

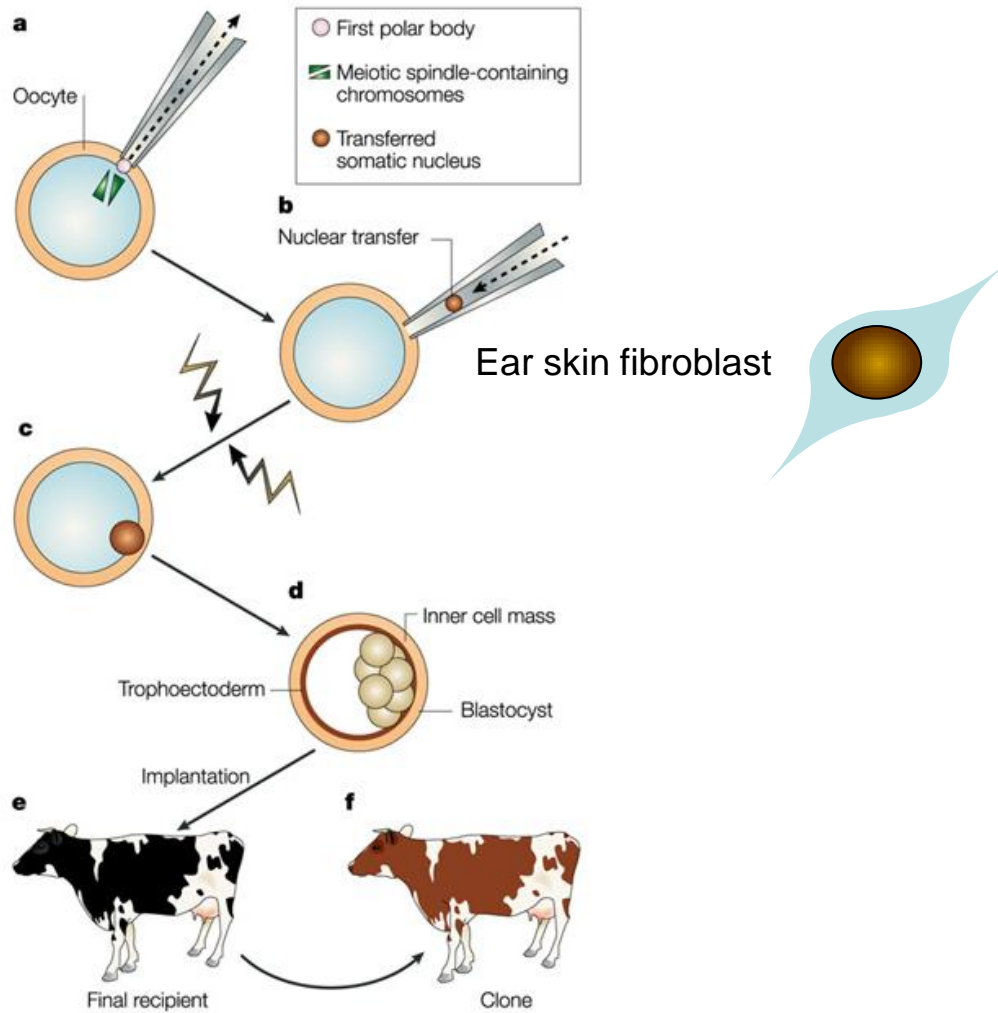


# Investigation of inter-individual epigenetic variability in bovine clones: a high throughput study

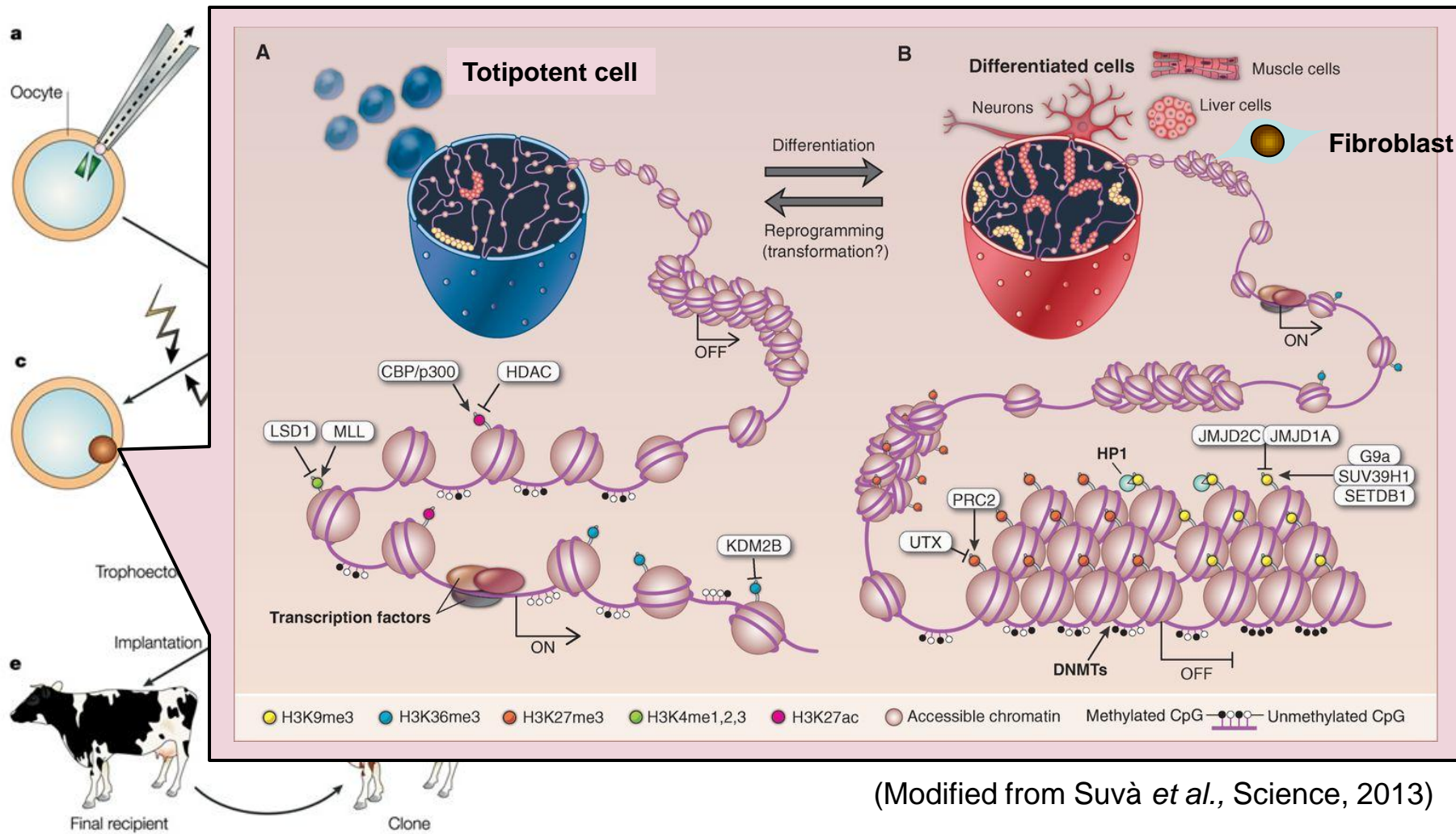
Hélène Kiefer *et al.*,

EAAP 2013-08-29

# Cloning is an epigenetic phenomenon with low efficiency

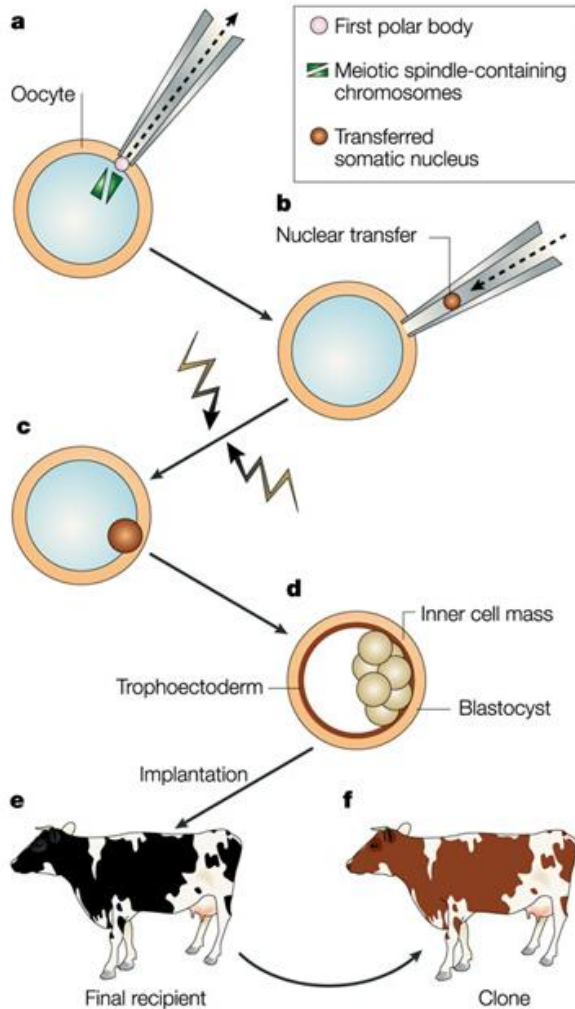


# Cloning is an epigenetic phenomenon with low efficiency

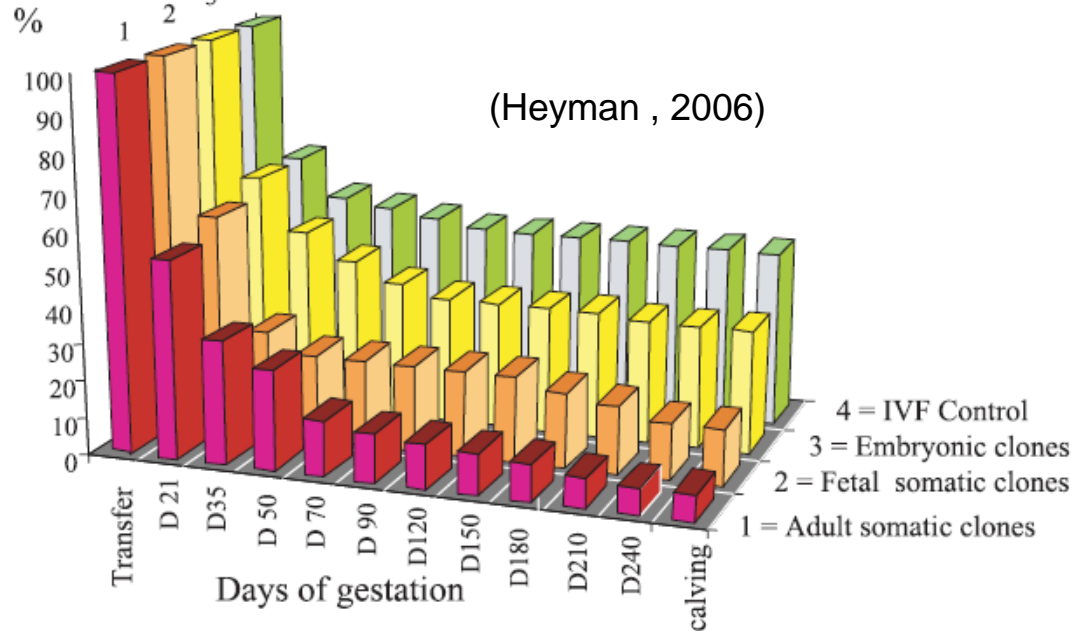


(Modified from Suvà *et al.*, Science, 2013)

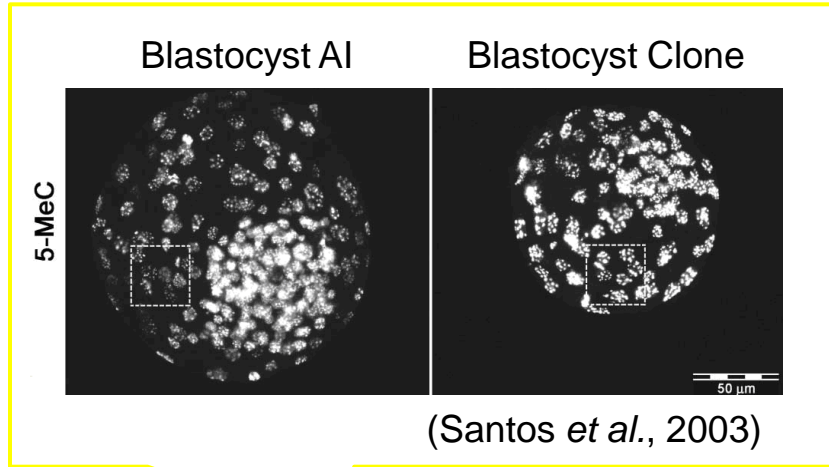
# Cloning is an epigenetic phenomenon with low efficiency



