

Expression analysis at different reproductive stages in a F₂ crossbred

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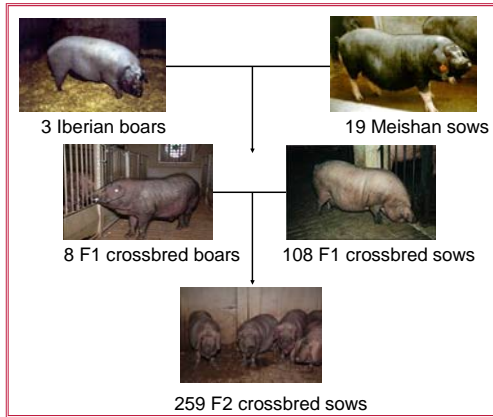


Figure 1. Generation of our experimental F₂ crossbred population.

Table 1. Distribution of samples analyzed by microarrays by tissue, physiological condition and prolificacy of sows (high ↑ or low ↓)

Physiological condition	Ovary		Uterus		Pituitary Gland	
	↑ prolificacy	↓ prolificacy	↑ prolificacy	↓ prolificacy	↑ prolificacy	↓ prolificacy
Heat	4	4	4	4	4	4
15 days pregnancy	3	3	3	3	2	2
30 days pregnancy	6*	6*	6*	6*	6**	6**
45 days pregnancy	3	3	3	3	3	3

* Pool of 6 samples of each group. (Total: 4 microarrays)

** Pituitary gland at 30 days pregnancy are pending of being processed.

INTRODUCTION

We have used an F₂ crossbred between Iberian and Meishan pigs (Figure 1) to study genes affecting reproductive traits with economical interest such as prolificacy, maternal capacity and piglet survival. These two breeds differ in their phenotypic performance for reproductive traits. Meishan is characterized by high prolificacy, while Iberian has a much smaller litter size.

MICROARRAY EXPERIMENT

RNA of ovary, uterus and pituitary gland from F₂ sows at different reproductive stages has been hybridized in oligonucleotide porcine microchips (GeneChip® Porcine Genome Array, Affymetrix) with the objective to characterize changes in gene expression profile between tissues and times (Table 1).

Data analysis was performed with RMAExpress package^[1] and experimental home-developed software using Bayesian statistics. Significant differences (posterior probability < 10⁻¹¹) have been found between expression at different reproductive stages: 708 genes in uterus, 281 genes in ovary and only two genes in pituitary gland (presynaptic cytomatrix protein and oligodendrocyte-myelin glycoprotein).

Clustering analysis^[2] showed that arrays are clearly separated by reproductive stages (Figure 2). Heat map^[3] representation of gene clusters showed the different expression profiles (Figure 2 and 3). In the near future, we will perform a qPCR validation of those genes with bigger ratio between time points for each expression profile. In addition, we aim to compare expression profiles in sows differing in prolificacy.

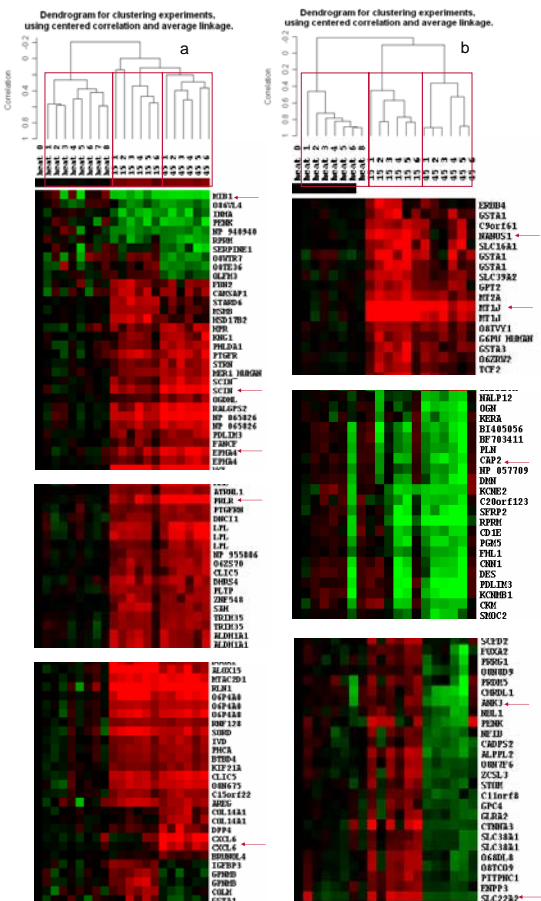


Figure 2. Cluster by microarray and heat map representation of some significant genes in ovary (a) and uterus (b). Arrows mark some of the genes selected for validation.

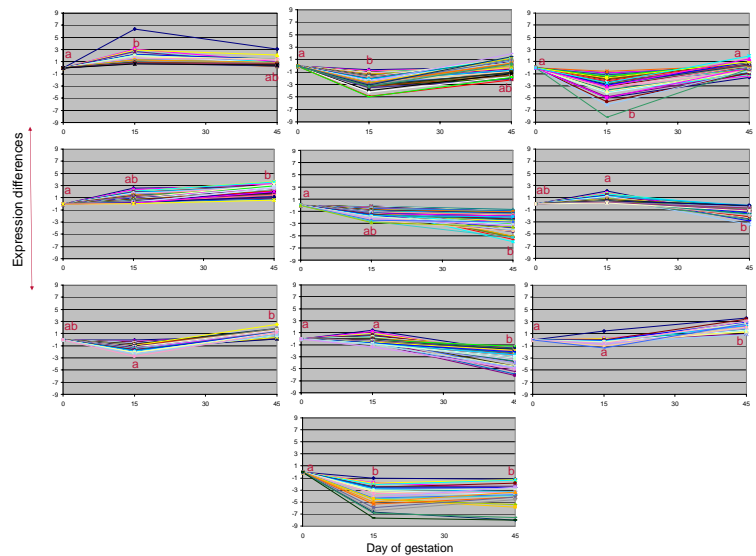


Figure 3. Expression profiles for differentially expressed gene clusters in uterus time course analysis. Different letters show significant differences between time points.

WORK FORWARD

- Validation of differentially expressed genes by qPCR
- Study of the Correlation between RNA and Protein levels
 - Analysis of protein content and modification by Western blotting
 - Analysis of protein distribution by ImmunoHistochemistry
 - Cellular distribution of the protein in ovary
 - Time-course analysis of protein accumulation