



**74<sup>th</sup> EAAP ANNUAL MEETING, Lyon – France**  
**Session 10 - "Impact of epigenetics and genetics in determining animal physiology"**

**METAPROGRAMME**

SANTé et Bien-être des  
Animaux en élevage  
(MP-SANBA)

➤ **Epigenetic biomarkers for environmental enrichment and parity in pregnant sows**

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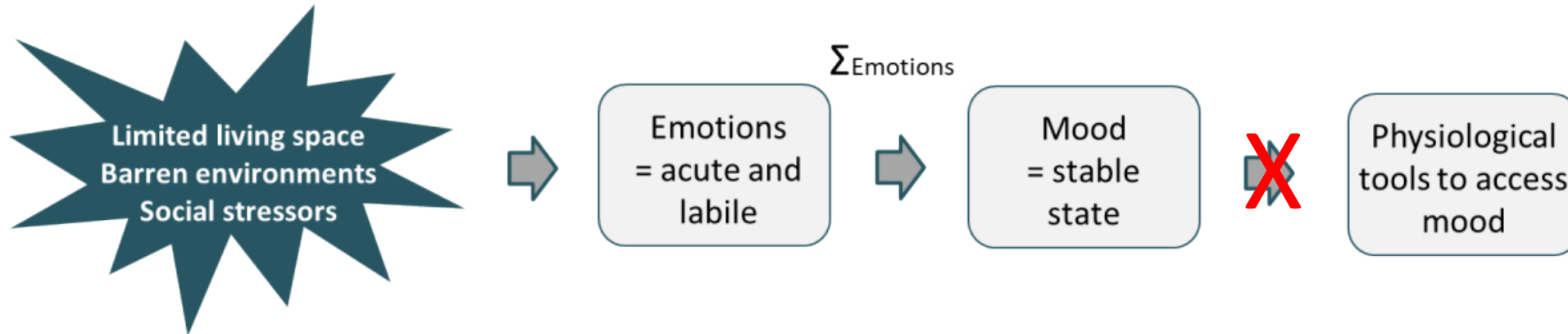
# Introduction: welfare definition and challenges

## Animal welfare definition

“The state resulting from the **positive mental** and physical state related to the satisfaction of its physiological and behavioral needs, as well as its expectations” *ANSES, 2018*



Need for valid, reliable and feasible animal-based welfare biomarkers



Better welfare solutions

Properly understand welfare needs

Easy to implement physiological tools

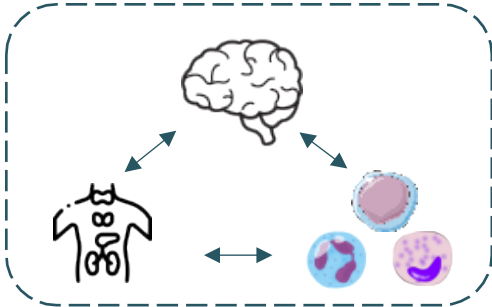


# Introduction: DNA methylation

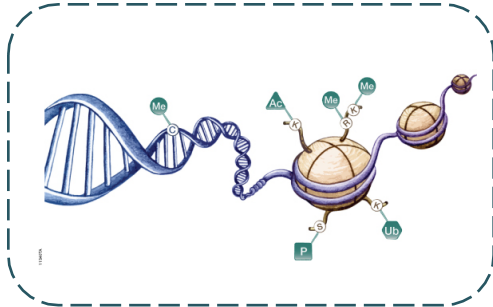
Social-environmental determinants



Brain x endocrine x immunity



Epigenetic modifications



Other factors influencing DNA methylation:

- Nutrition
- Diseases (cancer, autoimmune, cardiovascular...)
- Toxic exposure
- Climatic influence
- Aging (↑ hypomethylation in specific DNA regions)



*Tyrka et al, 2016*  
*Roy et al, 2017*  
*Blaze and Roth, 2017*  
*Andrews and Neises, 2012*  
...



Is it also possible to observe blood methylome differences when comparing animals with contrasted welfare states?



# Objective



**Evaluate the variations in DNA methylation in blood cells of farm animals as a welfare indicator**

Use of pregnant sows housed in contrasted welfare conditions and of different parities



Why pregnant sows?