Pregnancy rate



# Epigenetic perturbations induced by cloning are maintained throughout the life

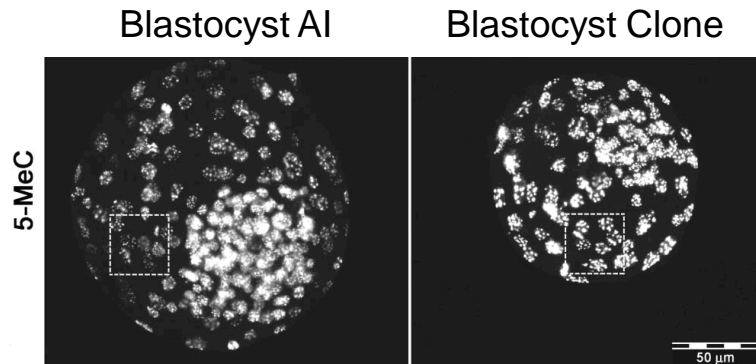


Nuc. Transfert

Birth

Adulthood

# Epigenetic perturbations induced by cloning are maintained throughout the life

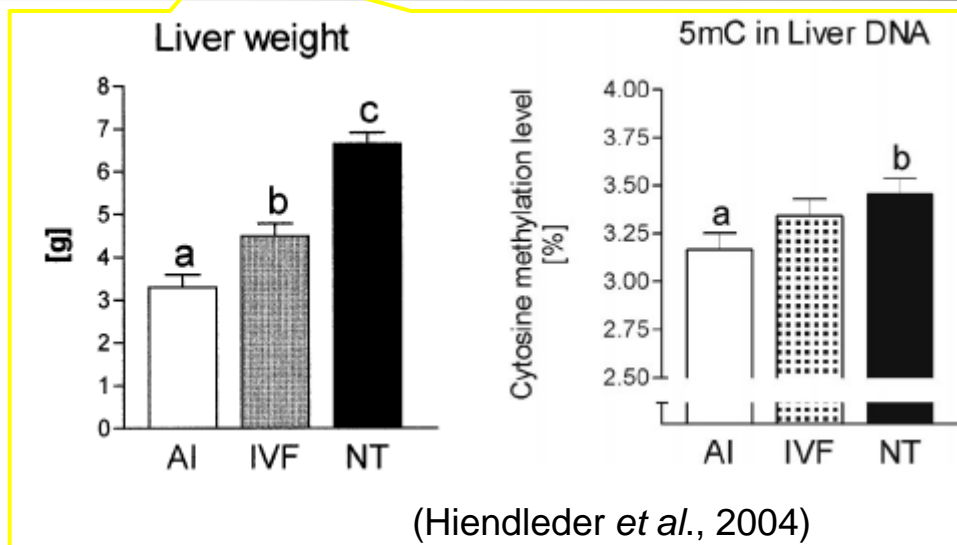


(Santos *et al.*, 2003)

Nuc. Transfert

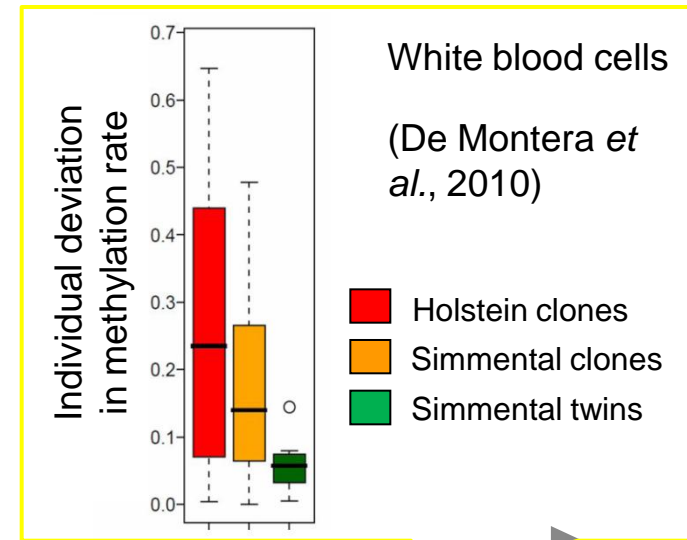
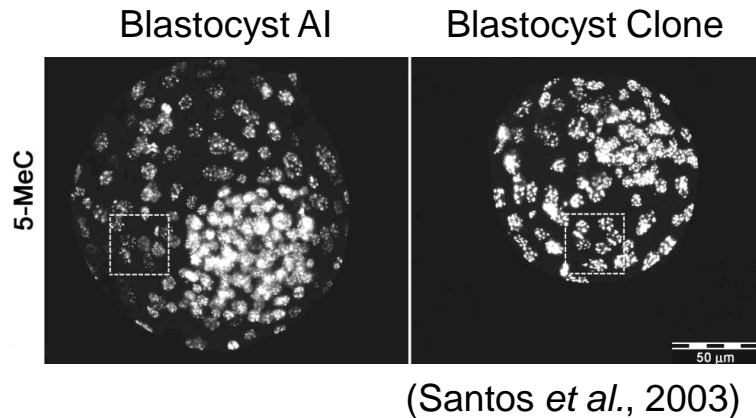
Birth

Adulthood



(Hiendleder *et al.*, 2004)

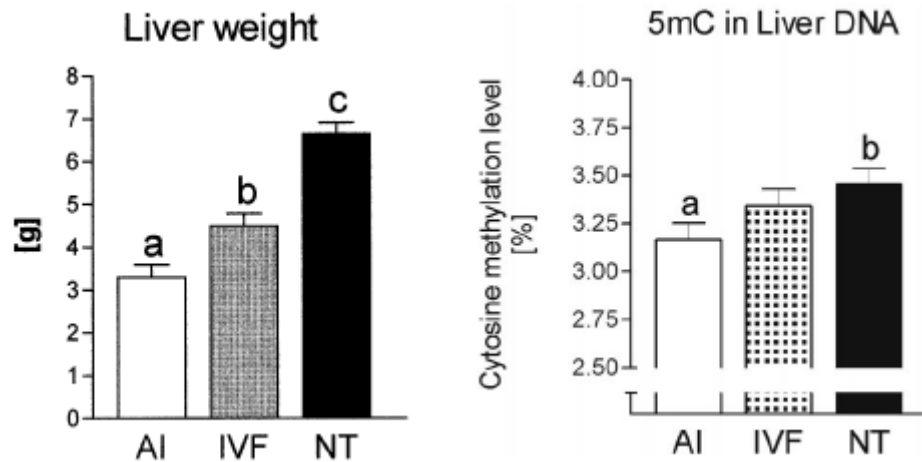
# Epigenetic perturbations induced by cloning are maintained throughout the life



Nuc. Transfert

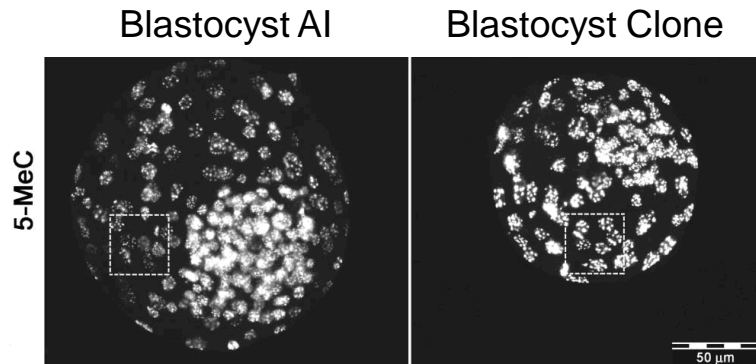
Birth

Adulthood

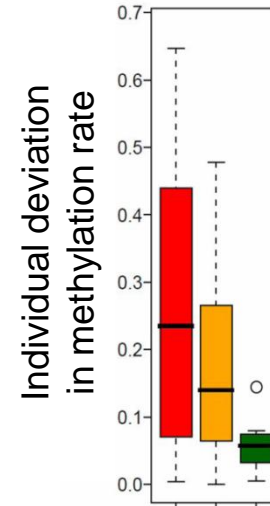


(Hiendleder *et al.*, 2004)

# Epigenetic perturbations induced by cloning are maintained throughout the life



(Santos *et al.*, 2003)

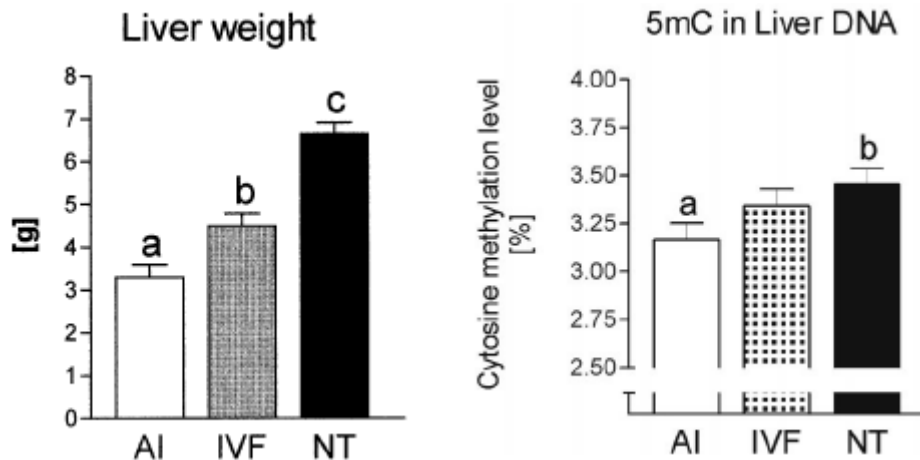


White blood cells

(De Montera *et al.*, 2010)

- Holstein clones
- Simmental clones
- Simmental twins

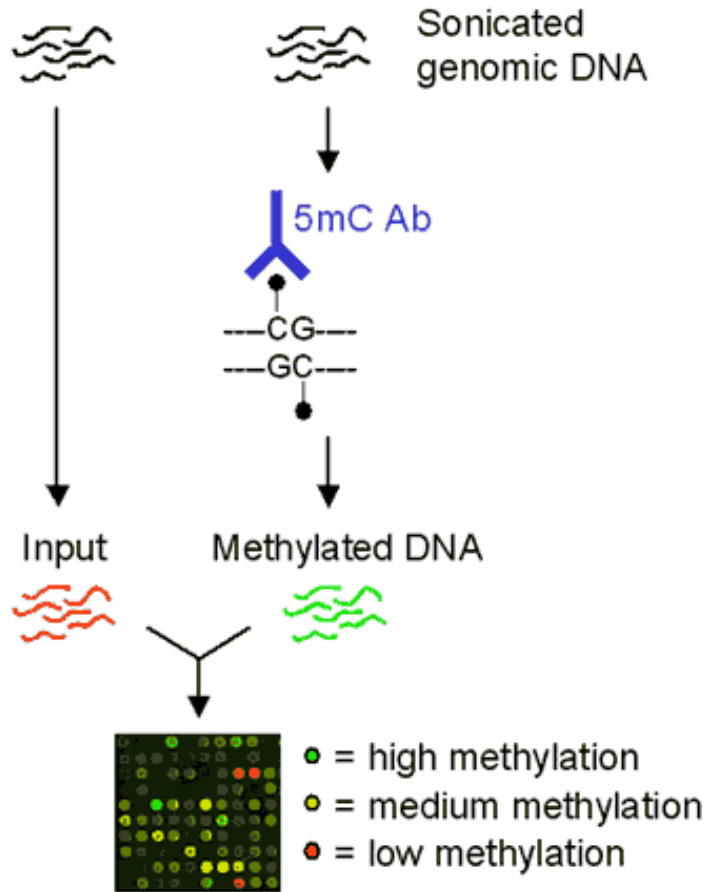
Are there genes specifically affected by epigenetic perturbations in clones ?



