

# Genome-wide DNA methylation profiles of pig testis reveal epigenetic markers/genes for boar taint

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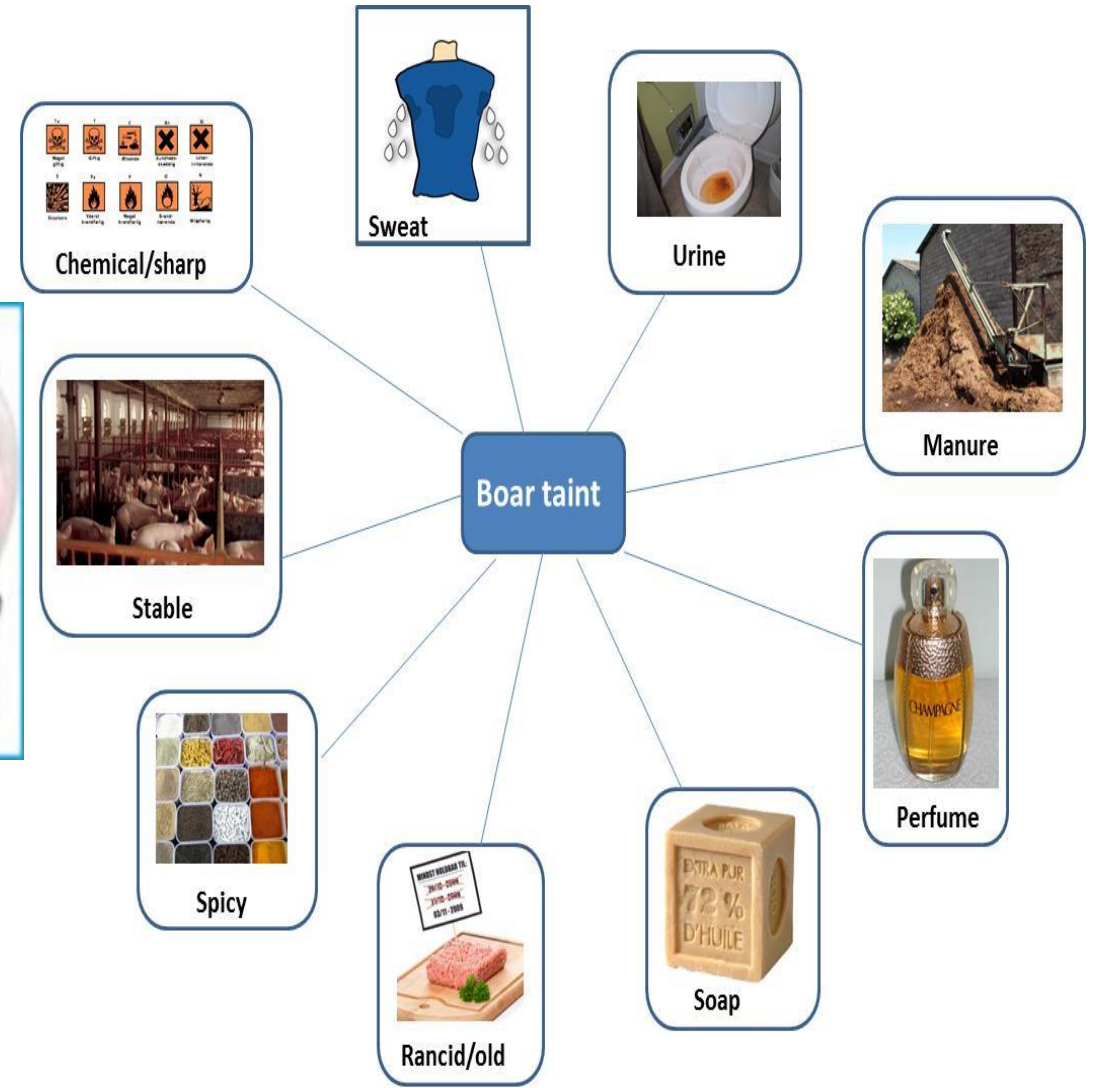
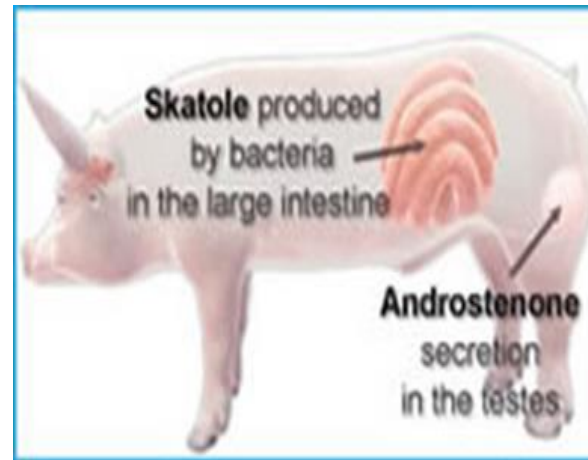
# Background - Boar taint (BT)