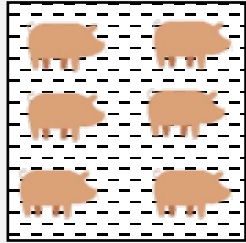
- Diverse housing systems → ≠ welfare levels
- Stable condition → moods?
- Several gestations in the production system → ≠ parities



# Experimental design

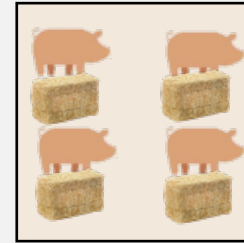
- Environmental enrichment with a known positive impact on the sows' welfare (*Merlot et al, 2022*)

During successive gestations:



## Conventional (C)

- Concrete floor
- 2.4 m<sup>2</sup>/sow



## Enriched (E)

- Accumulated straw floor
- 3.5 m<sup>2</sup>/sow

- 28 animals selected (2<sup>nd</sup> gestations or more)

14 Conventional (7 x 2 repetitions)

14 Enriched (7 x 2 repetitions)

15 Low Parity (LP; 2<sup>nd</sup> or 3<sup>rd</sup> gestation - 456 ± 71 days old)

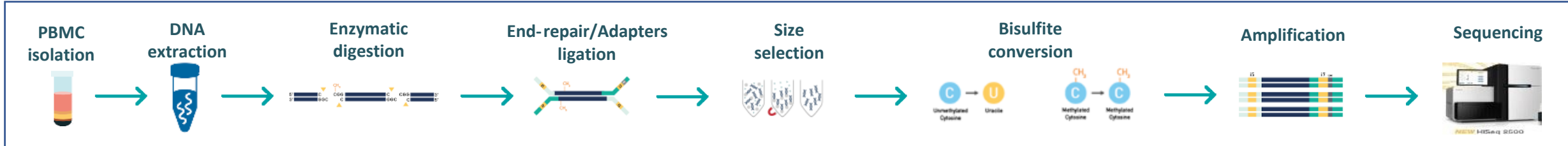
13 High Parity (HP; 4<sup>th</sup> or more gestations - 753 ± 80 days old)

# Methods: Reduced-representation Bisulfite Sequencing

## Library preparation and sequencing

Blood sampling at gestation day 98 (09:00 – 10:00)

RRBS Libraries performed by Aurélie Chaulot Talmon (UMR BREED)



## Bioinformatics pipeline

- ❑ Ensembl genome *Sus scrofa* (Sscrofa\_11.1)
- ❑ CpGs with minimum sequencing depth: 10 to 500x (CpG<sup>10-500</sup>)
  - SNP filtration (105171 SNPs list in Large White; *Julie Demars – INRAe, GenPhySE*)
- ❑ Adjusted p-value < 0.001 and minimum  $\Delta$  = 25% methylation difference
  - Differentially Methylated Cytosines (DMCs)
  - > 3 DMCs in 100bp = Differentially Methylated Regions (DMRs)

1 Adapted from Perrier et al (2018)

1 **Trimming**  
*Trim Galore*  
Babraham Bioinformatics

2 **Read Alignment**  
*Bismark*  
Babraham Bioinformatics

3 **Methylation extraction**  
*Bismark*

4 **Identification of DMCs**  
*MethylKit*  
Akalin, 2012

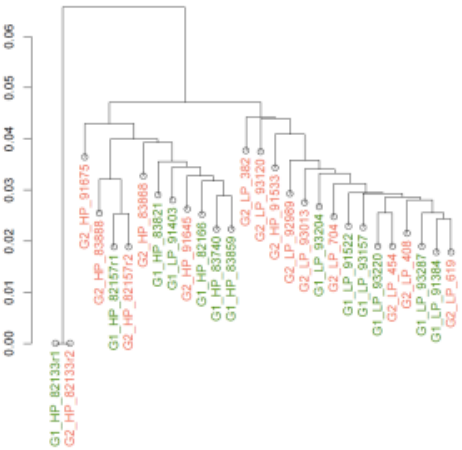
5 **Annotation of DMCs and DMRs**  
*Homemade script integrating*  
<http://homer.saik.edu/homer/ngs/annotation.html>