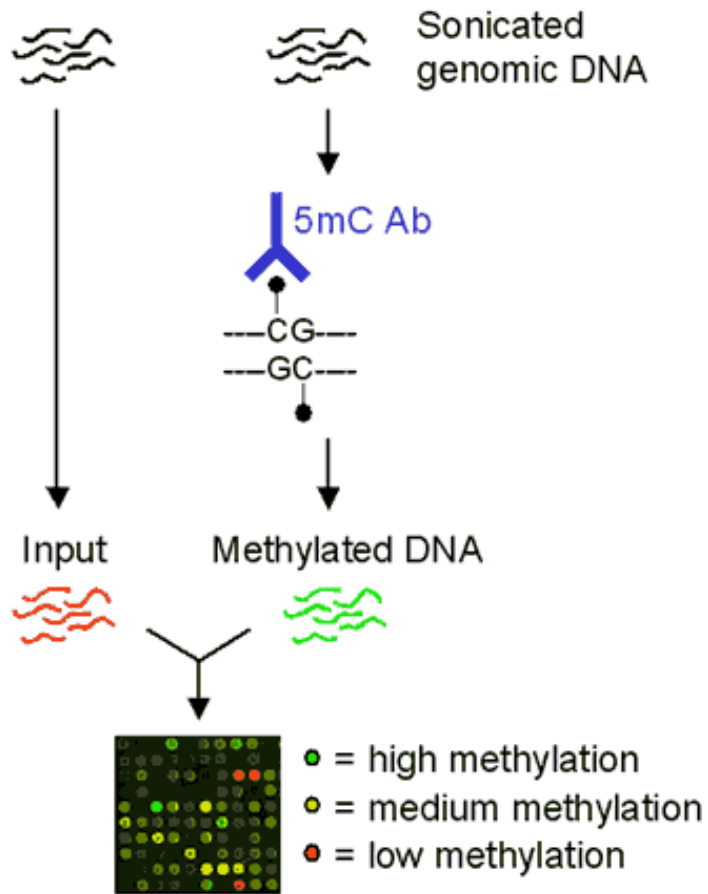
(Hiendleder *et al.*, 2004)



# Strategy : MeDIP-chip

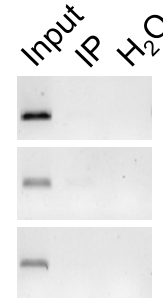
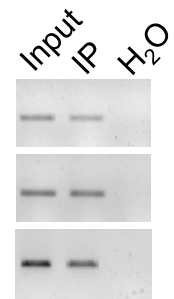


# Strategy : MeDIP-chip

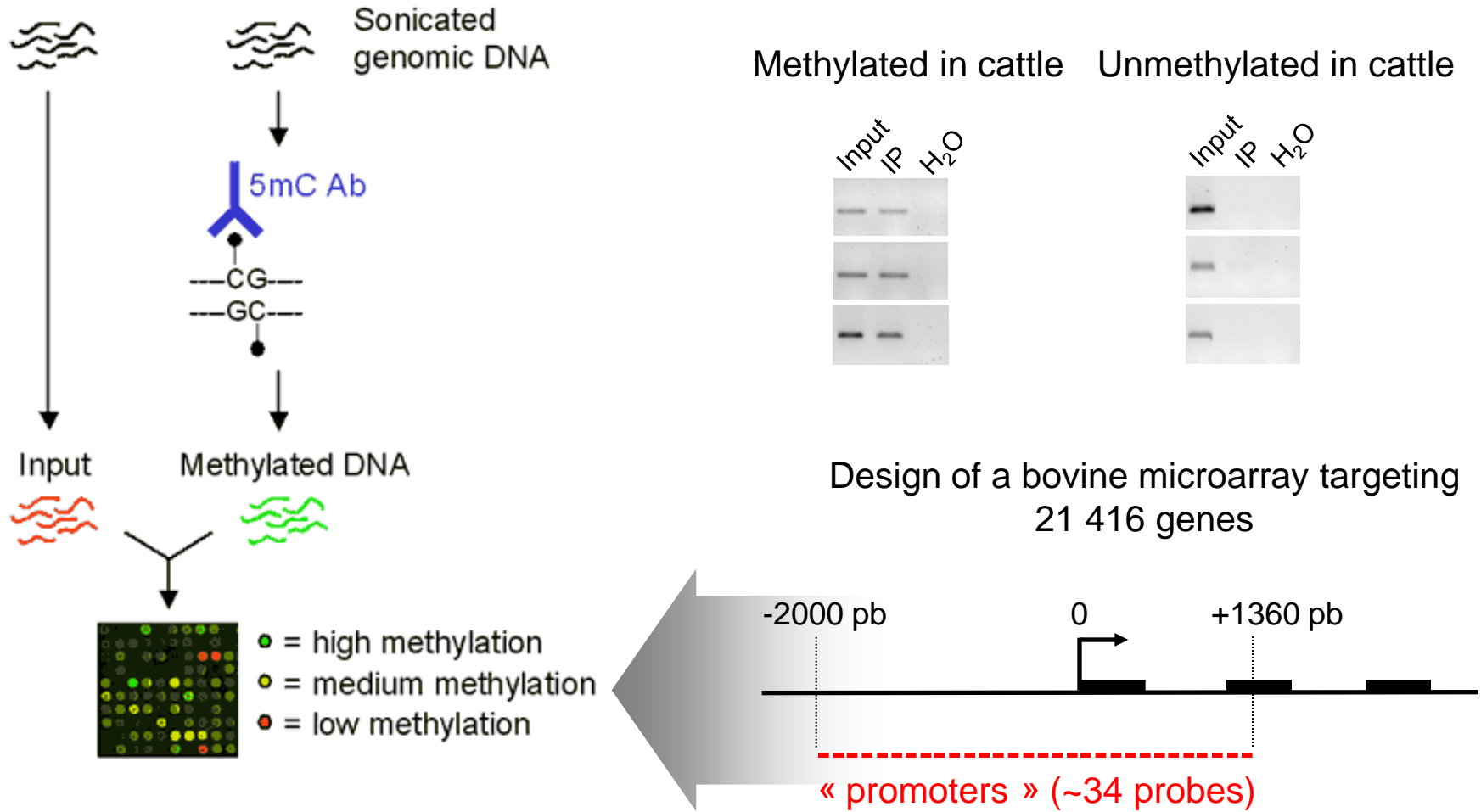


Methylated in cattle

Unmethylated in cattle



# Strategy : MeDIP-chip



# Animals

## CLONES

B0828 (GD 263)  
 ↓  
 411, 3302, 3303,  
 3304 (term)  
 ↓  
 406 (PD 1)  
 ↓  
 2263 (PD 4)



B512 (3 y)  
 ↓  
 477 (3.5 y)  
 ↓  
 439 (4 y)  
 ↓  
 447, 468 (5 y)  
 ↓  
 248, 437 (6 y)



Fertilization/  
Nuc. Transfert

Birth  
(caesarean section)

Adulthood

## ARTIFICIAL INSEMINATION (AI) CONTROLS

964, 972 (GD 266)  
 ↑  
 955, 979 (GD 267)  
 ↑



578 (5 y) ↑  
 428, 4498, 460 (6 y) ↑  
 2353 (7 y) ↑  
 229 (8 y) ↑  
 002 (10 y) ↑  
 5538 (15 y) ↑



Young clones



Adult clones



Young AI controls



Adult AI controls

# Animals

## CLONES

B0828 (GD 263)  
 ↓  
411, 3302, 3303,  
3304 (term)  
 ↓  
406 (PD 1)  
 ↓  
2263 (PD 4)



B512 (3 y)  
 ↓  
477 (3.5 y)  
 ↓  
439 (4 y)  
 ↓  
447, 468 (5 y)  
 ↓  
248, 437 (6 y)



Fertilization/  
Nuc. Transfert

Birth  
(caesarean section)

Adulthood

## ARTIFICIAL INSEMINATION (AI) CONTROLS

964, 972 (GD 266) ↑  
955, 979 (GD 267) ↑



578 (5 y) ↑  
428, 449, 460 (6 y) ↑  
2353 (7 y) ↑  
229 (8 y) ↑  
002 (10 y) ↑  
Cell donor 5538 (15 y) ↑



Genotype 5538

Genotype 2251

Genotype 029

Young clones

Adult clones

Young AI controls

Adult AI controls

# Animals

## CLONES

B0828 (GD 263)  
 ↓  
411, 3302, 3303,  
3304 (term)  
 ↓  
406 (PD 1)  
 ↓  
2263 (PD 4)



B512 (3 y)  
 ↓  
477 (3.5 y)  
 ↓  
439 (4 y)  
 ↓  
447, 468 (5 y)  
 ↓  
248, 437 (6 y)



Fertilization/  
Nuc. Transfert

Birth  
(caesarean section)

Adulthood

## ARTIFICIAL INSEMINATION (AI) CONTROLS

964, 972 (GD 266) ↑  
955, 979 (GD 267) ↑



578 (5 y) ↑  
428, 449, 460 (6 y) ↑  
2353 (7 y) ↑  
229 (8 y) ↑  
002 (10 y) ↑  
5538 (15 y) ↑