➤ The boar taint (BT) is primarily caused by the accumulation of skatole and androstenone ~ high heritability (0.33 and 0.59)

➤ Surgical castration for BT

- ⊖ Animal welfare
- ⊖ Labour intensive (use of anesthesia)
- ⊖ Consumer acceptance

➤ Selection of low BT boars can be an effective approach to avoid BT and other disadvantages of surgical castration



Patterson, R. L. S. (1968), Gower, D. B. (1972), Strathe, A. B. *et al.*, (2013)

# Previous Work on Multiomics of Boar Taint

Quantitative Genetics → GWAS/GP → Transcriptomics → eQTLs → **Epigenetics**

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Genetic parameters for androstenone and skatole as indicators of boar taint and their relationship to production and litter size traits in Danish Landrace

A. B. Strathe, I. H. Velandar, T. Mark and H. N. Kadarmideen

*J ANIM SCI* 2013, 91:2587-2595.

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Genetic parameters for male fertility and its relationship to skatole and androstenone in Danish Landrace boars

A. B. Strathe, I. H. Velandar, T. Mark, T. Ostensen, C. Hansen and H. N. Kadarmideen

# SCIENTIFIC REPORTS

**OPEN** Differential expression and co-expression gene networks reveal candidate biomarkers of boar taint in non-castrated pigs

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Markus Drag<sup>1,4</sup>, Ruta Skinkytė-Juskienė<sup>1</sup>, Duy N. Do<sup>2</sup>, Lisette J. A. Kogelman<sup>1,3</sup> & Haja N. Kadarmideen<sup>1,4</sup>

**PLOS** ONE

RESEARCH ARTICLE

## Systems genomics study reveals expression quantitative trait loci, regulator genes and pathways associated with boar taint in pigs

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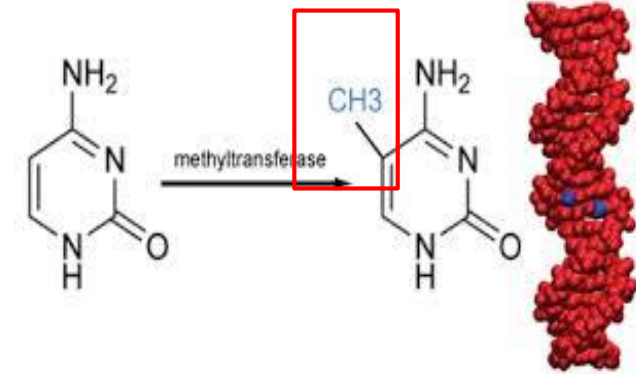
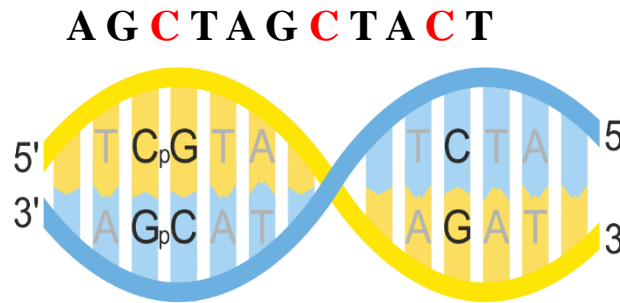
Haja N Kadarmideen ✉ \* Show less Authors ^

02 AUG 2019 // <https://doi.org/10.1152/physiolgenomics.00125.2018>

# Epigenetics – DNA methylation

➤ **Epigenetics:** No change in DNA sequence, but changes in gene function that are heritable changes