# Results: descriptive analysis

## Analyzing 2 560 073 identified CpG10-500 (without SNP)

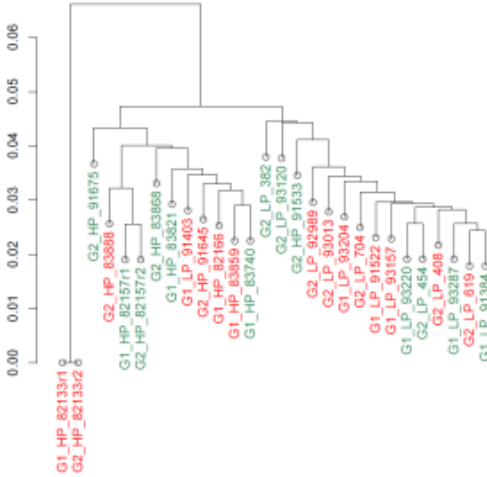
- 3 major branches in hierarchical clustering
- Explication by following variables:

### Rep 1 vs Rep2



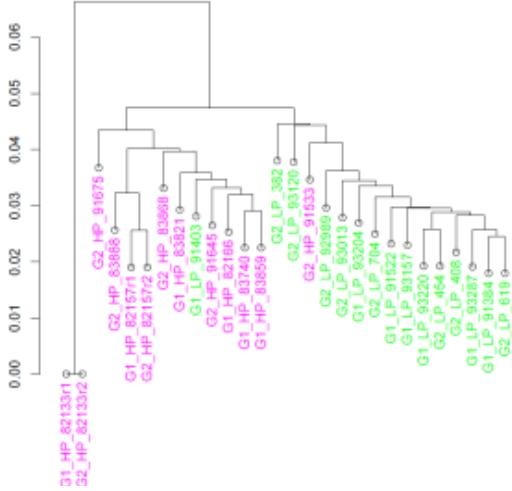
- No clear sample separation
  - R1 and R2 samples can be analyzed together

### Conventional vs Enriched



- No clear sample separation
  - ↓ housing impact on sample clustering

### Low Parity vs High Parity



- Clear sample separation
  - ↑ parity impact on sample clustering

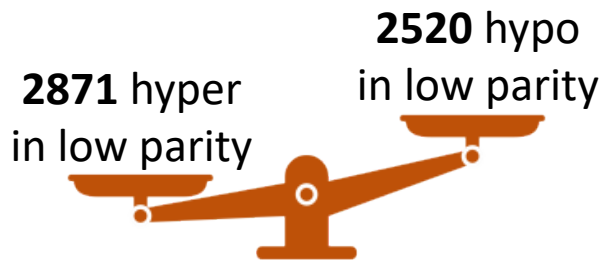
Differential analysis based on the interaction:  
1<sup>st</sup>: How do animals of ≠ parity respond to the = housing?  
2<sup>nd</sup>: How do animals of = parity respond to ≠ housing?