Males

Females



Young clones



Adult clones

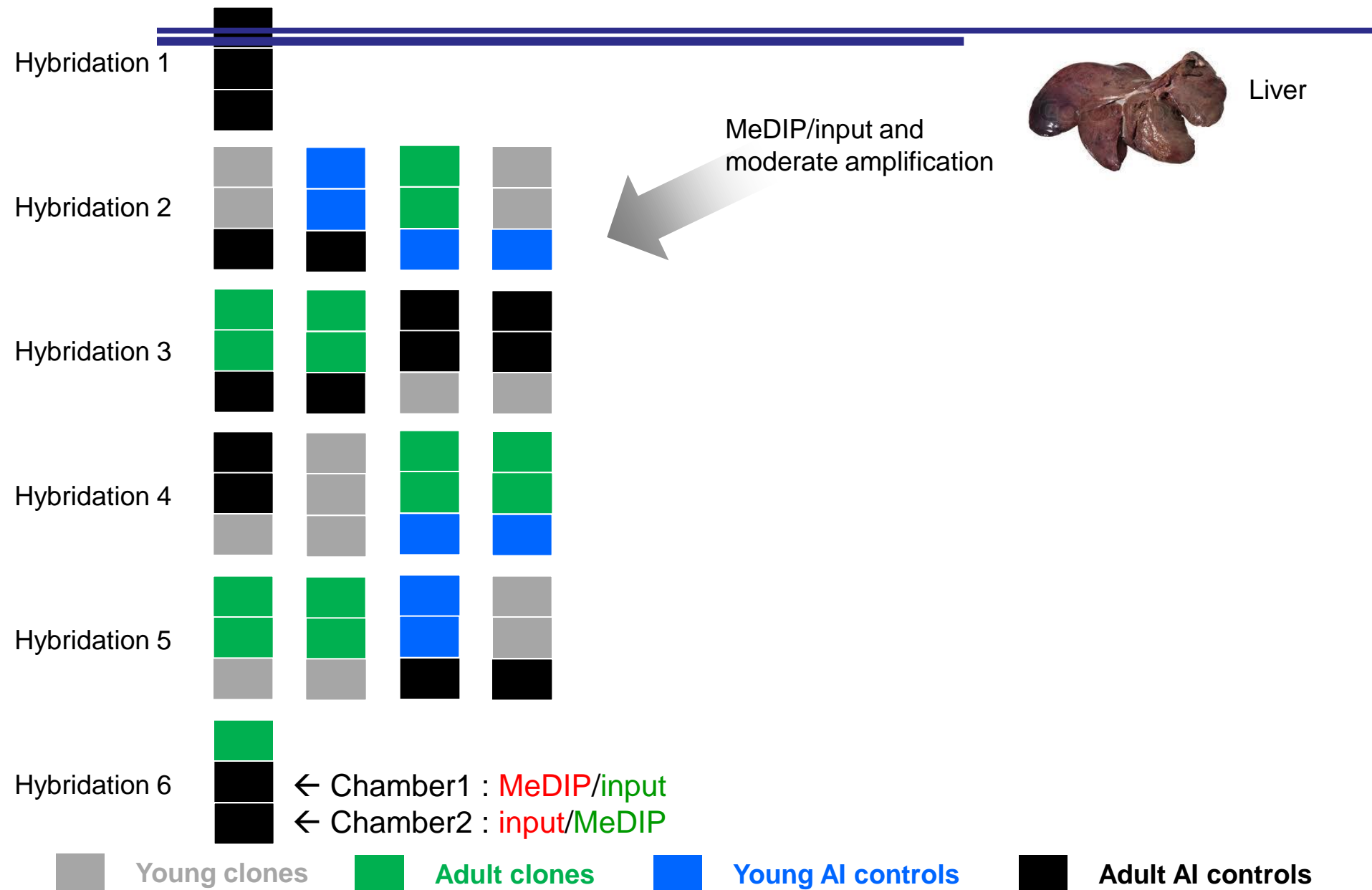


Young AI controls

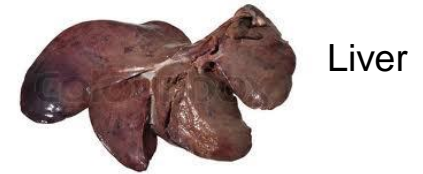


Adult AI controls

# Experimental design



# Experimental design



Hybridation 1



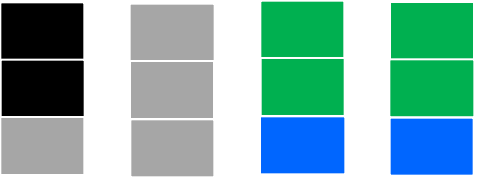
Hybridation 2



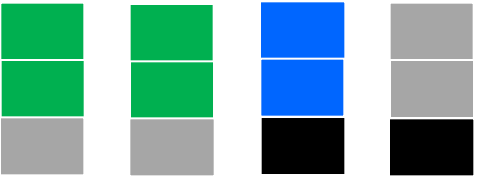
Hybridation 3



Hybridation 4



Hybridation 5

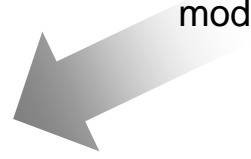


Hybridation 6

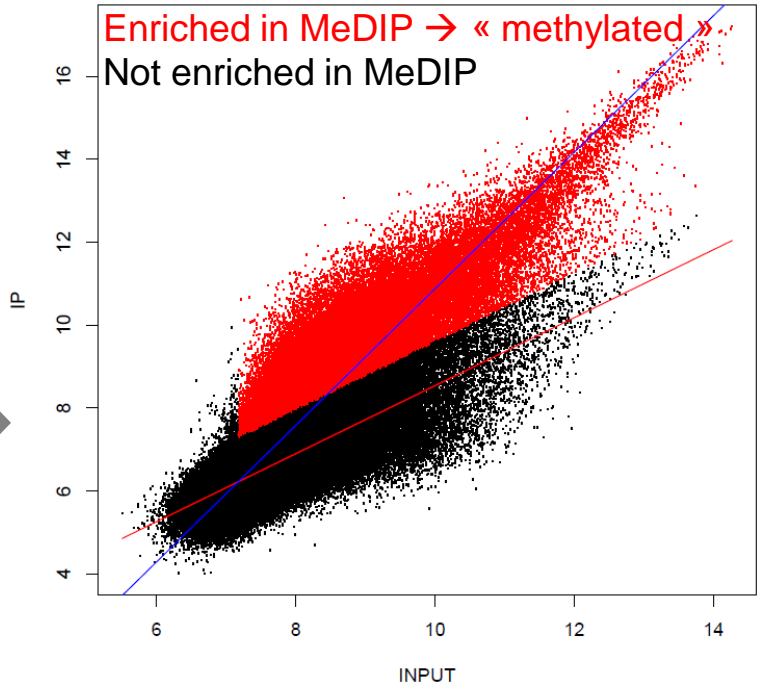


← Chamber1 : MeDIP/input  
 ← Chamber2 : /MeDIP

MeDIP/input and moderate amplification



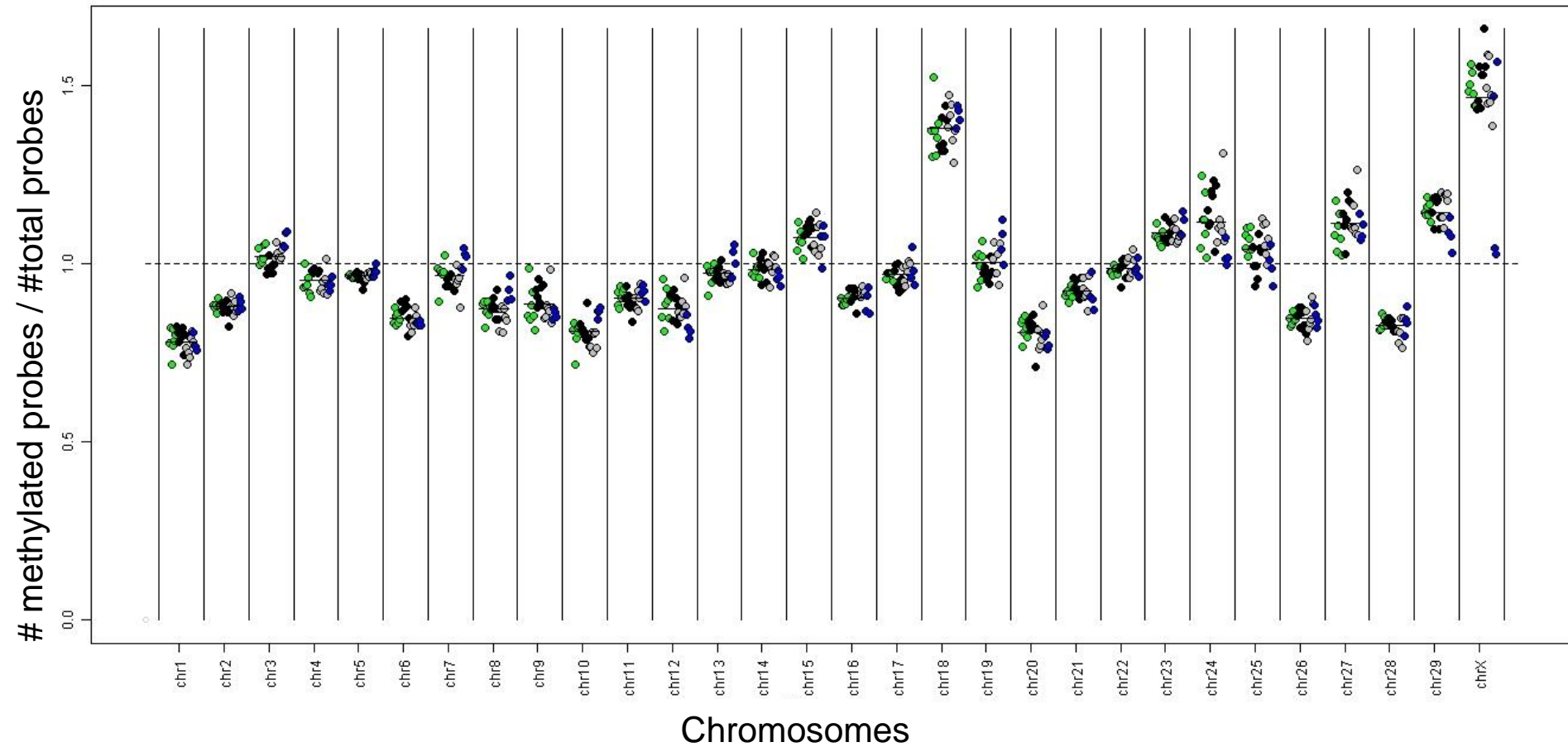
ChIPmix R package



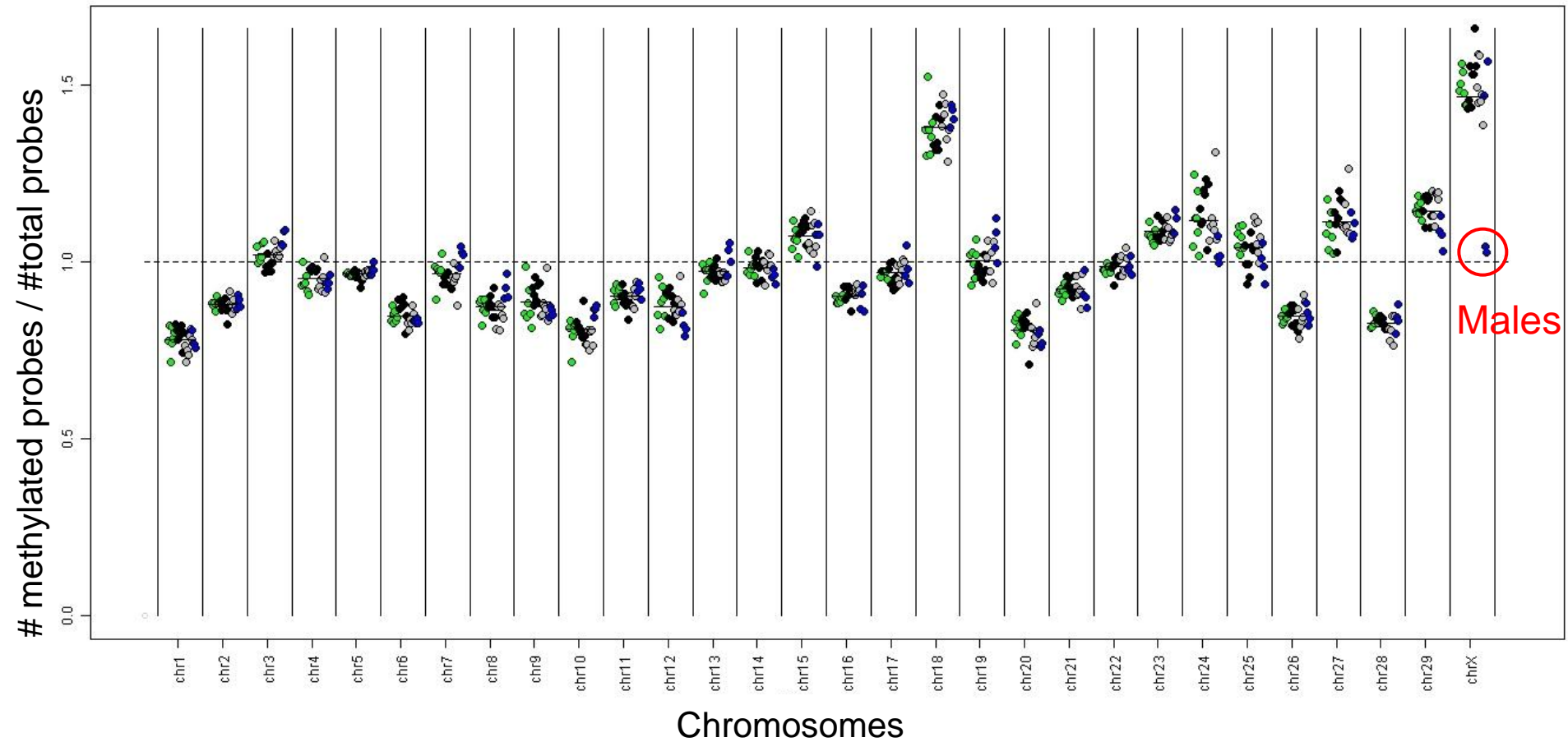
Young clones    
  Adult clones    
  Young AI controls    
  Adult AI controls