➤ **DNA methylation:**



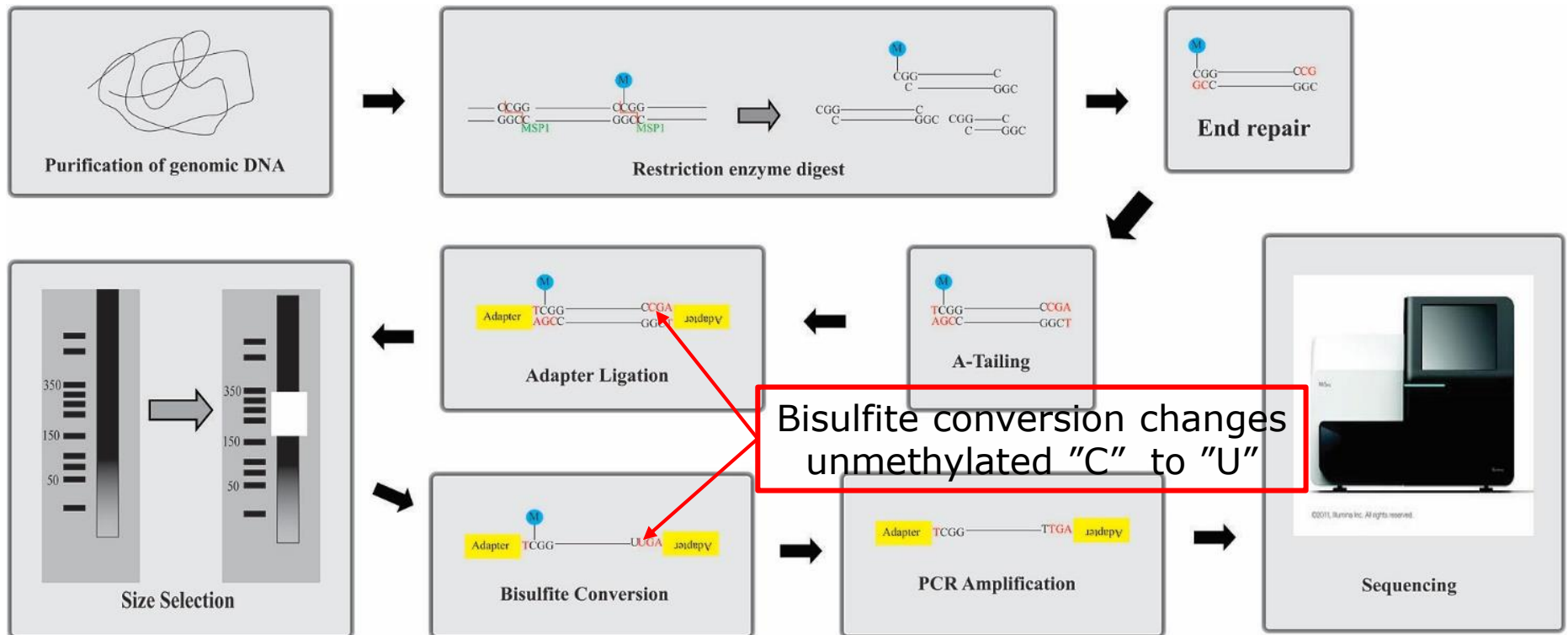
- DNA methylation has been examined to be associated with growth, immune response and reproduction traits in pigs. Our questions were:
  - *Does DNA methylation affect boar taint (BT) levels?*
  - *Are candidate biomarkers (differentially expressed / co-expressed genes) and eQTL-genes differentially methylated in high vs low BT boars ?*

Wu, C. T. and J. R. Morris. (2001)

# Materials - 9 testis sample for RRBS

- Summarised BT  
EBV = Skatole  
EBV + Human  
nose score EBV

<b>Low BT EBV</b>	3 testis sample	9 RRBS (Reduced representation bisulfite sequencing)
<b>Medium BT EBV</b>	3 testis sample	
<b>High BT EBV</b>	3 testis sample	



Meissner *et al.*, (2005)

# Methods – QC, alignment and methylation rates

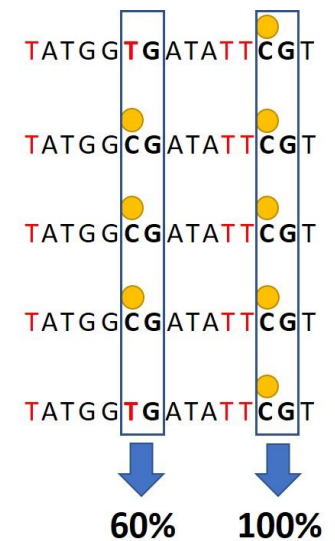
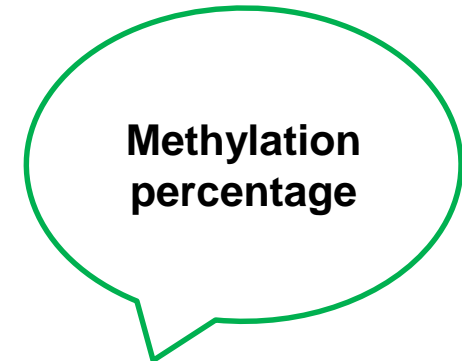
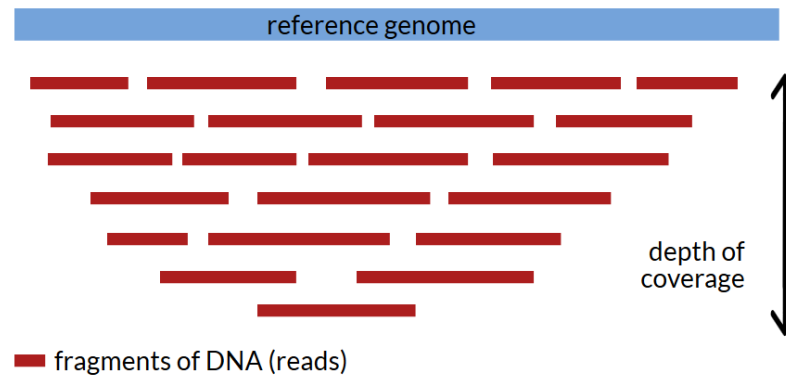
➤ **QC trimming by Trimmomatic software (version 0.36)**