# Results: how do animals of ≠ parity respond to the = housing?

## Differential analysis

DMCs result from **Low parity** vs **High Parity**

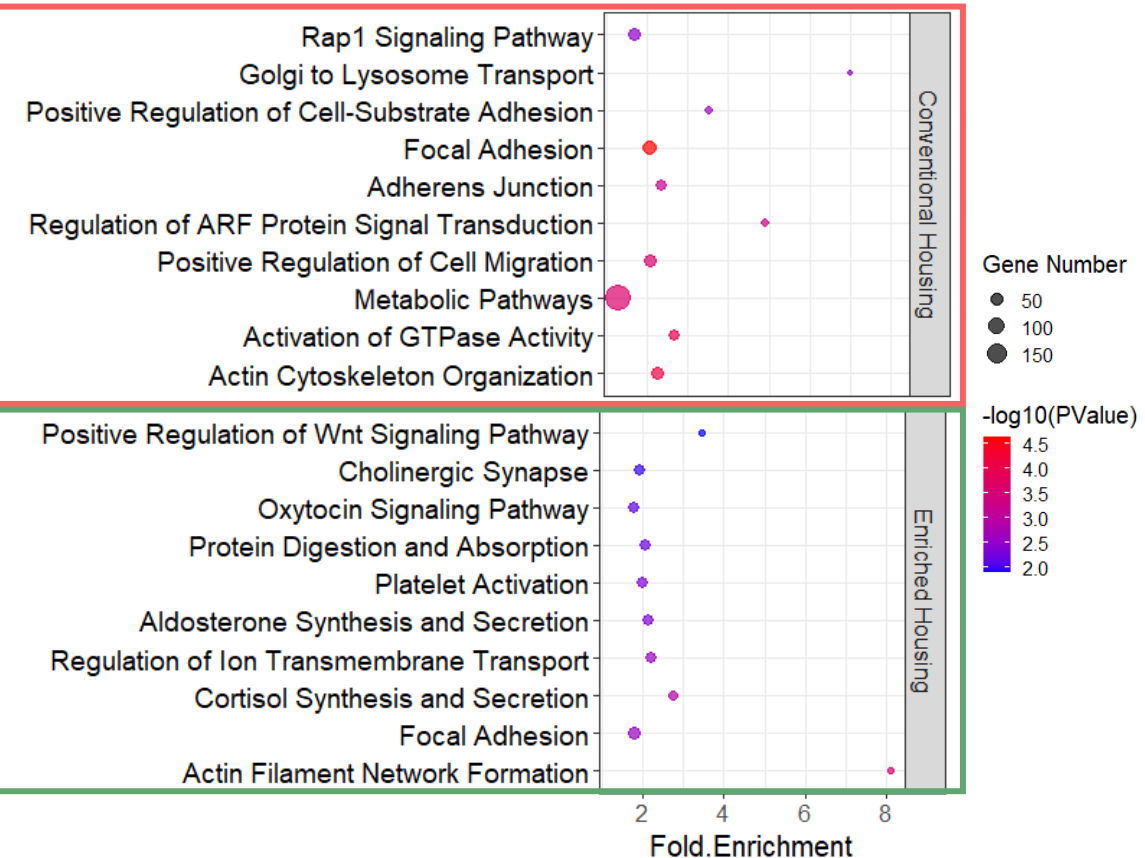
### Conventional



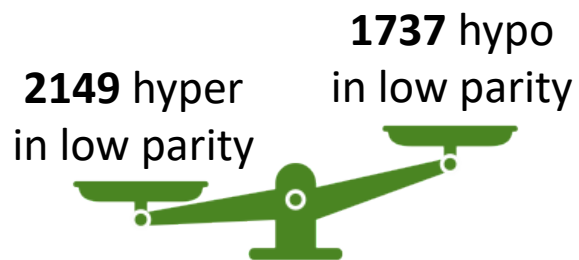
- ☐ 5391 DMCs (Low vs High Parity)
- ☐ 2046 genes related to:
  - Extracellular matrix
  - Migration
  - Metabolism
  - Signaling pathways

## Pathway analysis – top 10 pathways

DMCs list and genes covered by RRBS as background



### Enriched



- ☐ 3886 DMCs (Low vs High Parity)
- ☐ 1792 genes related to:
  - Extracellular matrix
  - Hormonal regulation
  - Ion transport
  - Platelet activation

Common DMCs	Hyper in C_LP	Hypo in C_LP
Hyper in E_LP	220	82
Hypo in E_LP	77	182

☐ Total **561** common DMCs

- ☐ + DMCs affected by parity in the conventional housing;
- ☐ Low Parity sows from both housings present + hypomethylated DMCs;
- ☐ ≠ DMCs/pathways affected by parity in each housing



# Results: how do animals of $\neq$ parity respond to the = housing?

## Differential analysis

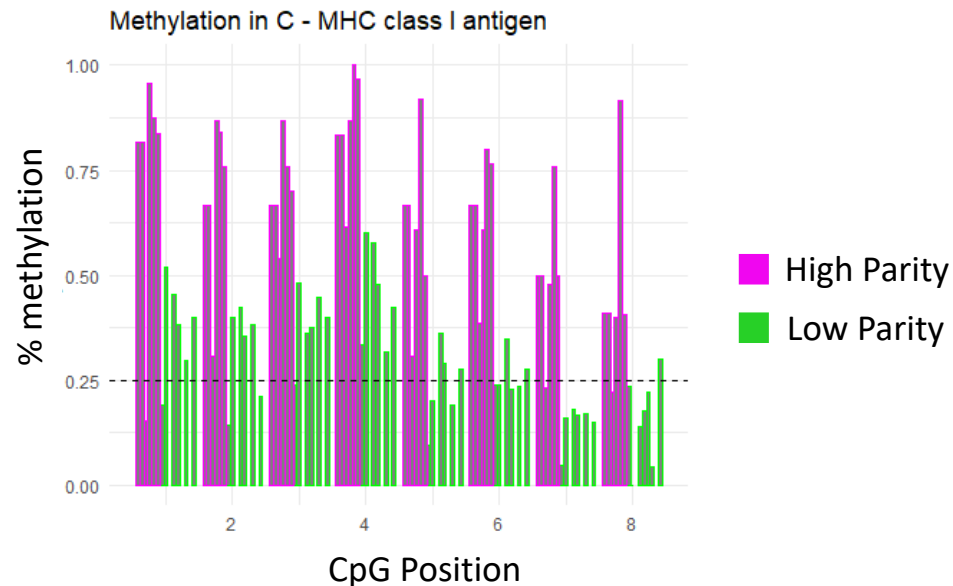
DMCs can be aggregated in Differentially Methylated Regions (DMRs; > 3 DMCs in 100bp)