# The proportion of methylated probes per chromosome is conserved across individuals



# The proportion of methylated probes per chromosome is conserved across individuals



Young clones



Adult clones

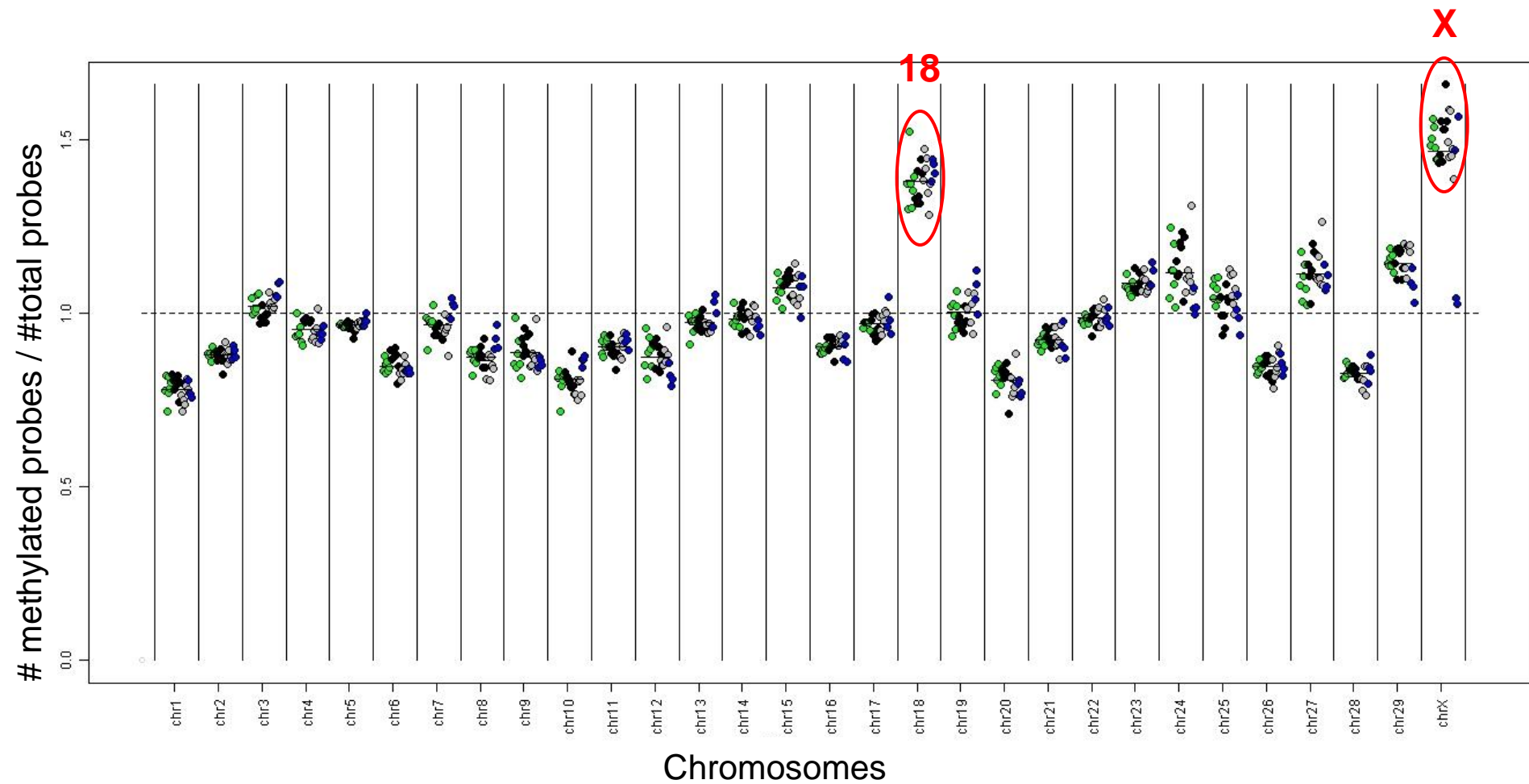


Young AI controls



Adult AI controls

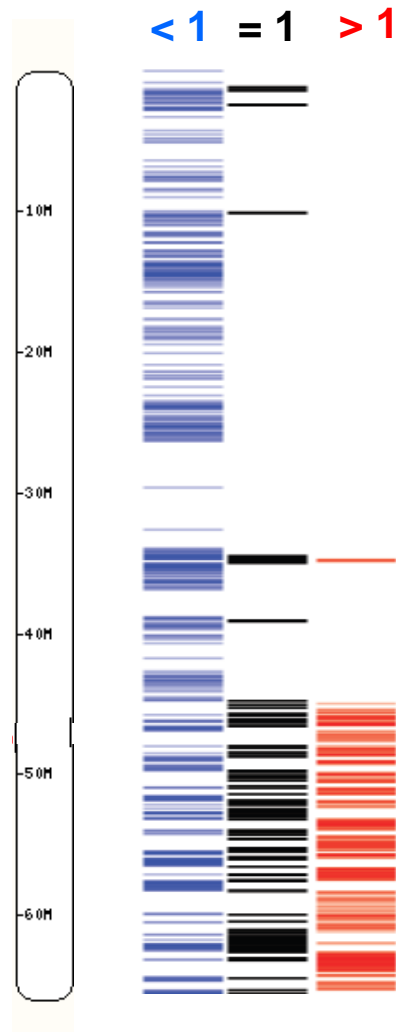
# The proportion of methylated probes per chromosome is higher in chromosomes 18 (and X in females)



Young clones
  Adult clones
  Young AI controls
  Adult AI controls

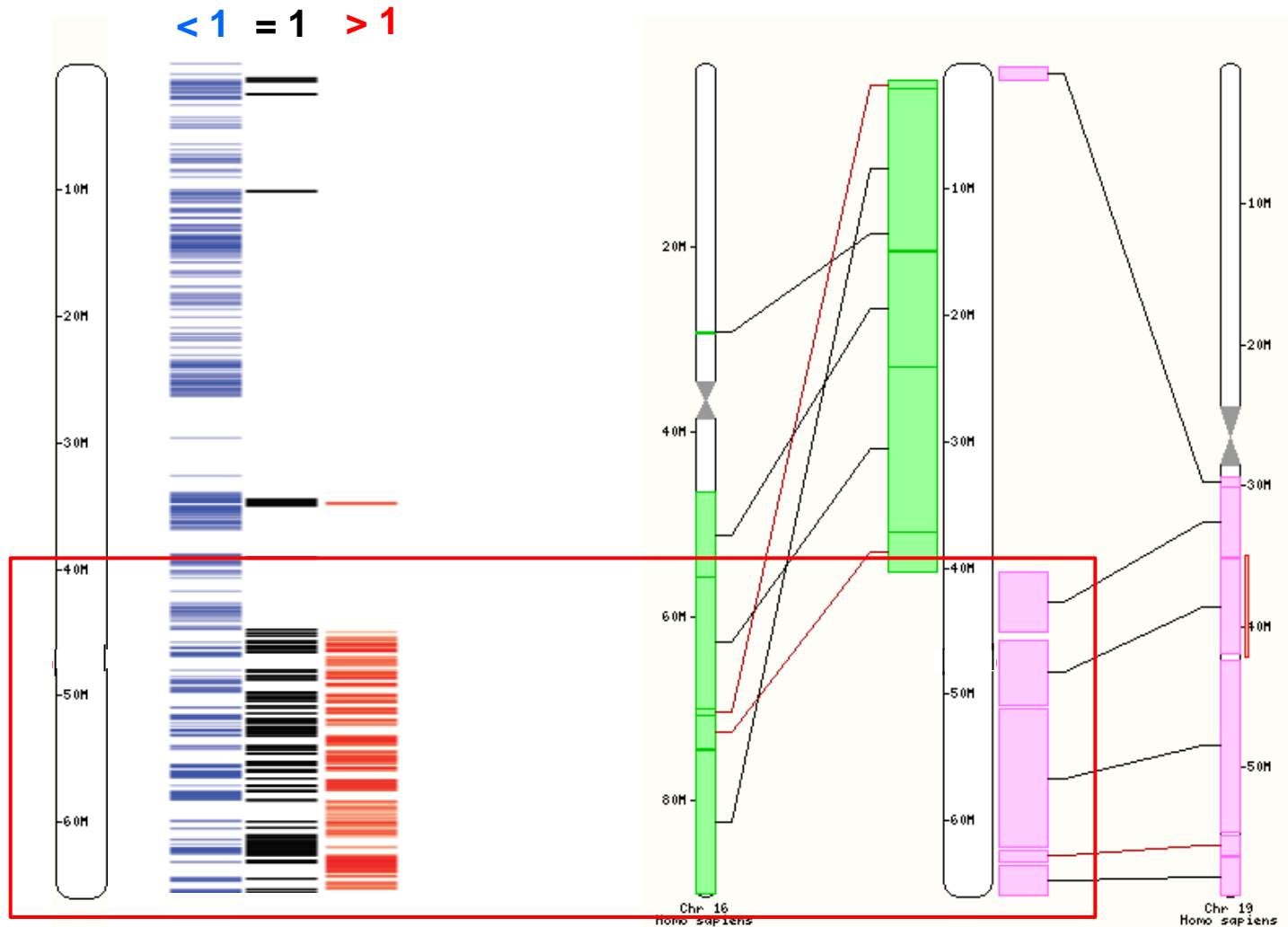
# Local methylation of chromosome 18

Local methylation / Global chr18 methylation :

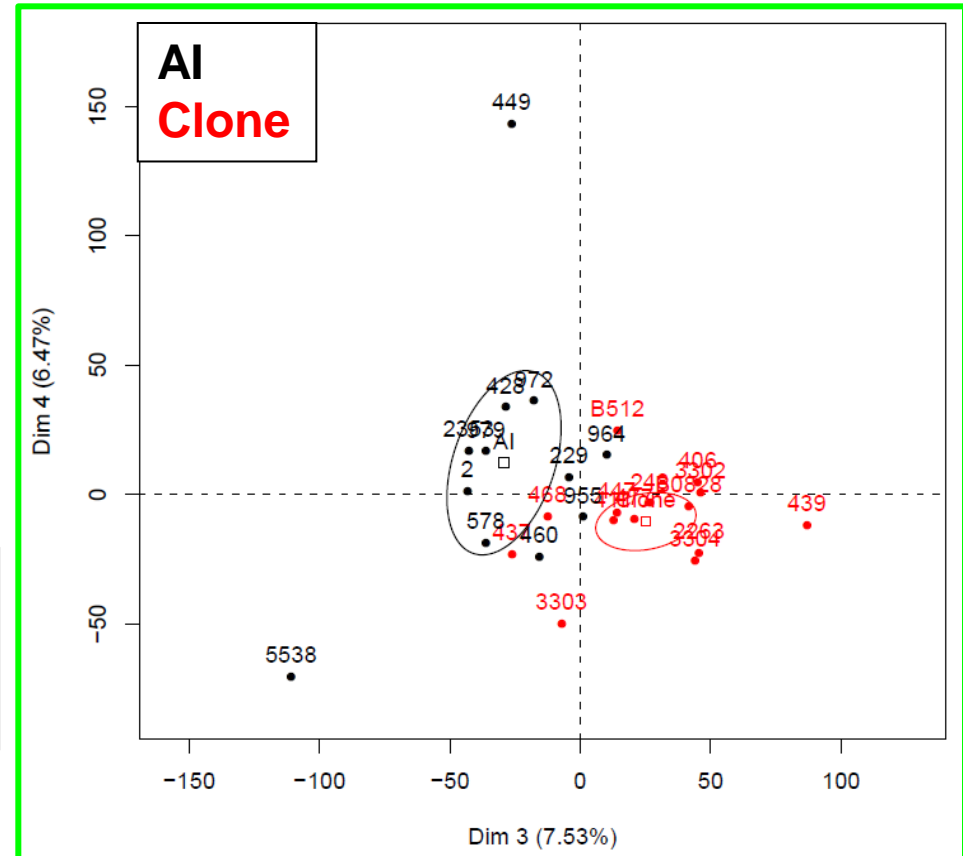
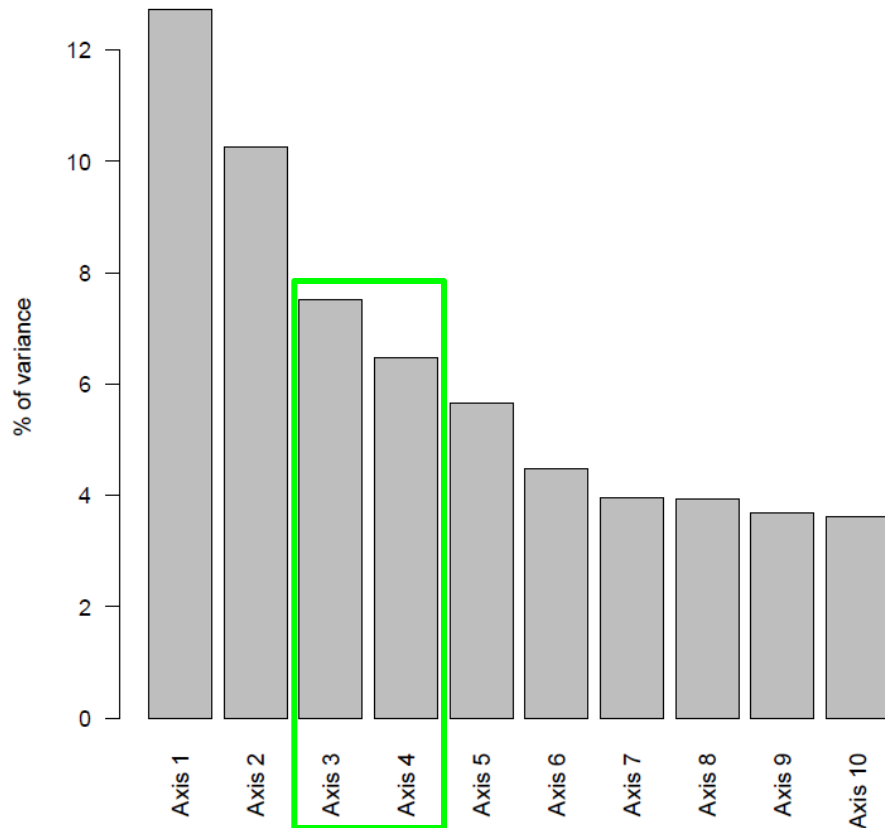


# Hypermethylation of chromosome 18 is restricted to the portion syntenic to Human chr19

Local methylation / Global chr18 methylation :

