

ANNUAL PROGRESS REPORT
NATIONAL RESEARCH SUPPORT PROJECT — NRSP-8
Year Ending 2004
Preliminary Information — Not for Publication

Michigan Station Report to the NRSP-8 Swine Genome Committee
Submitted by Catherine W. Ernst

I. PROJECT TITLE:

National Animal Genome Research Program (NAGRP)

II. COOPERATING AGENCIES AND PRINCIPAL LEADERS:

- A. Agencies and Departments: Michigan Agricultural Experiment Station and Department of Animal Science, Michigan State University
- B. Project Leader: Catherine Ernst
- C. Cooperating Investigators: R.O. Bates, M.E. Doumit, N.E. Raney, V.D. Rilmington, D.B. Edwards, R.J. Tempelman and G.J.M. Rosa,, Michigan State University; U. DeSilva, Oklahoma State University; M.F. Rothschild, U.S. Swine Genome Coordinator

III. NATURE OF WORK AND PRINCIPAL RESULTS OF YEAR:

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

A. Pig Resource Population

The propagation of a resource population has been completed. The foundation animals consisted of RYR1 normal Pietrain females and Duroc males. F₁ progeny were produced from two different farrowing groups during the summer and fall of 2000. From F₁ litters, 6 boars from 3 foundation Duroc sires were kept for breeding purposes. There were 60 F₁ females retained of which 51 farrowed F₁ litters. All other F₁ animals were slaughtered and carcass measurements and meat quality characteristics determined. The F₁ breeding animals were retained until 958 F₂ progeny were produced and evaluated for carcass and meat quality traits. The initial F₂ progeny were farrowed in November 2001 and groups of approximately 88 F₂ pigs were slaughtered every 8 weeks, with completion of the final group in February 2004. The number of progeny per F₁ sire ranged from 149 to 307 pigs born. Data collected were: birth weight, weaning weight, 6 week weight, 10 week weight and weight before slaughter near 110 kg. At 3 week intervals, weight, and ultrasonic tenth rib and last rib backfat thickness and loin muscle area at the tenth rib were measured, beginning at 10 weeks of age through 22 weeks of age. At slaughter further data collection included: carcass weight, carcass temperature and loin muscle pH at 45 min. After 24 hr chilling, subsequent data collection included: carcass backfat depth (1st rib, last rib, last lumbar, tenth rib, off midline), loin muscle area, carcass length, carcass temperature and pH. Additional carcass data collection included: closely trimmed wholesale cut weights of the ham, loin, belly, Boston butt and picnic. A loin section from the tenth to last rib was partitioned into 2.54 cm chops with the following data collected: firmness (1-5 scale) and marbling scores (1-10 scale), Japanese color scores (1-6 scale), CIE L*, a* and b*, 24 hour post-processing drip loss, cooking loss, Warner Bratzler shear force values and percent intramuscular lipid. In addition, a trained sensory panel is determining tenderness, juiciness and off-flavor. Samples of *longissimus dorsi*, subcutaneous fat and liver were collected from 16 animals per slaughter group for RNA isolation. Using DNA samples taken from the founder animals along with the F₁ and F₂ pigs, segregating polymorphisms at microsatellite marker loci are being identified. Marker loci genotypes will be compared with phenotypic data to determine if there are significant phenotypic differences among animals with different allelic complements.

B. Transcriptional Profiling

Fetal myogenesis and postnatal skeletal muscle hypertrophy are critical yet poorly understood processes in growing pigs. Global gene expression analyses can be used to increase understanding of these processes by identifying key genes and pathways controlling skeletal muscle development. This experiment examined differential expression of genes in hind limb skeletal muscle tissue of pigs at 60 d of gestation and 7 wk of age. Oligonucleotide microarrays used for this study consisted of 13,297 70mer oligos (Pig Array-Ready Oligo Set v.1.0, Qiagen, Inc., Valencia, CA) and were printed at the University of Minnesota Advanced Genetic Analysis Center. Total skeletal muscle RNA from three pigs at 60 d of gestation and three pigs at 7 wk of age were reverse transcribed and labeled with both Cy3 and Cy5. For microarray screening, each 60 d sample was randomly paired with two 7wk samples for a total of six slides. The fluorescence intensity data was LOESS normalized and analyzed using a mixed model. Sixty-two genes were revealed to be significantly differentially expressed (fold change ≥ 1.5 , p-value ≤ 0.01) with 36 genes found to be more highly expressed at 7 wk of age and 26 more highly expressed at 60 d of gestation. Differential expression of titin and titin-cap were validated by relative real-time RT-PCR analysis confirming higher expression in the 7 wk samples vs. the 60 d gestation samples. Thus, high density oligonucleotide arrays provide a powerful tool for examining gene expression patterns in developing pig skeletal muscle.

IV. APPLICATION OF FINDINGS:

Transcriptional profiling of skeletal muscle tissue reveals important genes in the pathways regulating skeletal muscle growth and development. In addition, development of a unique pig resource population provides a novel resource for identifying QTL associated with growth and carcass merit in pigs.

V. WORK PLANNED FOR NEXT YEAR:

The initial microsatellite genome scan for the resource population is currently underway and will be completed in 2005. There were 511 animals from 38 F₂ full-sib families selected from the 11 farrowing groups to complete this phase. Initially, each pig will be genotyped for 126 microsatellite markers that are approximately equally spaced across the 18 autosomes and the X chromosome. Once putative QTL are determined these chromosomal regions will be further saturated with additional markers to reduce the distance between informative flanking markers. In addition, transcriptional profiling studies will be initiated using RNA samples from the *longissimus dorsi* and fat tissue samples that were collected. Transcriptional profiling studies will also continue for skeletal muscle tissue at various developmental ages and myogenic satellite cells derived from these tissues.

VI. PUBLICATIONS FOR THE YEAR:

A. NRSP-8 Project Publications:

Ernst, C.W., N.E. Raney, V.D. Rilmington, G.A. Rohrer, J.A. Brouillette and P.J. Venta. 2004. Mapping of the FES and FURIN genes to porcine chromosome 7. *Anim. Genet.* 35:142-143.

Farber, C.R., N.E. Raney, V.D. Rilmington, P.J. Venta and C.W. Ernst. 2003. Comparative mapping of genes flanking the human chromosome 12 evolutionary breakpoint in the pig. *Cytogenet. Genome Res.* 102:139-144.

Wesolowski, S.R., N.E. Raney and C.W. Ernst. 2004. Developmental changes in the fetal pig transcriptome. *Physiol. Genomics.* 16:268-274.

B. Other Publications:

Martínez, M.M., G.M. Hill, J.E. Link, N.E. Raney, R.J. Tempelman and C.W. Ernst. 2004. Impact of pharmacological zinc and phytase on liver metallothionein concentration and mRNA abundance in the young pig. *J. Nutr.* 134:538-544.