Enriched housing: 23 DMR-associated genes

Conventional housing: 39 DMR-associated genes

- DMR-associated genes involved in immunity (ex: MHC class I antigen 3)

### MHC class I antigen 3: antigen presentation



Chromosome	Status in LP	Gene name	Gene feature	Interval shift
1	Hypo	SKOR1		island
10	Hypo	ADARB2	intron	island
11	Hypo	SPATA13	utr5	
11	Hypo	COL4A2	intron	shore
12	Hypo	SEPTIN9	intron	
12	Hypo	TMEM104	utr3	island
12	Hypo	U2		island
12	Hypo	NTN1	intron	shore
13	Hypo	TOPAZ1	tss	island
13	Hypo	MYLK	utr5	shelves
13	Hypo	COL6A1		island
14	Hypo	MYL2	utr3	
14	Hypo	ADRA2A	tts	island
15	Hypo	LNPB	intron	
15	Hypo	SPAG16	intron	
15	Hypo	AGAP1	intron	island
15	Hypo	KIF1A	intron	shelves
17	Hypo	NFATC2	intron	
3	Hypo	SERTAD2	utr5	
3	Hypo	RPS7	utr5	shore
4	Hypo	BOP1	intron	
5	Hypo	ITGB7	exon	island
6	Hypo	KLHL36	intron	shore
6	Hypo	FOXJ3	intron	
7	Hypo	<b>MHC class I antigen 3</b>	<b>intron</b>	<b>shore</b>
7	Hypo	LEMD2	exon	
7	Hypo	CCDC88C	utr5	island
8	Hypo	DGKQ	exon	island
9	Hypo	DENND2B	intron	island
AEMK02000682.1	Hypo	NOTCH1	promoter	shelves
X	Hypo	CHST7	exon	island
X	Hypo	PLXNA3	intron	island
2	Hyper	ANO1	utr3	shore
3	Hyper	SDK1	exon	shore
4	Hyper	RAP1A	utr5	
7	Hyper	DICER1		
11	Hyper	ATP11A	intron	island
13	Hyper	DSCAM	intron	shelves
AEMK02000682.1	Hyper	LOC110258087	tss	island

# Conclusion and perspectives

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## The housing condition:

- Does not have a major impact on the DNA methylation of blood cells from pregnant sows

## The parity:

- Has a major impact on DNA methylation of blood cells from pregnant sows
- Higher hypermethylation in low parity sows in both systems → successive gestations or age (epigenetic clock)?
- Different-parity animals respond distinctly to the same housing condition



## Next steps

- DNA methylation at lactation day 11: = or ≠ responses?
- Offspring follow-up: ≠ signatures depending on the maternal group?
- Transcriptomic data (presented at session 80): data integration to elucidate the observed effects and determine the best welfare biomarkers!