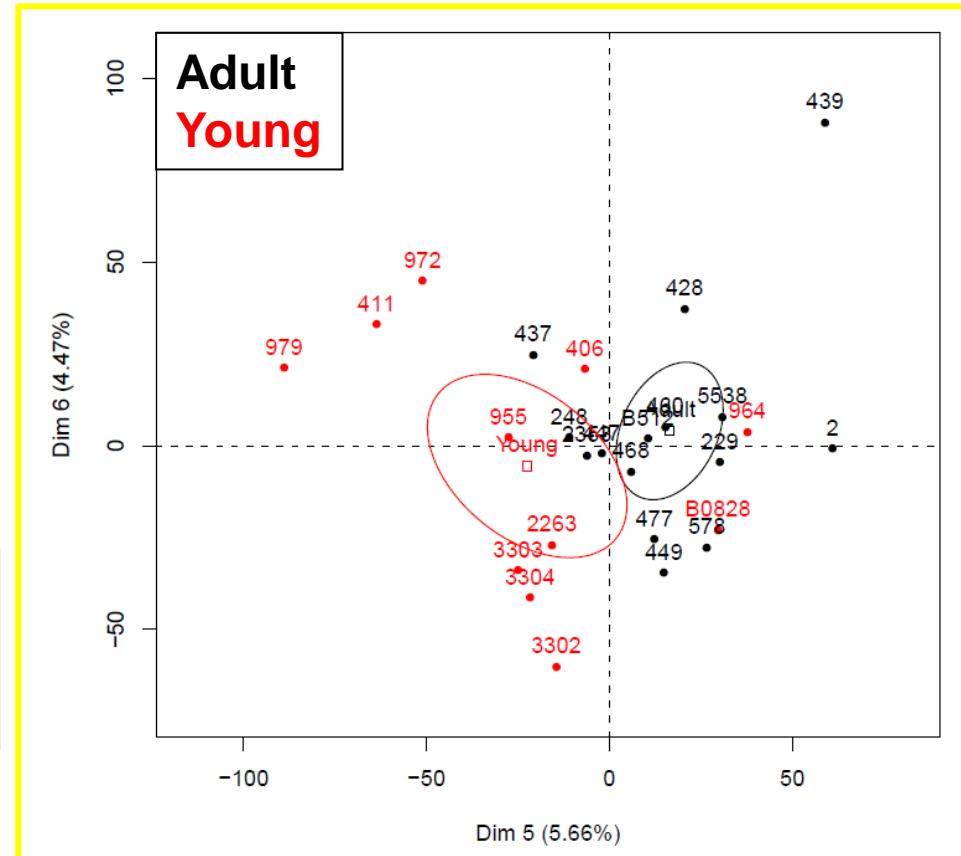
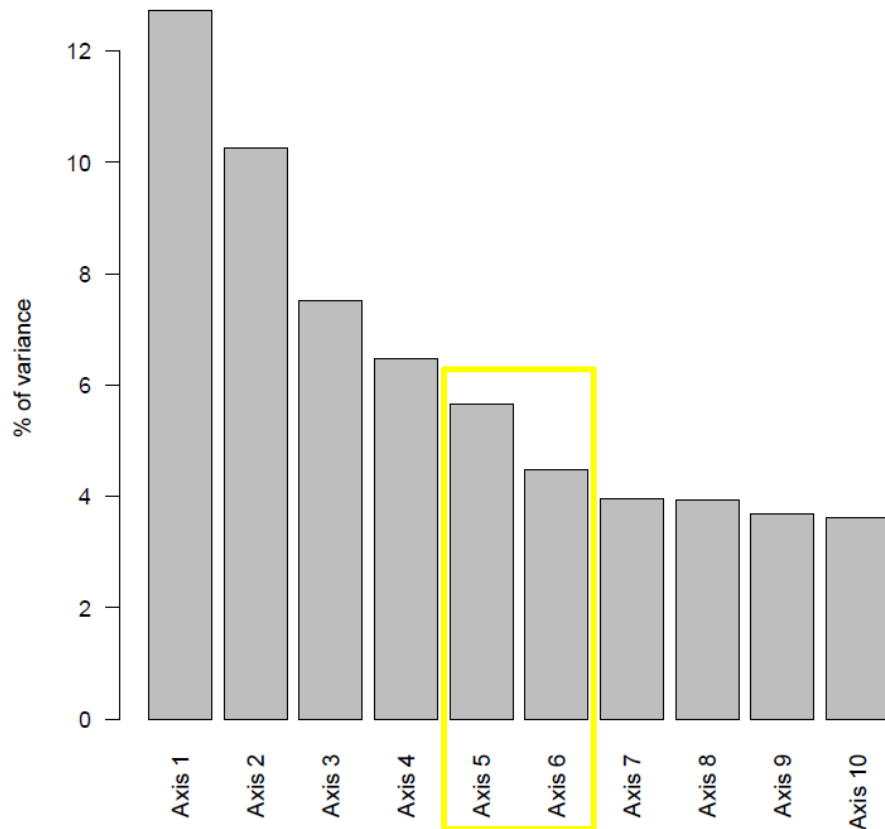


# Cloning and age are components of the variance observed among animals



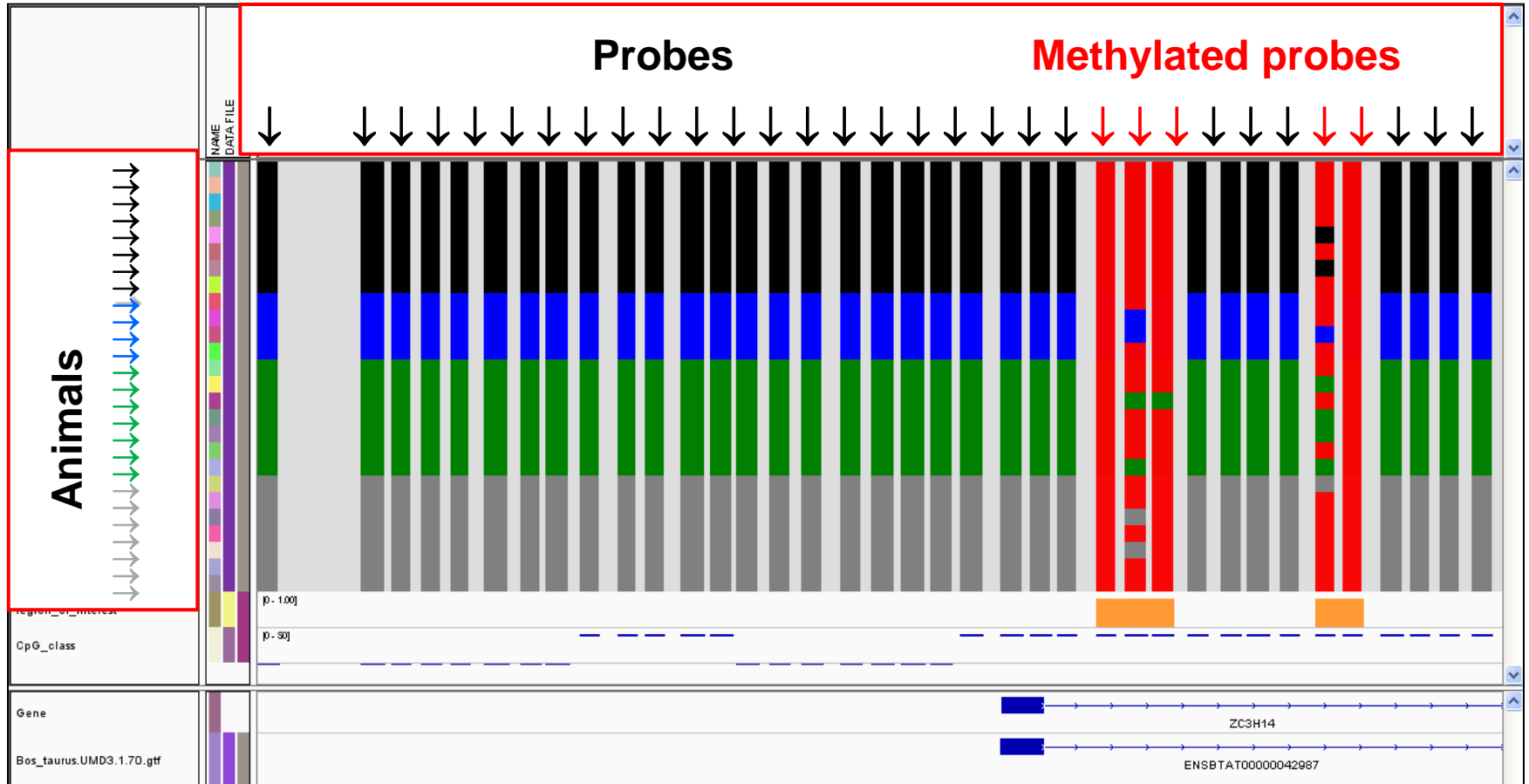
Principal Component Analysis (PCA) :  
# methylated probes per promoter

# Cloning and age are components of the variability observed among animals



Principal Component Analysis (PCA) :  
# methylated probes per promoter

# Spatial analysis : 96% of the promoters show clusters of enrichment (Spatstat R package)

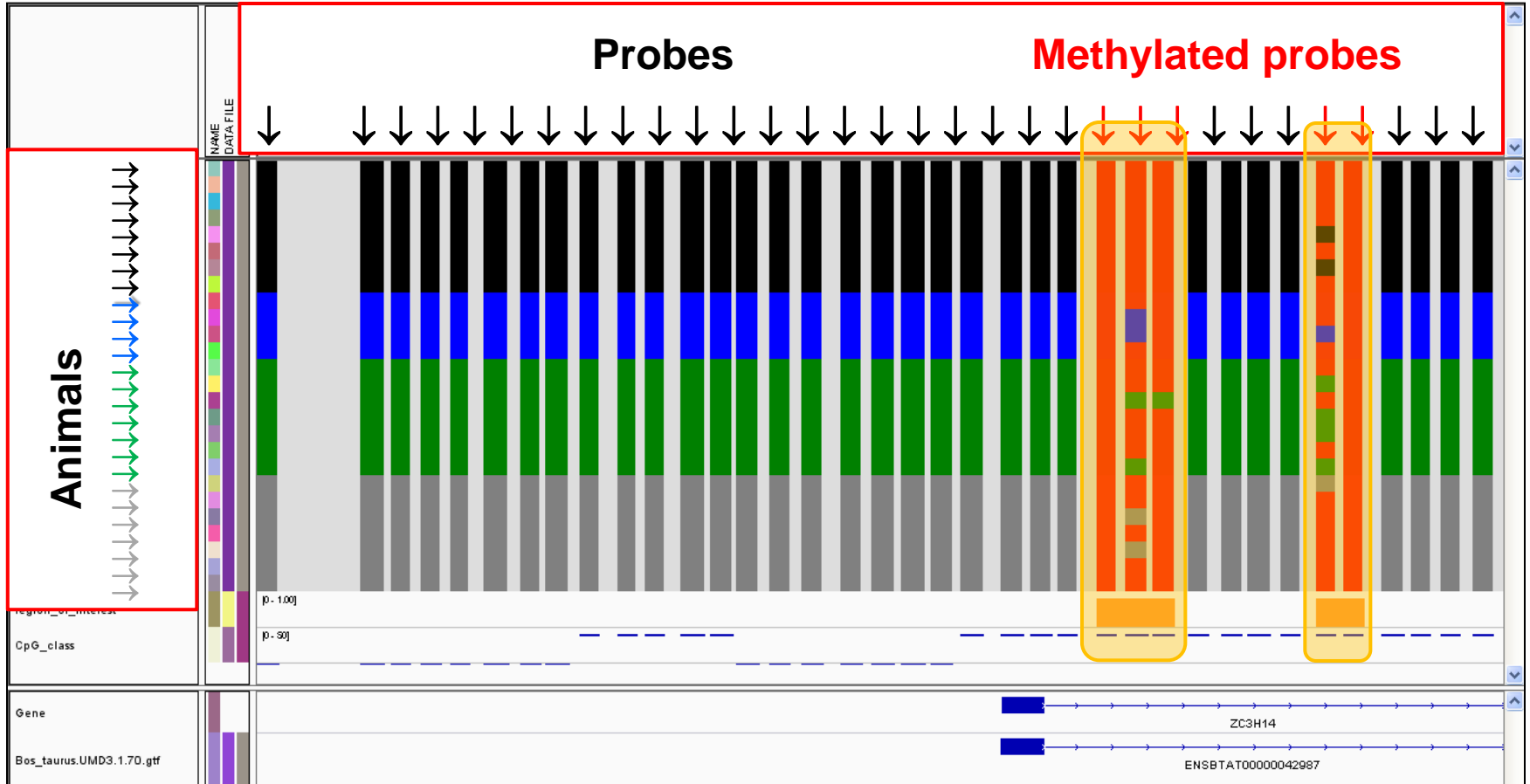


**Promoter**





# Spatial analysis : 96% of the promoters show clusters of enrichment (Spatstat R package)



➔ Identification of 16432 regions of interest of  $\geq 2$  consecutive probes

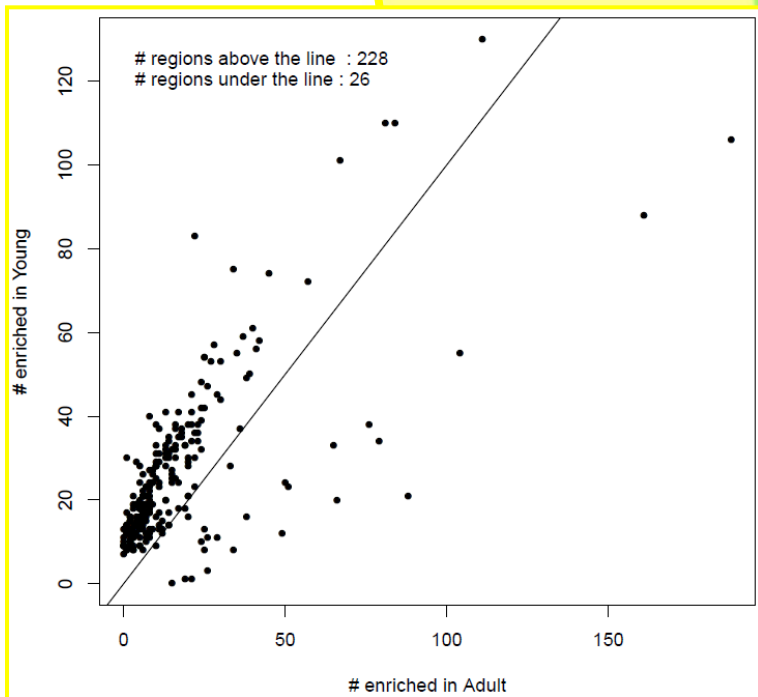
# Identification of Differentially Methylated Regions (DMR) (Spatstat R package)