- RRBS adapters
- reads less than 20 bases long

➤ **Alignment by Bismark Bisulfite Mapper (version 0.19.0)**

- map clean reads to the porcine reference genome (Sscrofa11.1/susScr11)
- Determine the cytosine methylation status, i.e. methylated/unmethylated cytosine

➤ **Methylation percentage** = read number of Cs / (read number of Cs + read number of Ts)



Bolger *et al.*, (2014), Krueger, F. and Andrews, S. R. (2011)

## Methods – Weighted methylation mean

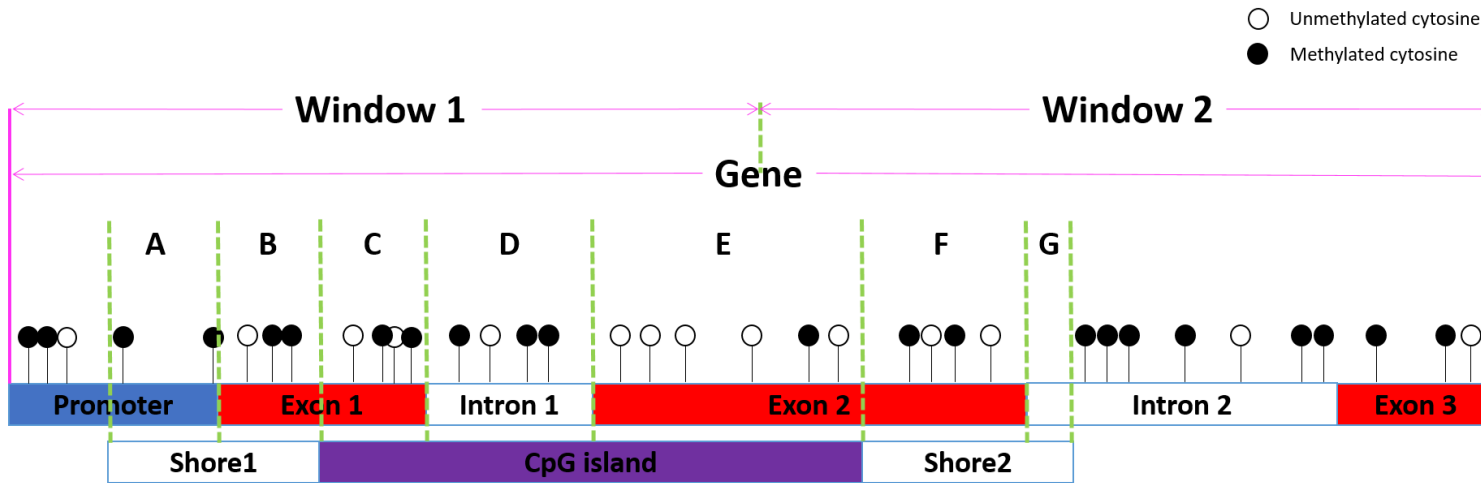
➤ The **weighted methylation mean** ( $\pi$ ) of a CpG site for each BT group was:

- $\sum_1^n \frac{MR_i}{TR_i} * W_i$  and  $W_i = \frac{TR_i}{\sum_1^n TR_i}$ ,
- where  $MR_i$  and  $TR_i$  are **methyated** and **total reads number** at a given CpG site of individual  $i$ ,  $n$  is the total individual number of each BT group and  $W_i$  is the **weight of reads** of individual  $i$ .

➤ **Differentially methylated cytosine (DMC)** through the logistic regression model in *methyKit* package:

