST membrane array, we identified 9 genes that were differentially expressed during pig embryo elongation and we confirmed differential expression for four genes (of four tested) by quantitative RT-PCR. These four genes were Steroidogenic acute regulatory protein, Interleukin 1 beta, Transforming growth factor beta 3, and thymosin beta 10. Our study further showed that RNA amplification is useful for transcriptional profiling with limiting porcine embryonic RNA, and that this novel targeted array can detect differential gene expression during trophoblastic elongation. A manuscript on this work has been accepted for publication (Lee et al., 2005).

ii) In a study to validate a novel 13,000 oligonucleotide set, designed and synthesized by Qiagen-Operon, Inc., we have hybridized RNA from four adult tissues (lung, liver, small intestine, and skeletal muscle) from six healthy pigs (9-12 weeks of age) to an array of this oligonucleotide set. After normalization for differences in hybridization conditions, and by using linear model ANOVA, we have identified 424 genes with differential expression amongst tissues, and used clustering software to group these genes into specific patterns that recapitulates the same tissue-specific pattern seen by statistical analysis. We are currently using quantitative RT-PCR to confirm a subset of these genes, but an *in silico* analysis indicates that 9 of the 10 highest expressed genes, as well as 8 of the 10 lowest expressed genes, predicted to be muscle-specific based on the microarray results have similar patterns in mouse or human tissues.

iii) We have initiated a USDA-NRI-funded project to determine the genes expressed in both the endometrium and embryo/conceptus during the elongation and implantation phase of reproduction, in both the Yorkshire and the Meishan breeds. The questions being addressed are as follows: 1) Which genes are expressed in specific tissues, at specific times, and in a specific breed? 2) Which genes have significantly different expression in these tissues, times and breeds? 3) Which genes are expressed only in pregnant (versus cycling) endometrium? 4) What sets of genes are co-expressed during conceptus elongation and interaction with the dam? 5). What sets of genes have different expression patterns in pregnant versus cycling dams? 6) What sets of maternal immune genes respond to the presence of the conceptus? We

will use both the Qiagen oligonucleotides and quantitative RT-PCR to identify such genes, which can be used in the future as candidate genes to improve reproduction traits through identifying polymorphisms and association analysis. We have begun to collect the Yorkshire and Meishan conceptus/endometrial tissues required.

G. Identification of genes responding to *Salmonella* infection (Objective 2)

We have used two molecular techniques to identify some of the genes which increase or decrease expression levels in the early response to *Salmonella choleraesuis* or *S. typhimurium* infection. These are subtractive suppression hybridization (SSH) and microarray. We have found 10 genes that are differentially regulated by using SSH to compare RNA from mesenteric lymph nodes from uninfected pigs and from pigs infected for 48 hours with *S. choleraesuis*. We have confirmed this differential expression for all 10 genes through quantitative RT-PCR, and have begun to map these genes as well. Overall, the pattern of genes whose expression increases during *S. choleraesuis* infection indicates a significant response by the heat-shock pathway. We have begun to analyze the lungs of pigs infected with *S. choleraesuis* by using the oligonucleotide microarray, and find 57 genes with some statistical evidence ($P < 0.001$, false discovery up to 20%) for differential expression; of the 40 genes from this group with human functional annotation, 40% are related to the immune system.

H. Database development (Objective 3)

Database development is continuing. An EST database has been developed and is quite useful. A QTL database is under development and will be presented in a poster this year at PAG.

VI. Additions to the Project

None.

VII. Applications of Findings

A. A large number of genes continue to be identified and mapped by ISU researchers. An emphasis has been made (and will continue to be made) on genes that improve the comparative map as well as in connecting the genetic and physical pig genome maps.

B. Several new genes that may be important QTL are being mapped. These include genes associated with cured meat quality and sow longevity

C. QTL for several meat quality traits have been discovered. Additional fine mapping is underway and positional candidate genes are being considered.

D. Mapping of over 400 comparative loci to pig chromosomes SSC1, 4, 7, 8 and X adds additional information to comparative maps. These results also revealed possible homologies for SSC1, 3, 5, 6, 12 and 14 with the human genome and refine synteny breakpoints for one chromosome, SSC7. This information allows comparative information from human to be used for genome analysis in the pig.

E. Comparative mapping between human and pig chromosomes corroborates chromosome painting results in that approximately 85-90% of loci map to expected locations, but also demonstrate that pig gene order cannot be predicted from the order of human genes within conserved syntenic groups.

F. Low-cost transcriptional profiling results were verified by quantitative techniques. Genes involved in signal transduction and steroid biosynthesis are involved in the embryo elongation process.

VIII. General Project Plan

A. Objective 1

Experiment A. Accelerate polymorphism identification and linkage mapping of comparative anchor loci.