Age-related DMR (257/16432)

206

51

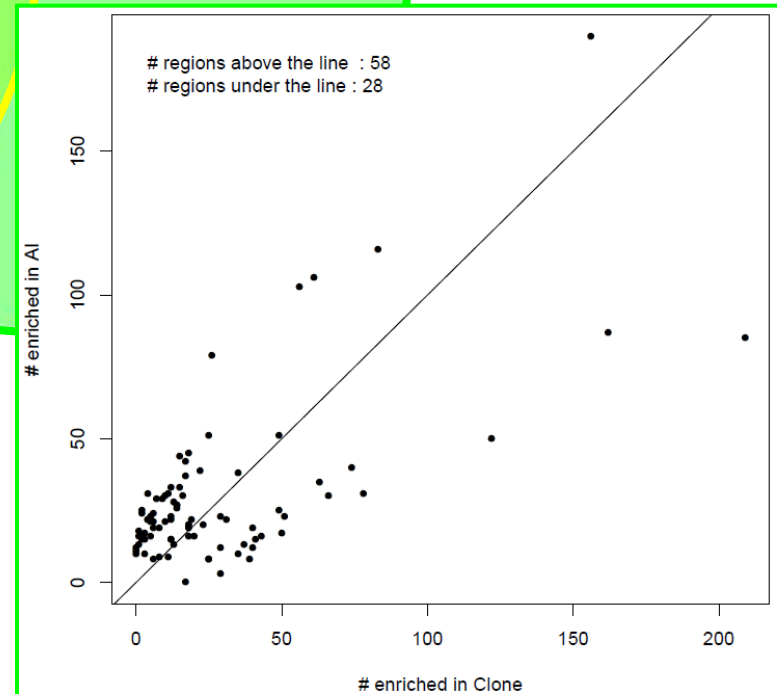
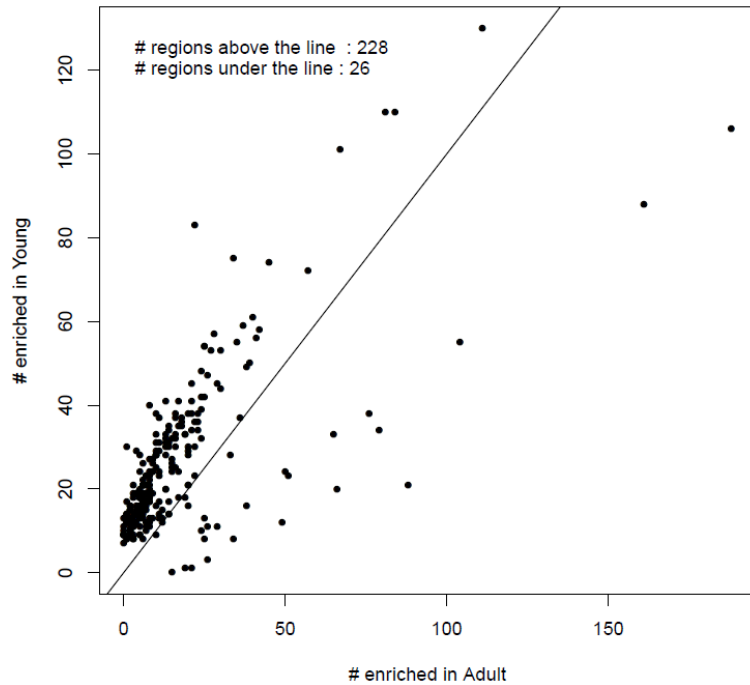
36



# Identification of Differentially Methylated Regions (DMR) (Spatstat R package)

Age-related DMR (257/16432)

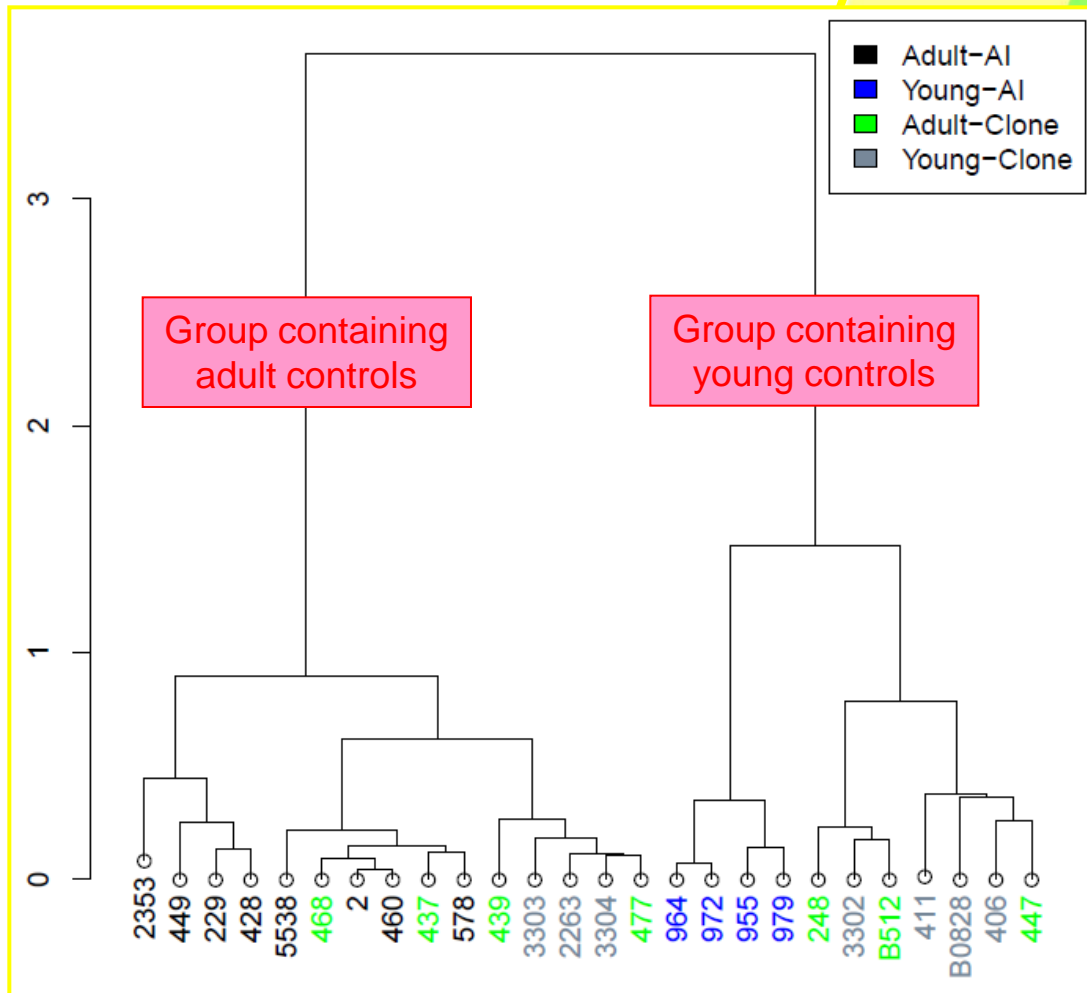
Cloning-related DMR (87/16432)



# Age-related DMR : cloning erases age-related differences observed in controls

Age-related DMR (257/16432)

Cloning-related DMR (87/16432)

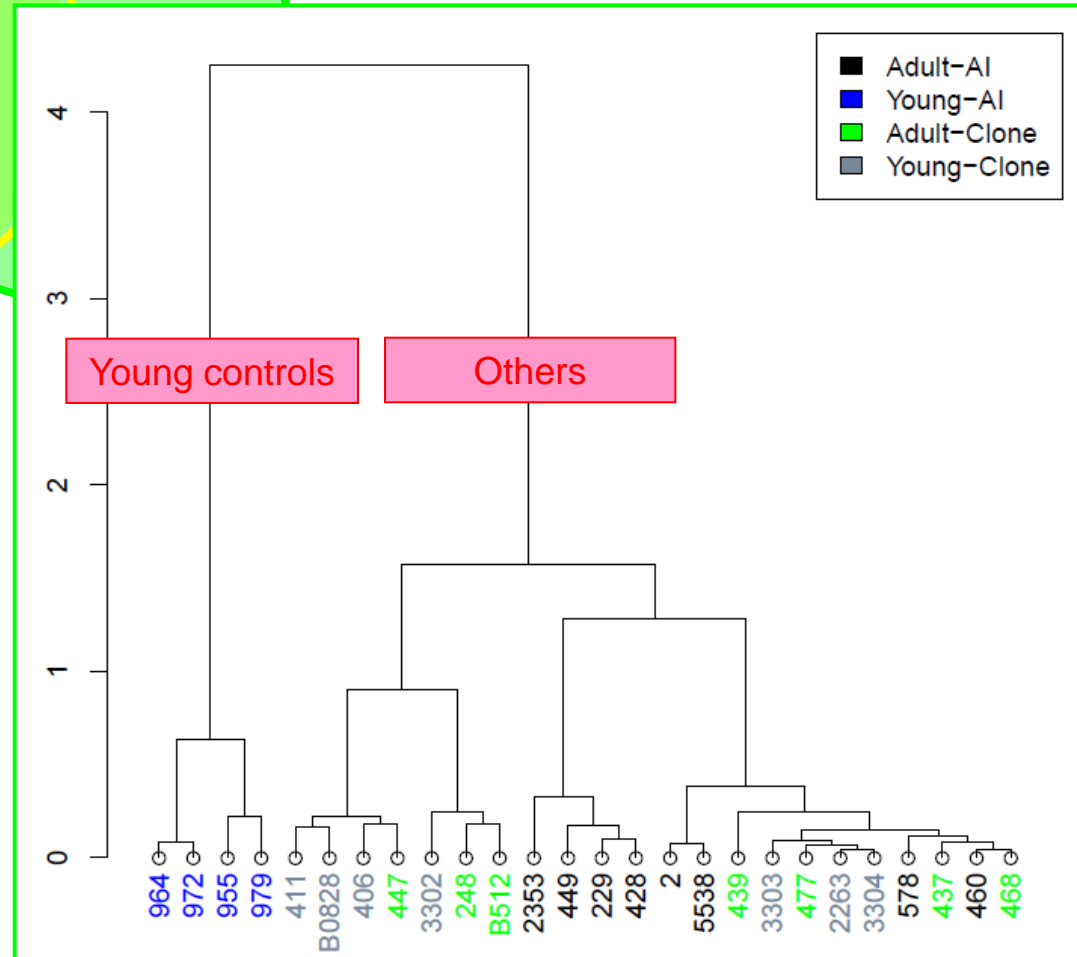
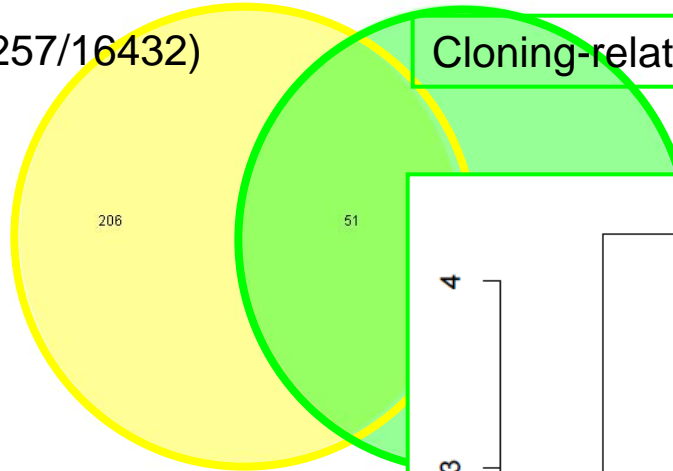


Correlation clustering :  
#methylated probes per region

# Cloning-related DMR : clones behave like adults independently of their age

Age-related DMR (257/16432)

