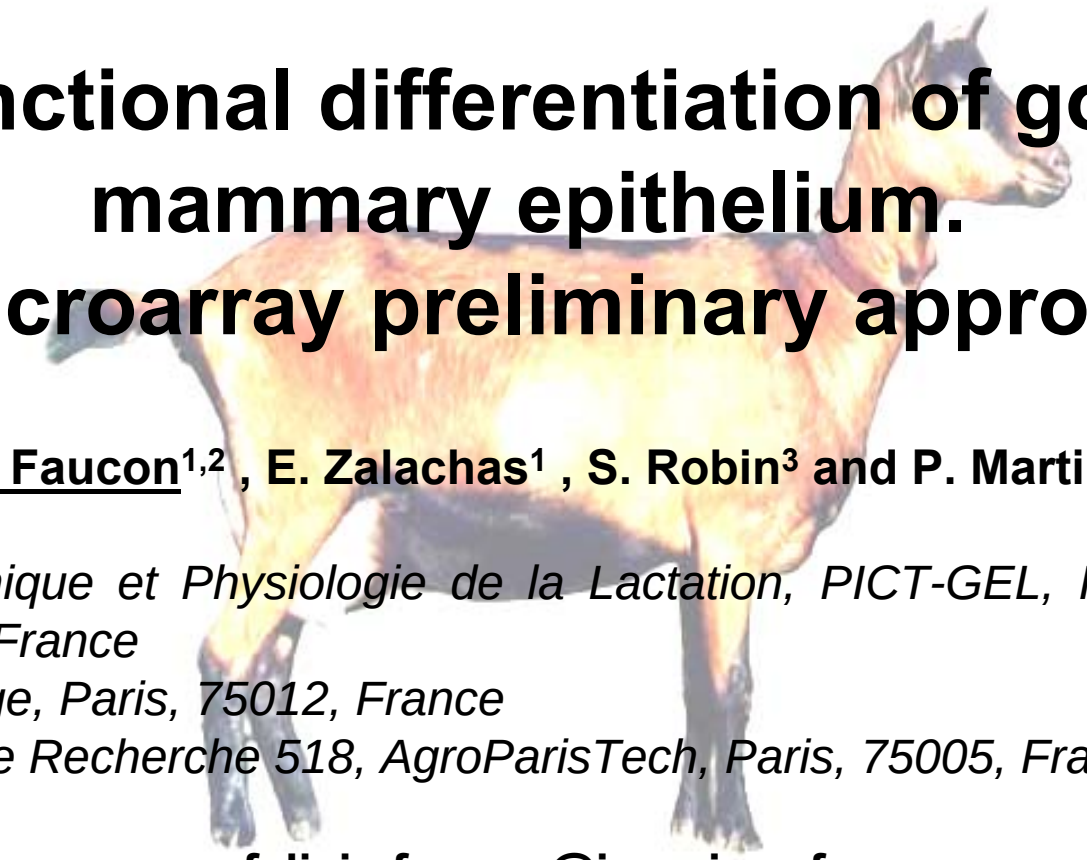

Functional differentiation of goat mammary epithelium. A microarray preliminary approach