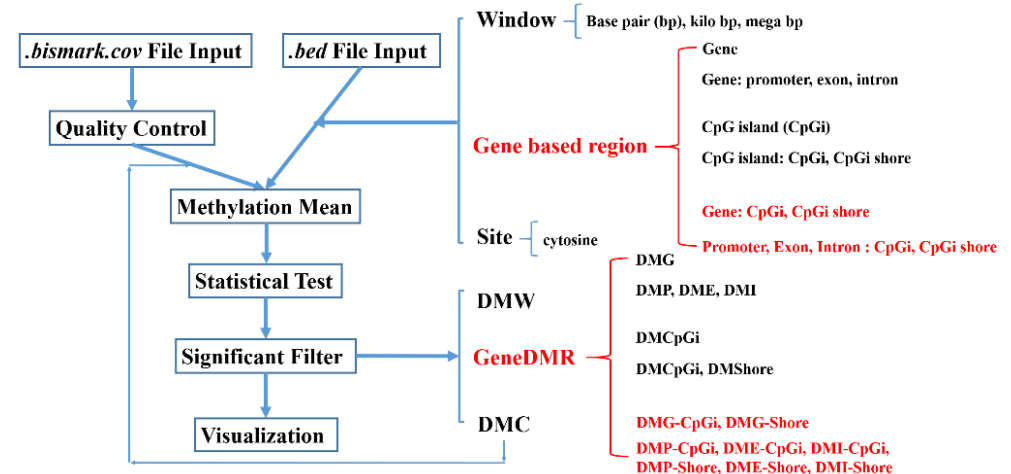
- $\log\left(\frac{\pi_i}{1-\pi_i}\right) = u + \beta T_i + e$ ,
- where  $\pi_i$  is the **weighted methylation mean** at a given CpG site of group  $i$  and  $T_i$  is the **BT group**

# Methods – Gene based weighted methylation - R package



$$\sum_1^n \frac{\sum_1^m MR_{ij}}{\sum_1^m TR_{ij}} * W_{ij} \text{ and } W_{ij} = \frac{\sum_1^m TR_{ij}}{\sum_1^n \sum_1^m TR_{ij}}$$

- $MR_{ij}$ : Methylated reads number
- $TR_{ij}$ : Total reads number
- $W_{ij}$ : Weight of reads