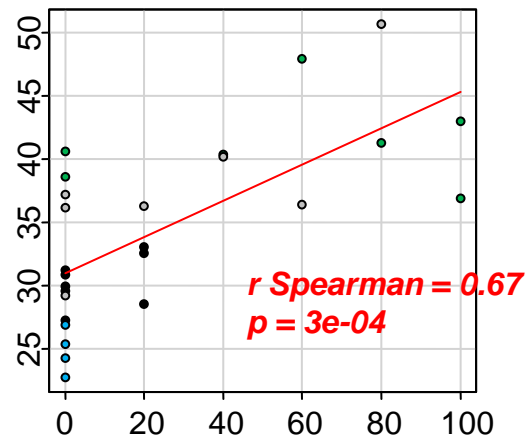
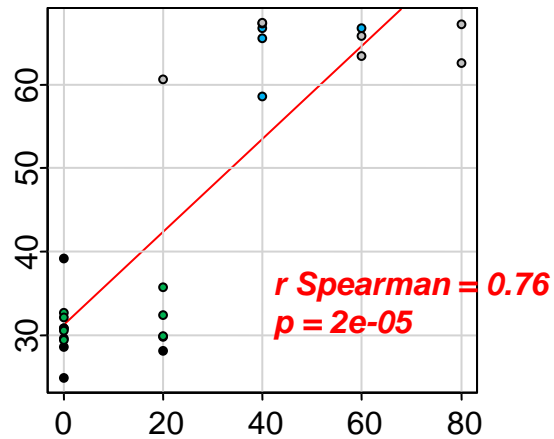
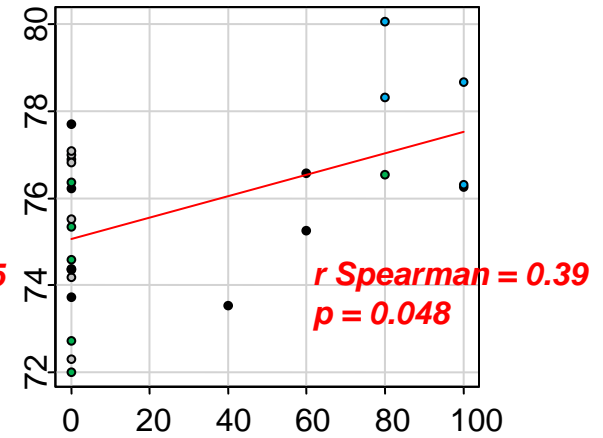
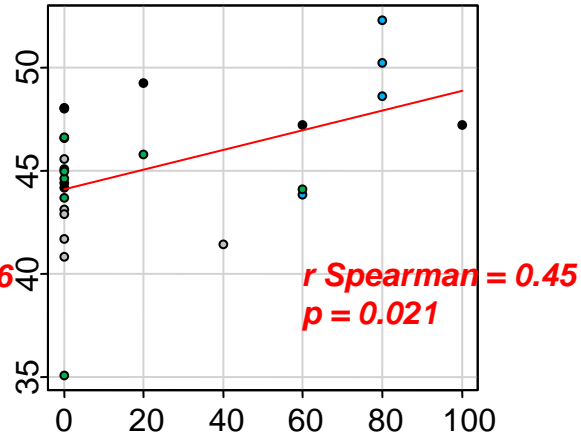
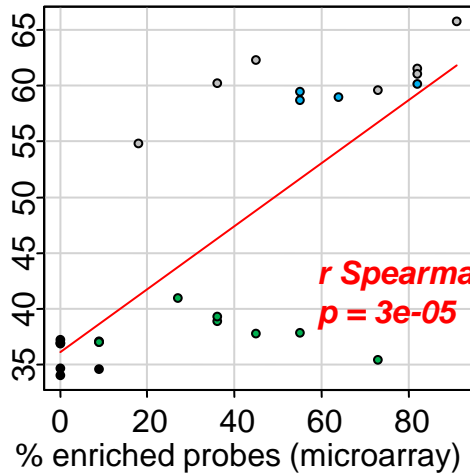
Cloning-related DMR (87/16432)



Correlation clustering :  
#methylated probes per region

# Validation by pyrosequencing on 5 DMR representing 49 CpG (average methylation per DMR)

% methylation (pyrosequencing)



Young clones



Adult clones

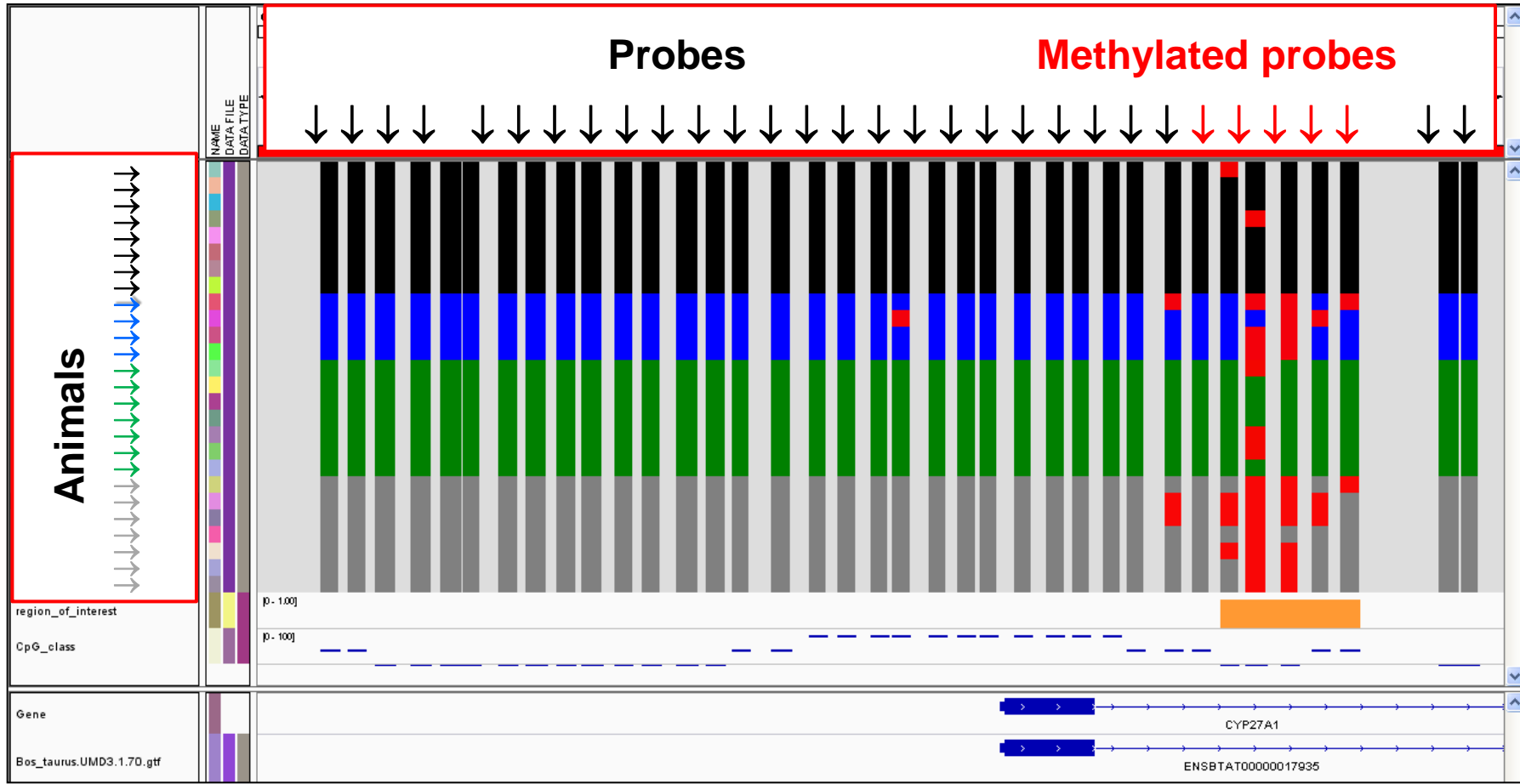


Young AI controls



Adult AI controls

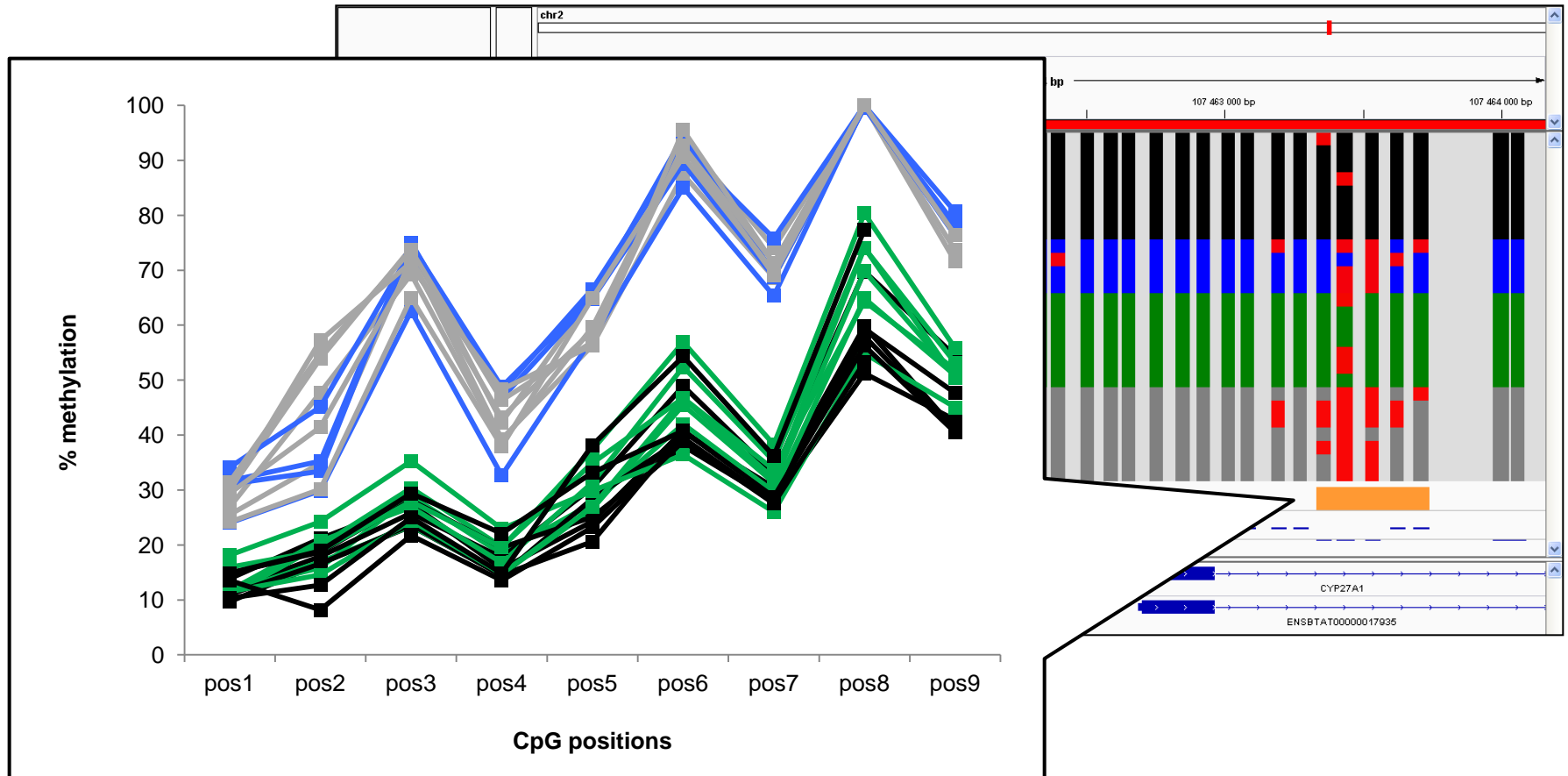
# Validation by pyrosequencing : chr2:107460687-107464047 (CYP27A1), age-related DMR (adj. P value = 0.004)



**Promoter**



# Validation by pyrosequencing : chr2:107460687-107464047 (CYP27A1), age-related DMR (adj. P value = 0.004)



Young clones
  Adult clones
  Young AI controls
  Adult AI controls



# Conclusion

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- **New tools** (microarray + bioinformatic pipeline) for **high throughput epigenetic analysis** in cattle
- New insights into the **bovine methylome** (hypermethylation of chromosome 18)
- Identification of regions with **epigenetic signatures** associated to **age and/or cloning**
- **Hypermethylation** of perinatal animals compared to adults
- Age-specific epigenetic signatures are **disrupted by cloning**

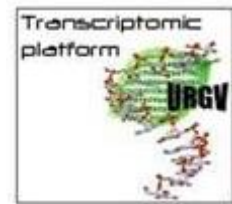
# Future directions

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- Relationships both with **gene expression** and **specific phenotypes** in liver ?
- Design of a **new generation microarray** integrating MeDIP-seq results (in progress)
- Analysis other **tissues** and other **environmental changes**



- Bioinformatic and statistical analysis : **Luc Jouneau**
- Pyrosequencing : **Evelyne Campion**
- Other members of our research group : H el ene Jammes, Michel Guillomot, Audrey Prezelin
- Production and care of the clones : Daniel Le Bourhis, Christophe Richard, Val erie Hall e (UCEA, INRA)
- Microarrays : Marie-Laure Martin-Magniette (INRA Evry), Sandrine Balzergue (INRA Evry)
- Liver collection : Pascale Chavatte-Palmer, Yvan Heyman (BDR, INRA)
- Histological studies on liver : Thibaut Larcher (INRA Nantes)
- Harris Lewin and Pablo Ross, UC Davis, USA
- Masahiro Kaneda and Takashi Nagai, Tokyo University of Agriculture and Technology, Japan
- Jean-Paul Renard



**EpigRAni**