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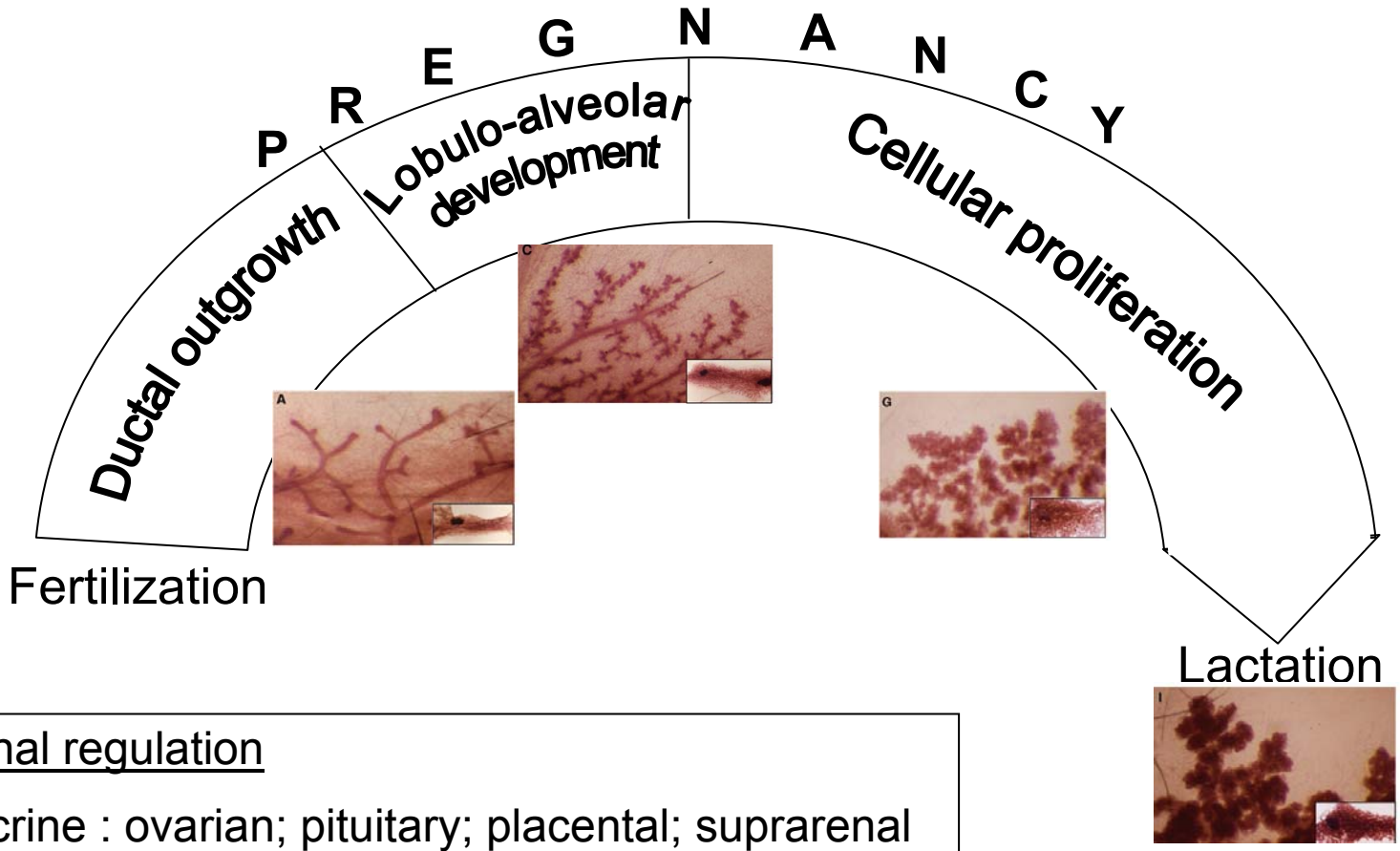
Introduction : mammary gland differentiation during pregnancy

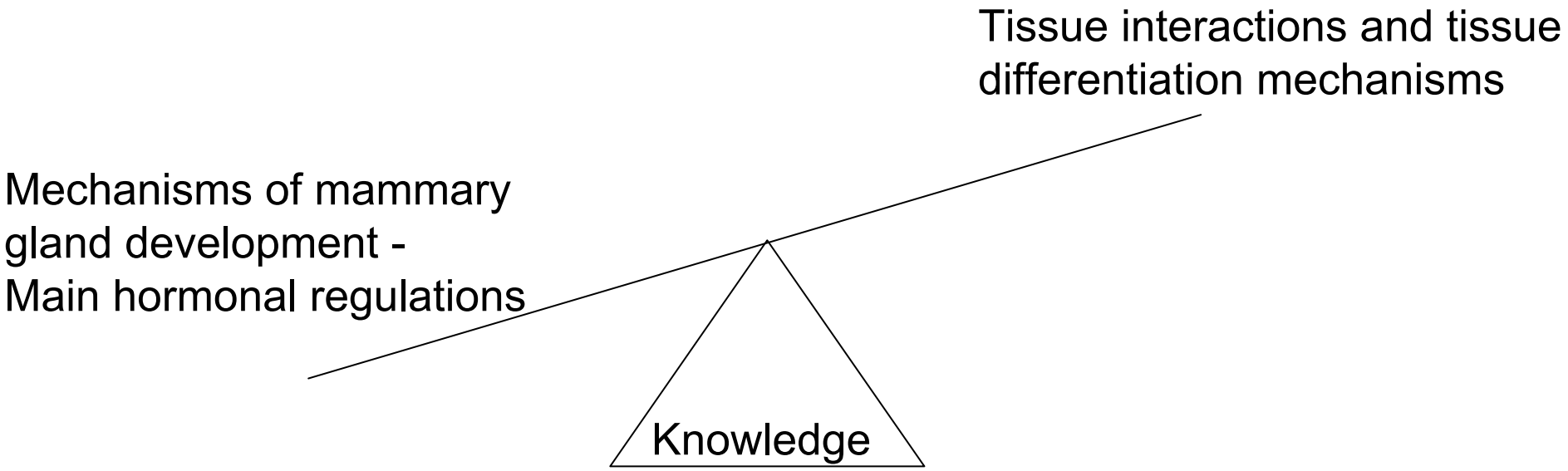


Hormonal regulation

- Endocrine : ovarian; pituitary; placental; suprarenal
- Paracrine

Adapted from « Cours sur la Biologie de la Lactation », Université de Sherbrooke, P. Lacasse ; Zhou et al., 2005, The EMBO Journal, 24, 635-644