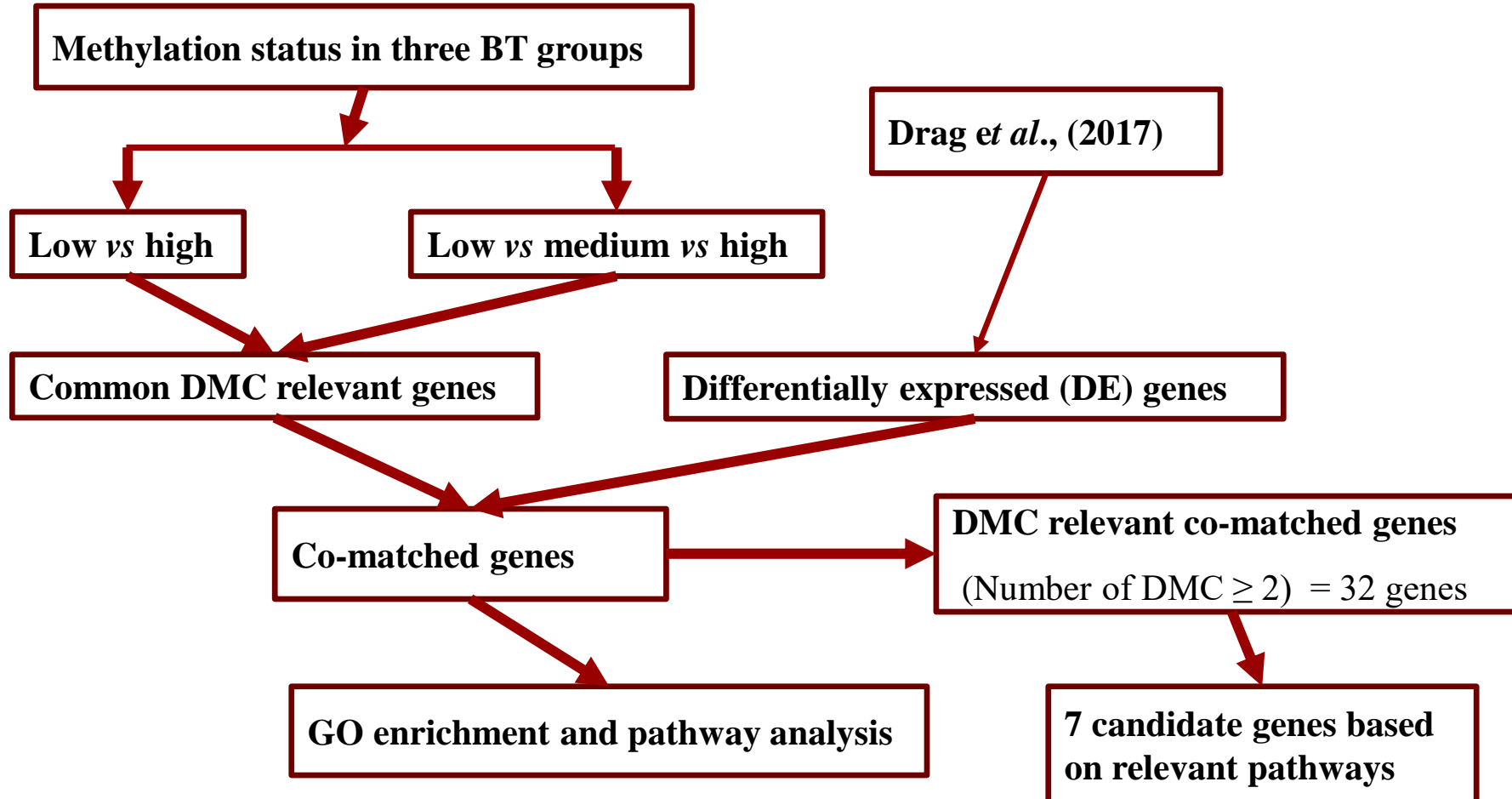


GeneDMRs is freely available at <https://github.com/xiaowangCN/GeneDMRs>

Wang, Dao and Kadarmideen, (2019)



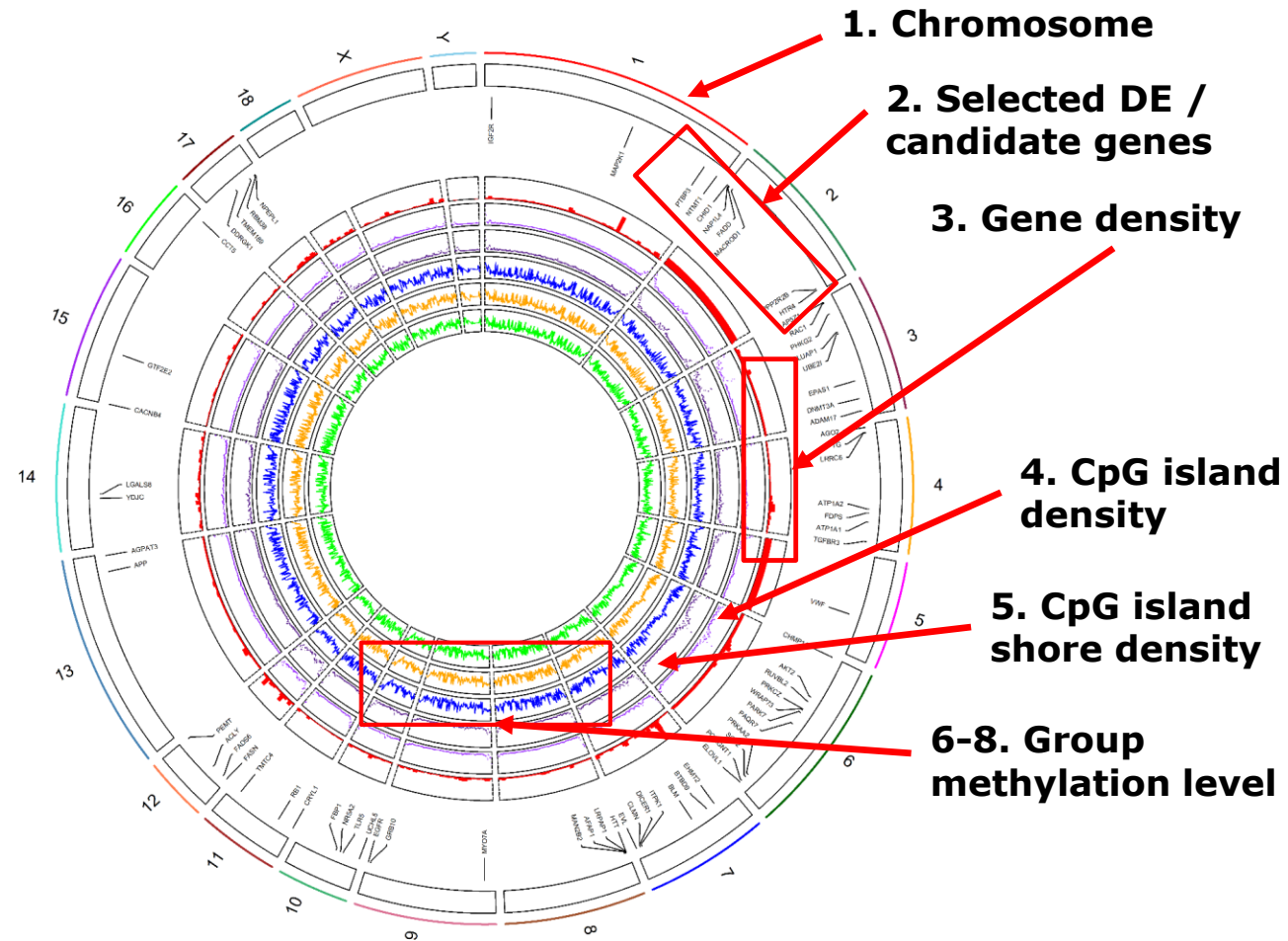
# Work flow to detect DMCs and DMG for Boar Taint and integration with Transcriptomics



