GENE EXPRESSION ANALYSIS OF PIG MUSCLE ASSOCIATED TO CHOLESTEROL AND FAT PARAMETERS.

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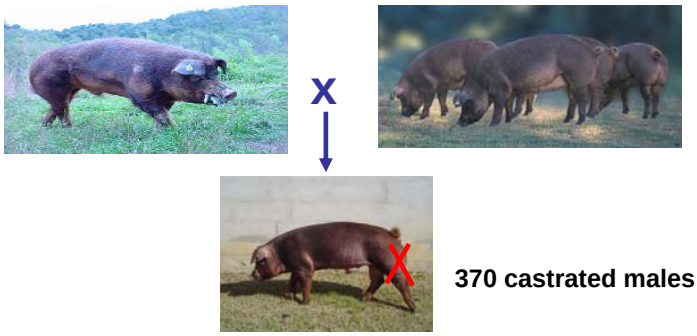
In order to **detect and identify genes involved in pig lipid metabolism** we have used a microarray approach over muscle samples from an experimental Duroc population of 370 castrated males distributed in five half-sib families.



370 castrated males

A total of 70 samples of the ***Gluteus medius*** (GM) were processed. They were selected from animals with the most extreme levels (**HIGH** and **LOW** lines; 35 animals per group) for an index composed by cholesterol and lipid metabolism parameters, such as plasma lipoprotein and triglyceride levels, percentage of intramuscular fat and fatty acid composition in muscle. In addition, *Longissimus Dorsi* (LD) muscle was processed for the 20 most extreme animals.

	ANIMALS	MUSCLE GM	MUSCLE LD
HIGH group for cholesterol and fat parameters	35	35	10
LOW group for cholesterol and fat parameters	35	35	10
Total (90 muscle samples)	*70*	*70*	*20*

Extraction, isolation and purification of total ARN from selected animals was carried out with RiboPure™ kit (*Ambion*). The integrity and purity of the extracted ARN was analyzed by means of electrophoresis in a Bioanalyzer equipment (*Agilent*).

Each sample was individually hybridized using *GeneChip Porcine Genome*® arrays (***Affymetrix***). Prior to data analysis,we performed a quality control of data with the function "qc" of the package *Simpleaffy* of Bioconductor (R).

After normalizing data with the *Robust Multichip Average* (RMA) algorithm, comparison between groups was performed with two different analyses: a standard t-test, and a Bayesian analysis by means of a mixed model with heterogeneous residual variances.

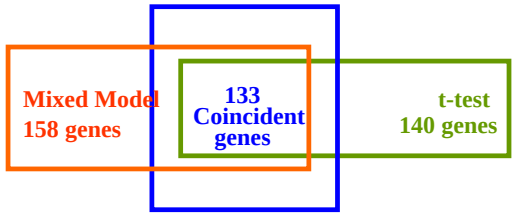
•The **t-test** results showed a total of 1007 genes whose expression levels differed significantly (p-value<10^{-7}) between the HIGH and LOW groups. Among these significant genes, 140 had a ratio of expression between the two lines superior to 1.5.

Nº genes with p-value < 10^{-7}	Nº genes with ratio>1.5
1007	140

•The **mixed model** analysis resulted in a total of 500 genes differently expressed at a significant level (p-value<10^{-9}), 158 of which showed a ratio between classes greater than 1.5.

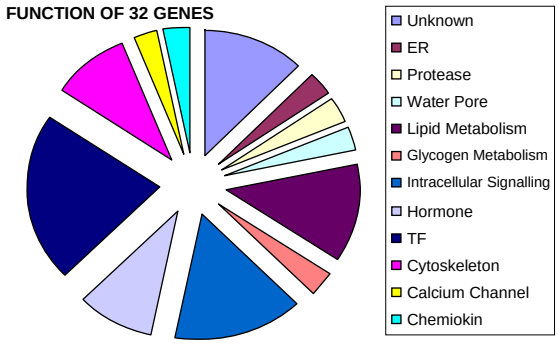
Nº genes with *prob.* < 10^{-9}	Nº genes with ratio>1.5
500	158

It is worth mentioning the high coincidence of significant genes detected with both analyses

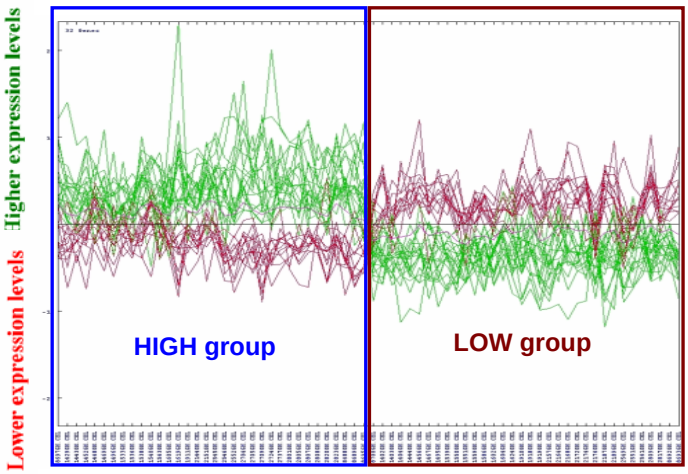


Taking into account the significance level, the ratio of variation and the function of the genes, we have selected a list of 32 genes to be validated by q-PCR.

The function of these 32 genes includes a variety of processes such as: transcription factor, lipid metabolism, intracellular signalling and hormones.



This image represent variation in expression levels for the 32 selected genes between individuals in both groups.



WORK FORWARD

→ Validation of the results: by real time **q-PCR**.
→ Analysis of ***LD*** information.
→Comparison between tissues (***LDvsGM***).
→**Sequencing** and **identification of polymorphisms** in promoter regions

REFERENCES
Tsai, S., Cassady, JP., Freking, BA., Nonneman, DJ., Rohrer, GA., Piedrahita, JA,. 2006. Animal Genetics, 37, 422-431

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