Main issue

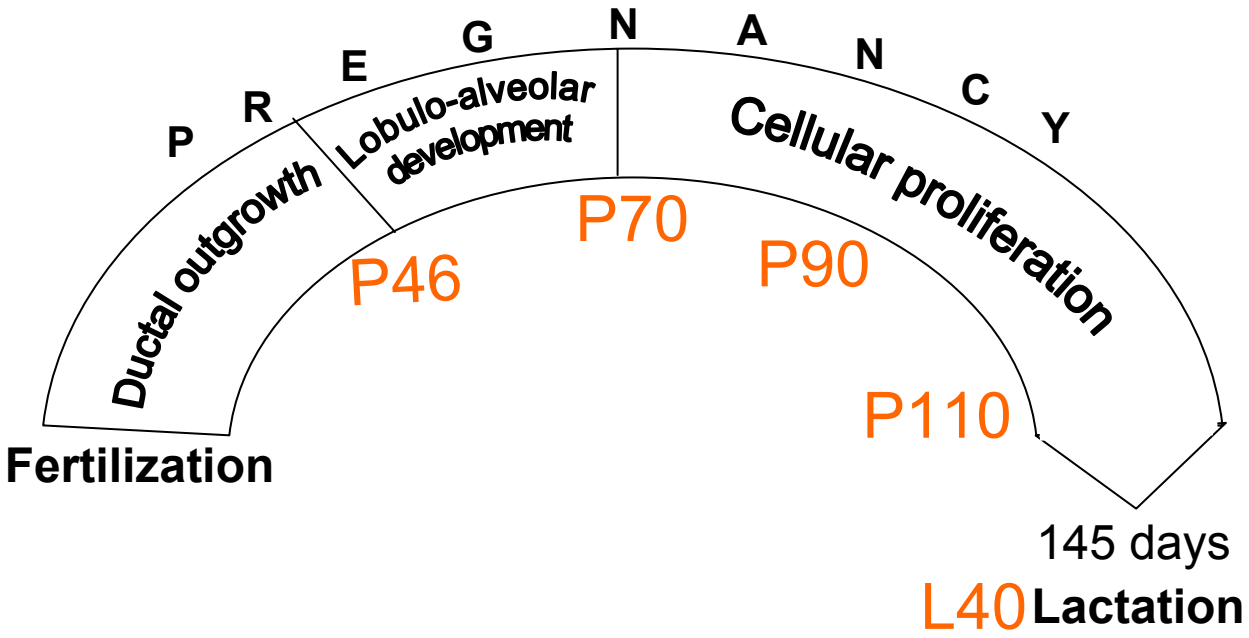


What are the main functional networks operating in the mammary tissue during its differentiation?

How to proceed ?

By drawing expression profile of genes during goat pregnancy

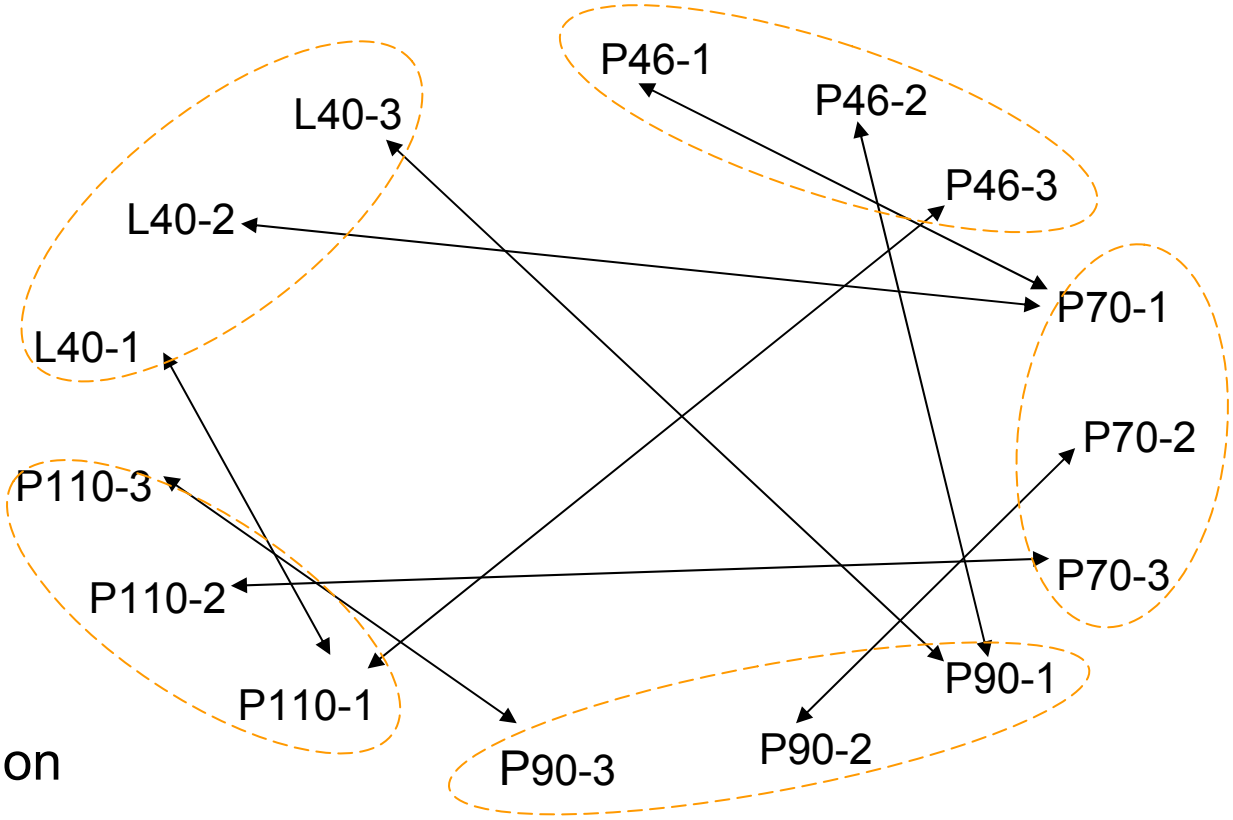
- 4 developmental stages (P46, P70, P90, P110)
- 1 lactation stage (L40)