# Thank you!

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### METAPROGRAMME

SANté et Bien-être des  
Animaux en élevage  
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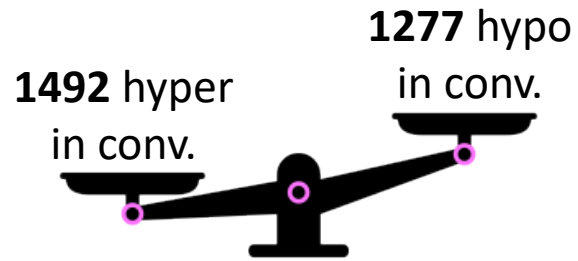


# Results: How do animals of = parity respond to ≠ housing?

## Differential analysis

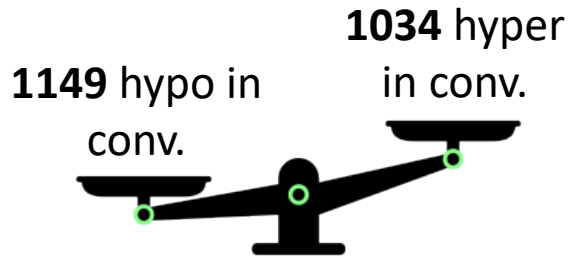
DMCs result from **Conventional** vs **Enriched**

### High parity



- ☐ 2769 DMCs (C vs E)
- ☐ 1346 genes related to:
  - Signaling pathways
  - Extracellular matrix
  - B cell function

### Low parity

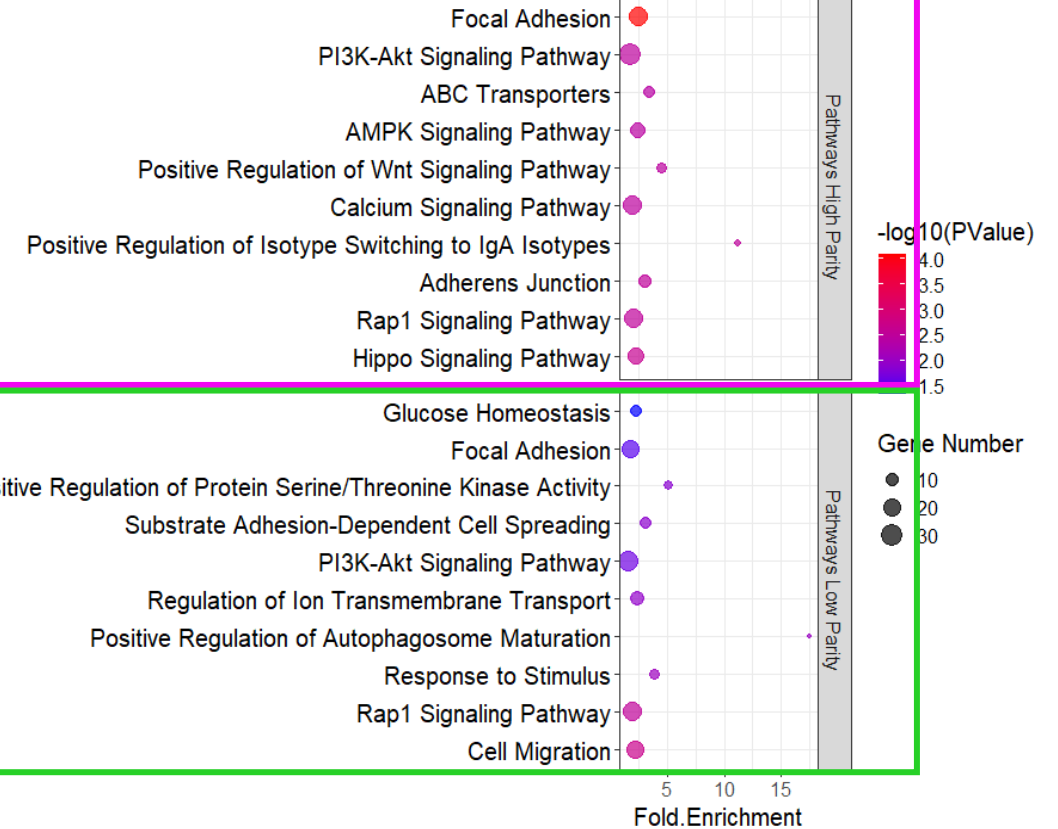


- ☐ 2183 DMCs (C vs E)
- ☐ 1114 genes related to:
  - Migration
  - Signaling pathways
  - Innate immunity
  - Extracellular matrix

☐ Total 255 common DMCs

## Pathway analysis – top 10 pathways

DMCs list and genes covered by RRBS as background



- ☐ + DMCs affected by the housing in high parity sows;
- ☐ ↑ hypomethylated DMCs in LP, however ↓ HP in the C housing
- ☐ ≠ DMCs/pathways affected by housing in each parity group