Experiment B. Continue physical mapping of genes using French somatic cell hybrid panel. Use of French Radiation Hybrid panel for high resolution physical mapping of new genes.

B. Objective 2

Experiment C. Continued identification of polymorphisms and mapping of interesting reproduction, growth, sow longevity, meat quality and performance genes.

Experiment D. Continue QTL research using several genes and markers. Continue to study role of candidate genes in traits of economic performance in the pig.

Experiment E. Use new transcriptional profiling tools to determine the gene expression profiles of adult tissues relevant to growth, reproduction, disease resistance and production; including liver, small intestine, placenta, muscle, embryo, endometrium, and lung.

Experiment F. Begin to study expression and function of additional new genes.

C. Objective 3

Experiment G. Continue database development and initialize QTL database.

VIII. Publications

A. Publications during the year

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 gene are associated with meat quality traits in pigs. J. Animal Sci. 82:2829-2839.

Ciobanu, D.C., Lonergan, S.M., Bastiaansen, J.W.M.,Mileham, Miculinich,, Schultz-Kaster, C., Sosnicki, A.A., Plastow , G.S. and M.F. Rothschild. 2004. Association of new Calpastatin alleles with meat quality traits in commercial pigs. 50th Int. Congress of Meat Science and Technology, Helsinki, Finland.

Gaboreanu, A.M., L. Grapes, A. M. Ramos, J.-J. Kim and M. F. Rothschild. 2004. Characterization of an X-chromosome PCR-RFLP marker associated with fat deposition and growth in the pig. Animal Genetics 35: 401-403.

Grapes, L., J.C.M. Dekkers, M.F. Rothschild, and R.L. Fernando. 2004. Comparing linkage disequilibrium-based methods for fine mapping quantitative trait loci. Genetics 166: 1561-1570.

Grapes, L., M. Z. Firat, J. C. M. Dekkers, M. F. Rothschild, R. L. Fernando. 2004. Optimal haplotype structure for linkage disequilibrium-based fine mapping of quantitative trait loci. American Association of Animal Science Midwest Region, Mar 15-17, Des Moines, IA

Grapes, L., S. Rudd, R. L. Fernando, M. F. Rothschild. 2004. *In silico* SNP identification from porcine EST sequences and comparative analysis with human SNP density. Plant and Animal Genome XII, Jan 10-14, San Diego, CA

Grindflek, E, N Hoen, H Sundvold, MF Rothschild, G Plastow and S Lien. 2004. Investigation of a Peroxisome Proliferator Activated Receptor gamma (*PPARG*) haplotype effect on meat quality and carcass traits in pigs. Anim. Genet 35:238-241.

Hu, Z-L, K. Glenn, A. M. Ramos, C. J. Otieno, and M. F. Rothschild. 2004. Expeditor: A Pipeline for Designing Pig Primers Using Human Gene Structure and Pig EST Information. Plant and Animal Genome XII, Jan 10-14, San Diego, CA

Kim, J.-J. and J. C. M. Dekkers. 2004. A combined line-cross and halfsib model to detect and characterize QTL in an F2 outbred cross population. American Society of Animal Science Annual meeting (Abstract). http://www.fass.org/2004/abstracts/414.PDF

Kim, K.S., J. J. Kim, J. C. M. Dekkers, and M. F. Rothschild. 2004. Polar overdominant inheritance of a DLK1 polymorphism is associated with growth and fatness in pigs. Mammalian Genome 15:552-559.

Kim, K.S., J.J. Kim, J.C.M. Dekkers, and M.F. Rothschild. 2004. Polar overdominance imprinting is associated with growth and fat deposition in pigs. PAG XII p240

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

Moller, M., F. Berg, J. Riquet, D. Pomp, A. Archibald, S. Anderson, K. Feve, Y. Zhang, M.F. Rothschild, D. Milan, L. Andersson and C.K. Tuggle. 2004. High-resolution comparative mapping across pig chromosome 4 (SSC4), emphasizing the FAT1 region. Mammalian Genome 15: 717-31.

Mote, B. E., J. D. Loy, M. F. Rothschild. 2004. Identification of SNPs in the insulin-like growth factor gene family and subsequent mapping of *IGF2R* and *IGFBP1* in pigs. Plant and Animal Genome XII, Jan 10-14, San Diego, CA

Rothschild, M.F. 2004. Porcine genomics delivers new tools and results: This little piggy did more than just go to market. Genetical Research 83:1-6.

Rothschild, M.F. 2004. DNA advances offer big payoffs. Pig Progress Magazine. 20:1-3.

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Rothschild, M.F., G.S. Plastow and S. Newman. 2004. Patenting in animal breeding and genetics. In: WAAP Book of the Year 2003, Eds: A. Rosati, A. Tewolde and C. Mosconi. Pgs 269-280

Stalder, K. J., M. Knauer, T. J. Baas, M. F. Rothschild, and J. W. Mabry. 2004. Sow Longevity. Pig News and Information. 25:53N-74N.