- 3 primiparous goats for each developmental stage
- Transcriptomic approach : microarray experiment

Materials and methods : animals and experimental plan

- 18 oligoarrays (22 k, Operon and Illumina bovine sets, CRB INRA Jouy-en-Josas, France)
- Mammary tissue harvesting
- Loop experimental design (kinetic approach)
- 9 dye-swaps



Optimization by variance minimization

Materials and methods : microarray experiment

Lab

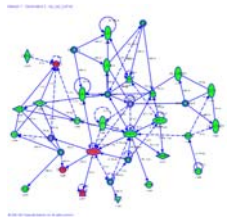
Data extraction

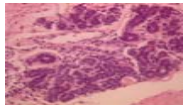
Data normalization

Statistical analysis

Clustering

Network detection





Crushing – Total RNA extraction

RNA

RNA

Reverse transcription

cDNA

cDNA

Cy3

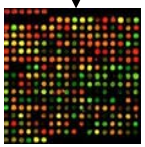
Labelling

Cy5

cDNA

cDNA

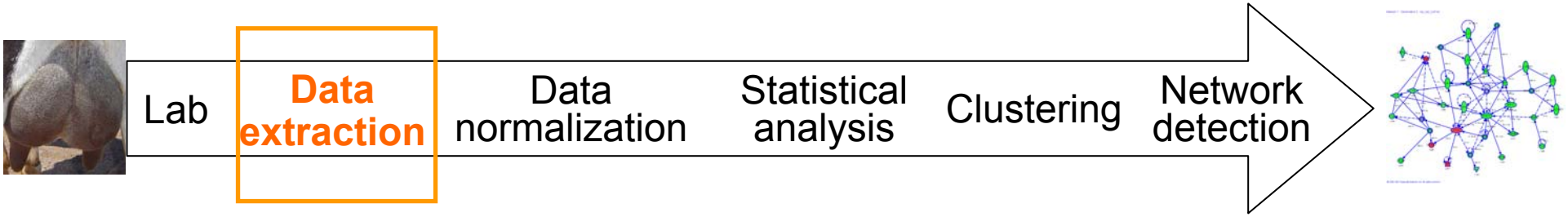
Hybridization



Scan

Swap = CyDyes inversion in the 2nd slide

Materials and methods : microarray experiment



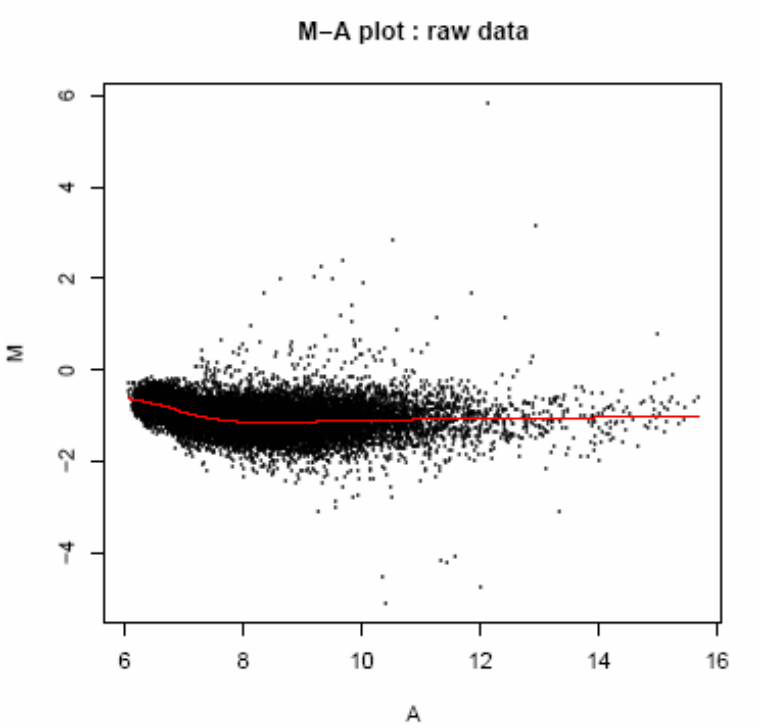
- Feature Extraction software (Agilent Technologies)