# Results – Circular genome methylation

➤ Variation between biological replicates was **low** when limited to 1 Mb window but more variation in smaller distances

➤ Coefficient of density of genes regression on methylation level: **-2.2** ( $P < 0.001$ )

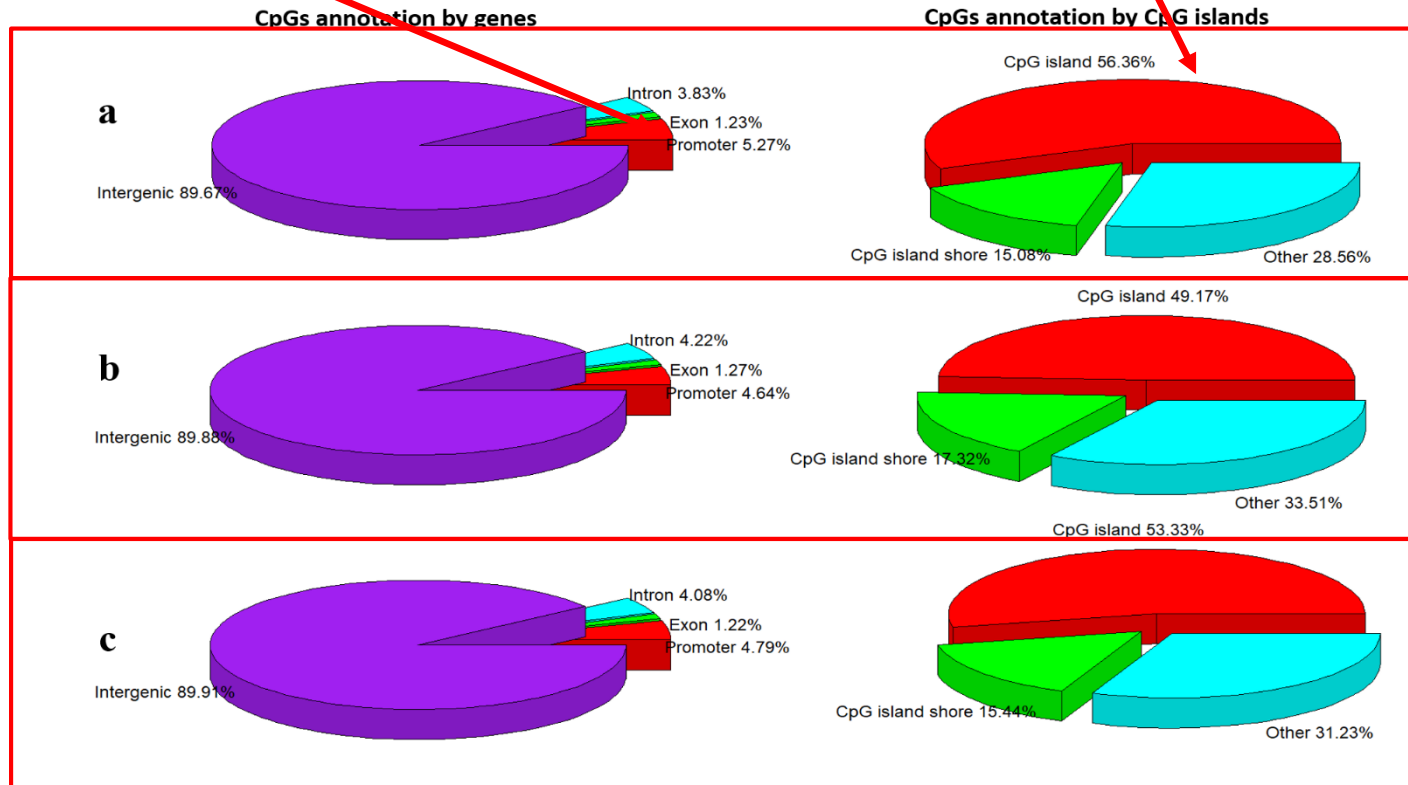


# Results – Distributions of CpGs in Porcine genome

Promoter: 4.64% ~ 5.27%

CpG islands: 49.17% ~ 56.36%

Low level