Thomsen, H., J. C. M. Dekkers, H. K. Lee, and M. F. Rothschild. 2004. Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine J. Anim. Sci. 82:2213-2228.

Tuggle, C.K., and Midwest Consortium. 2004. Finding the genes expressed in female reproductive tissues in pigs. Bulletin AS 650, January, 2004, Animal Industry Report 2004, Iowa State University, Ames, IA ASL-R1950.

Tuggle, C.K., X.W. Shi, L. Marklund, A. Stumbaugh, T.J. Stabel, M.A Mellencamp, L. Galina-Pantoja, and J. Bastiaansen 2004. Association of bacterial infection traits with genetic variation at candidate genes for porcine disease resistance. Bulletin AS 650, Animal Industry Report 2004, Iowa State University, Ames, IA ASL-R1952.

Tuggle, C.K., Y. Zhang, M.F. Rothschild, M.Moller, F. Berg, L. Anderson, J. Riquet, D. Milan, D. Pomp, A. Archibald, S. Anderson. 2004. A detailed gene map of pig chromosome 4, where the first quantitative trait locus in livestock was mapped. Bulletin AS 650, Animal Industry Report 2004, Iowa State University, Ames, IA ASL-R1951.

Zhao, S.-H. A. Erickson, and C.K. Tuggle. 2004. Physical and Linkage mapping of lymphocyte antigen 86 (Ly86) gene to porcine chromosome 7. Anim. Genet. 35:164.

Zhao, S.-H. and C.K. Tuggle. 2004. Linkage mapping and expression analyses during early gestation in the pig of a novel gene, PLacentally Expressed Transcript 1 (*PLET1)*. Anim. Genet. 35:72-74.

Zhao, S.-H., D. G. Simmons, J.C. Cross, T.E. Scheetz, T.L. Casavant, M. B. Soares, and C.K. Tuggle. 2004. PLET1, a highly expressed and processed novel gene in pig and mouse placenta is transcribed but poorly spliced in human. Genomics 84:114-125.

B. Publications planned.

Demeure, O., D. Pomp, M. F. Rothschild, D. Milan, and C. K. Tuggle. 2005. Large-scale EST mapping refines the comparative maps for SSC1 and SSC7 with the human genome. Mamm. Genome (submitted)

Grapes, L., A. Qu, L. Hittmeier, M. F. Rothschild and C. H. Stahl. 2005. Exploring the effect of dietary phosphorous levels on gene expression in two lines of pigs using microarrays. American Association of Animal Science Midwest Region, Des Moines, IA, Mar 21-23. (in press)

Grapes, L., M. Z. Firat, J. C. M. Dekkers, M. F. Rothschild, R. L. Fernando. 2005. Optimal haplotype structure for linkage disequilibrium-based fine mapping of quantitative trait loci. Genetics (Submitted).

Grapes, L., S, Rudd, R. L. Fernando, K. Megy, D. Rocha and M. F. Rothschild. 2005. Searching for mutations in pigs using the human genome. Animal Industry Reports (in press)

Hittmeier, L., R Lensing, L Grapes, M Rothschild, and C Stahl. 2005. Effect of phosphorous deficiency and genetics on bone characteristics and gene expression in young pigs. American Association of Animal Science Midwest Region, Des Moines, IA, Mar 21-23. (in press)

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Hu, Z.L., S. Dracheva, W. Jang, D. Maglott, J. Bastiaansen, J. Reecy, and M. F. Rothschild. 2005. PigQTLDB - A Pig QTL Database. Proceedings Plant and Animal Genome XIII, January 15-20, 2005, San Diego, CA. (in press)

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Mote, B.E., N. Deeb, O. Southwood and M. F. Rothschild. 2005.Using molecular marker technology for improvement in sow reproductive longevity. ISU Animal Industry Reports (in press)

Otieno, C. J., J. Bastiaansen, A. M. Ramos, M. F. Rothschild. 2004. Mapping and association studies of diabetes related genes in the pig. Animal Genetics (in press).

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Ramos, A.M., N. T. Nguyen, K. J. Stalder, and M. F. Rothschild. 2005. Molecular markers associated with improved yield and quality of dry-cured hams. Animal Industry Reports (in press)

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Vincent, A.L., B. J. Thacker, P. G. Halbur, M. F. Rothschild, and E. L. Thacker. 2005. Investigation of susceptibility to porcine reproductive and respiratory syndrome virus between genetically diverse lines of pigs using an in vitro flow cytometric assay. J of Virology (submitted)

Zhao, S.-H. C K Tuggle 2005. Mapping of the porcine suppressor of cytokine signaling 3 (*SOCS3*) gene to chromosome 12 by using somatic cell and radiation hybrid panels. Anim. Genet. (in press).