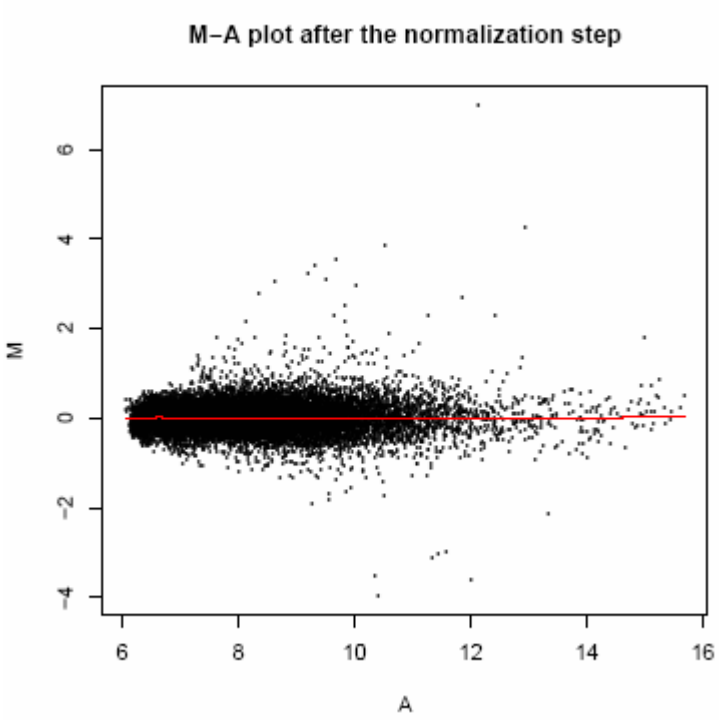
  All flag* settings were taken into account

* Flag = elimination

Materials and methods : microarray experiment

- Lowess normalization method (R, Anapuce library, J. Aubert)





M = log(Cyanine 5) – log(Cyanine 3)
A = (log[Cyanine 5+ log[Cyanine 3]) / 2

Materials and methods : microarray experiment

- Linear regression :

$$Y = X\Theta + E$$

Y : signal log(Cyanine 5/Cyanine 3)

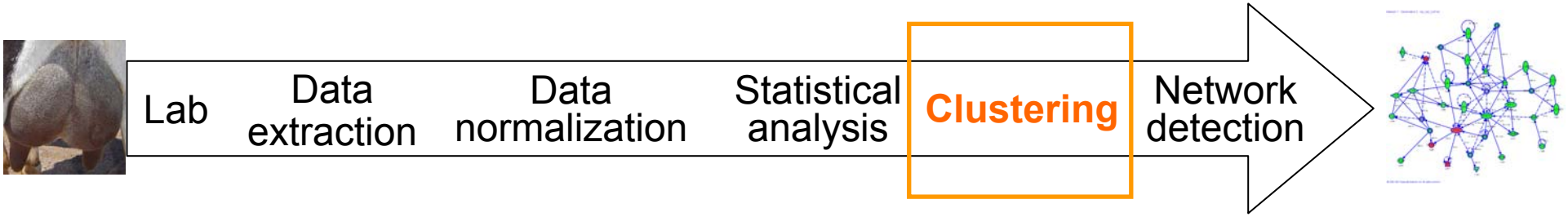
X : experimental design

Θ : difference between stage n+1 and stage n

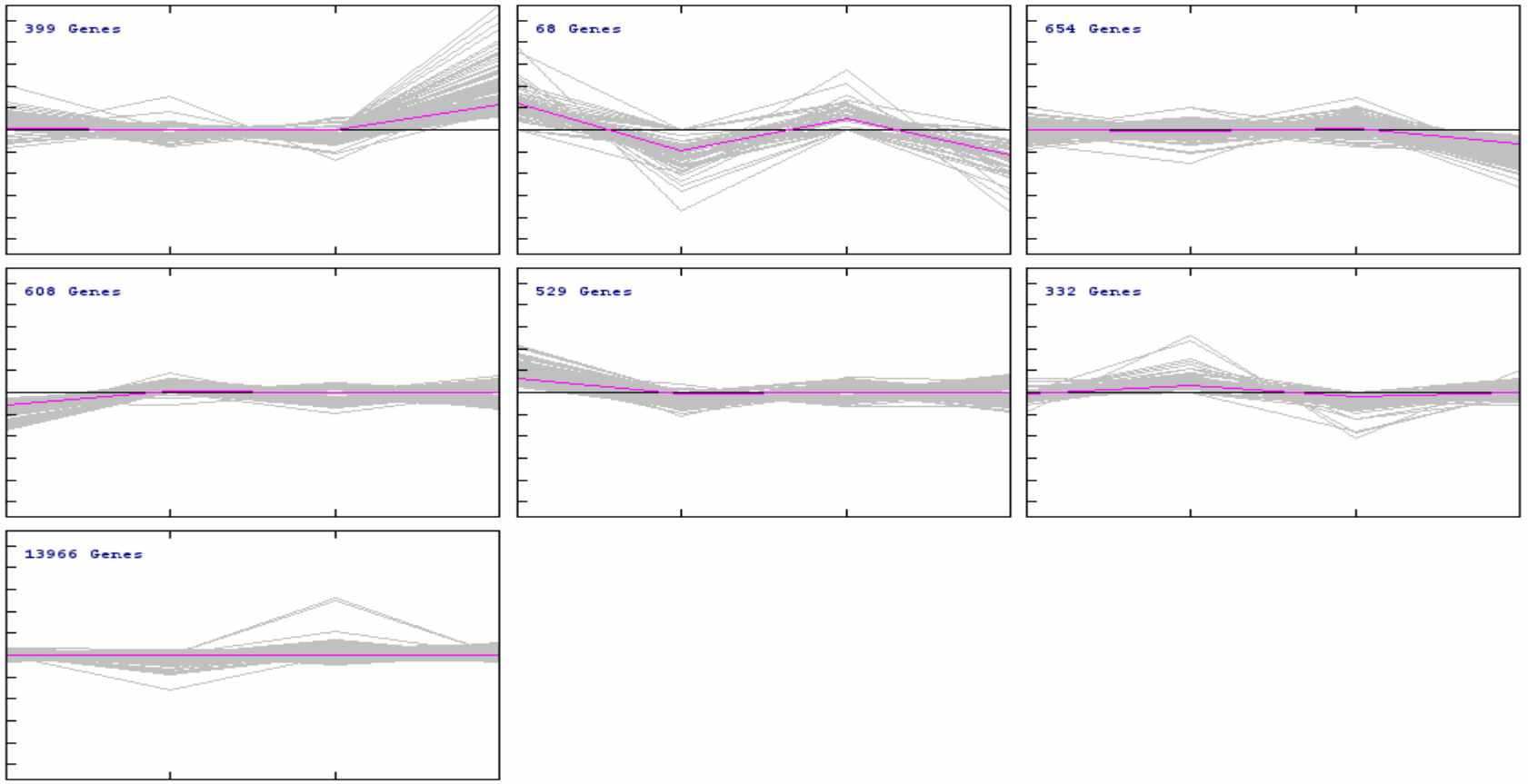
E : residual

Significance threshold : 0.05

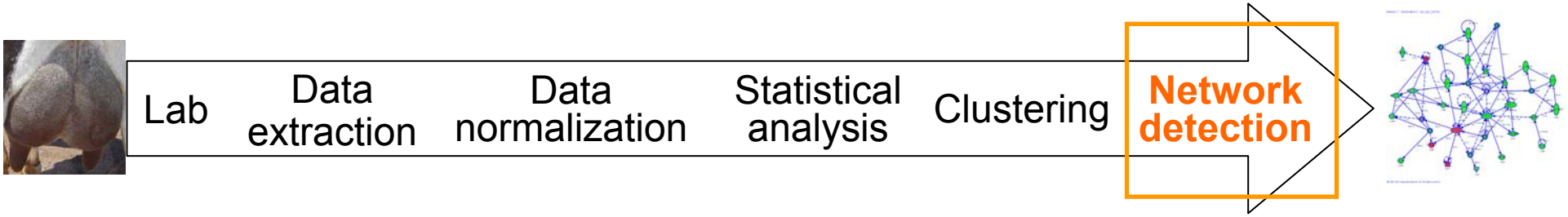
Materials and methods : microarray experiment



- K means clustering (selection of 7 clusters, TMeV software)



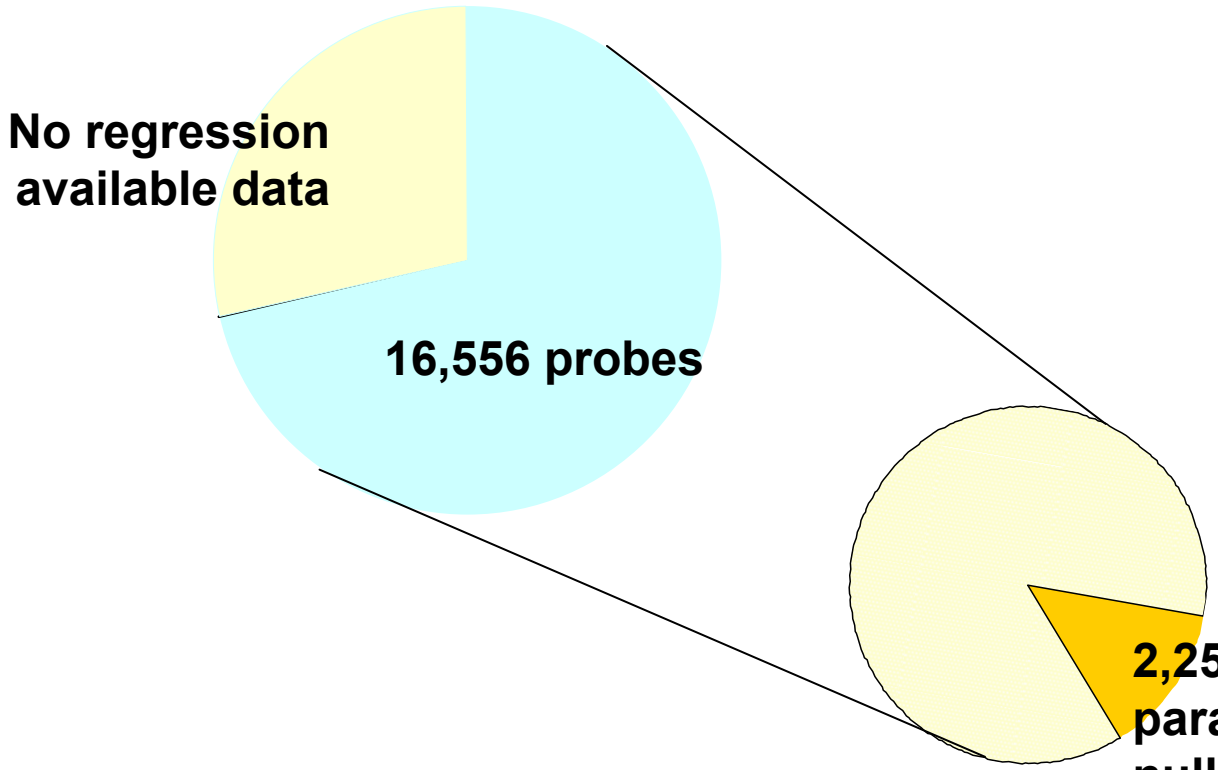
Materials and methods : microarray experiment



- Ingenuity Pathways Analysis web data base

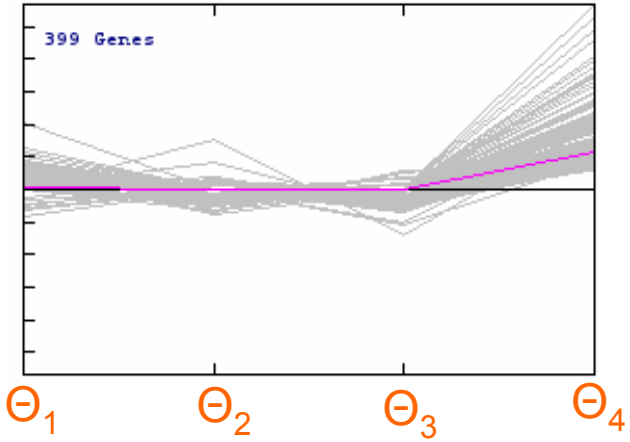
Results : general data information

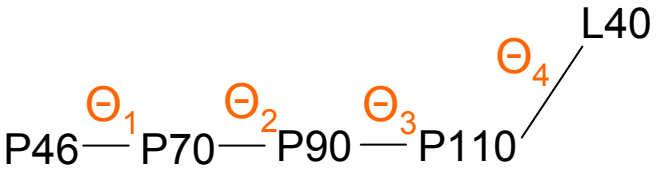
Amongst 23,232 probes



Results : overview of 5 clusters

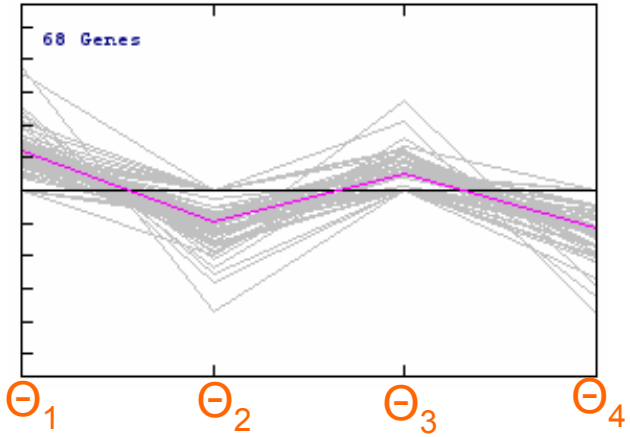
Cluster 1 (399 probes)

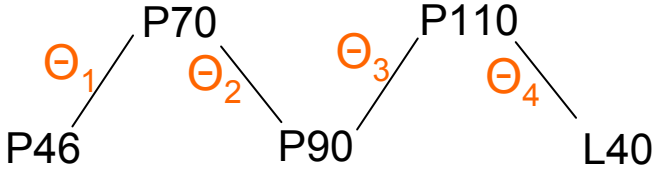




Lipid metabolism

Cluster 2 (68 probes)

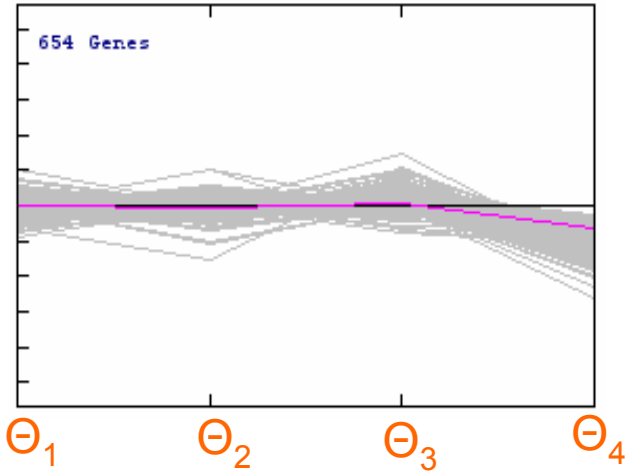




Immune response

Results : overview of 5 clusters

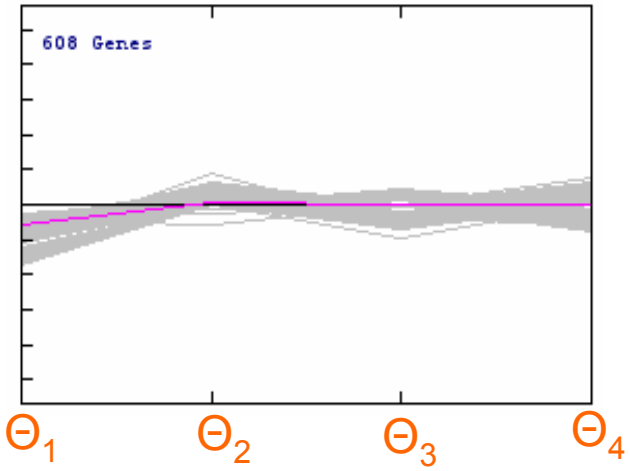
Cluster 3 (654 probes)



P46 —Θ1— P70 —Θ2— P90 —Θ3— P110
P110 —Θ4— L40

Cell cycle

Cluster 4 (608 probes)

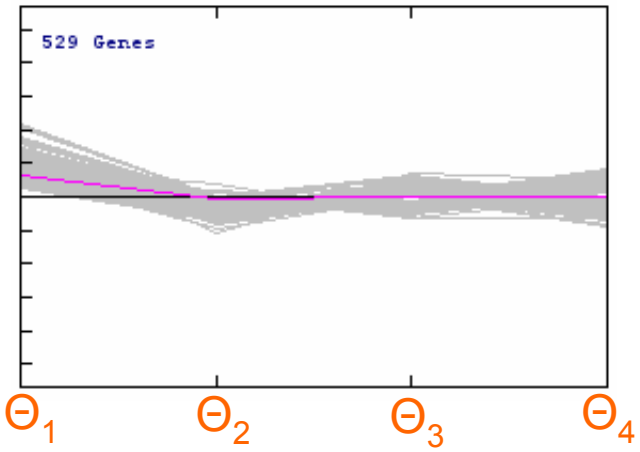


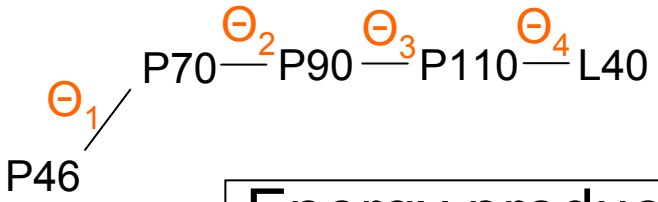
Tissue development
Organ development

P46 —Θ1— P70 —Θ2— P90 —Θ3— P110 —Θ4— L40

Results : overview of 5 clusters

Cluster 5 (529 probes)





Energy production

Carbohydrate metabolism

Discussion : results validation

- DAVID database using GO-Biological Process (Dennis et al., 2003, Genome Biology)

- Literature validation (Rudolph et al., 2003, Clarkson et al., 2004)

- Determination of key genes in functional networks and validation of their expression profile by RT-PCRq

Discussion : oncoming prospects

- Search tools to understand and characterize the **Immune response** observed in Ingenuity functional network at P70 and P110

- Proteomic study to validate the mRNA-protein transition

- **From tissue to cell** : Laser Capture Microdissection as tool to specifically characterize the mammary epithelial cell differentiation