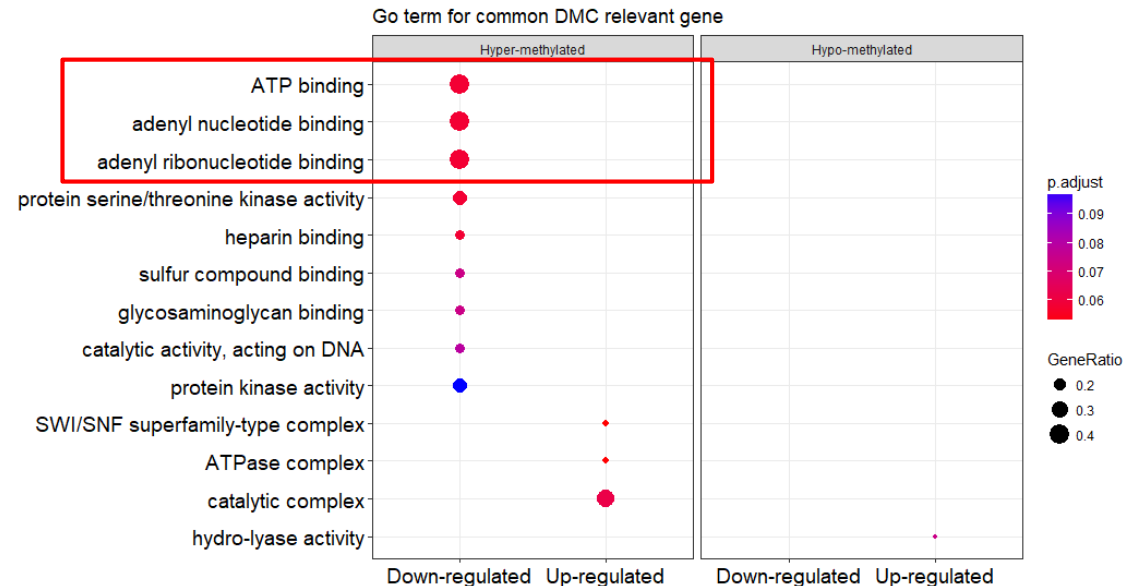


Medium level

High level

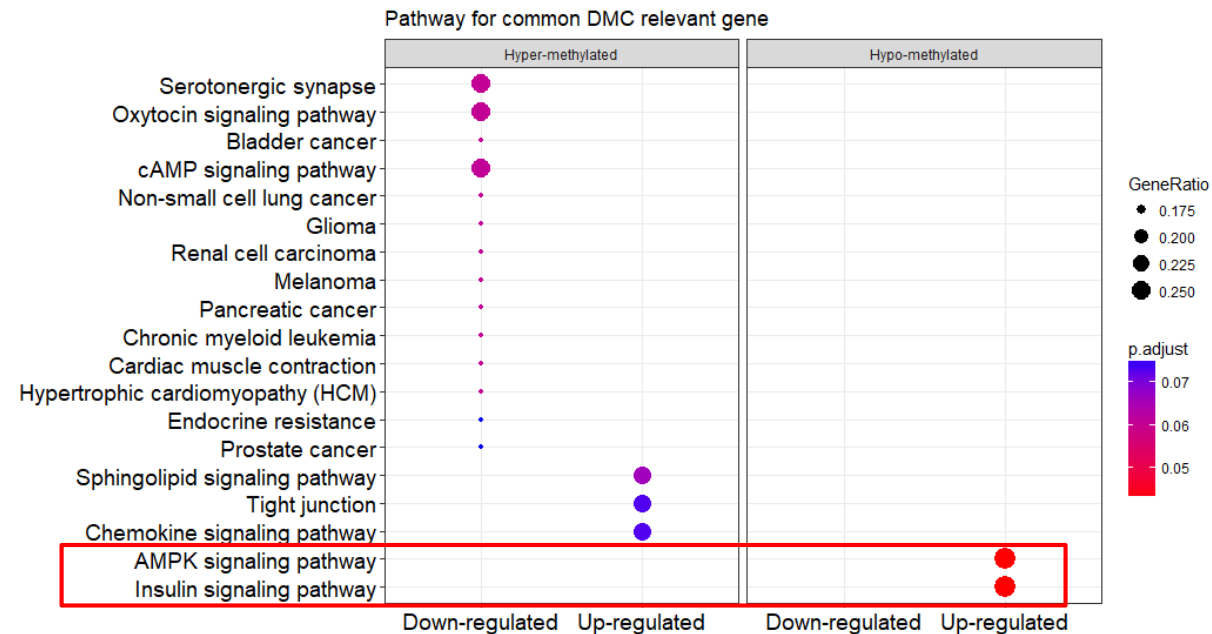
# Results – GO terms in hyper / hypo-methylation categories

- **Five downregulated genes** in the **hypermethylated** category: *ATP1A2*, *BLM*, *DICER1*, *MAP2K1* and *PRKAA2*
- **Three of the most significant GO terms:** ATP binding (GO:0005524), adenyly nucleotide binding (GO:0030554) and adenyly ribonucleotide binding (GO:0032559)



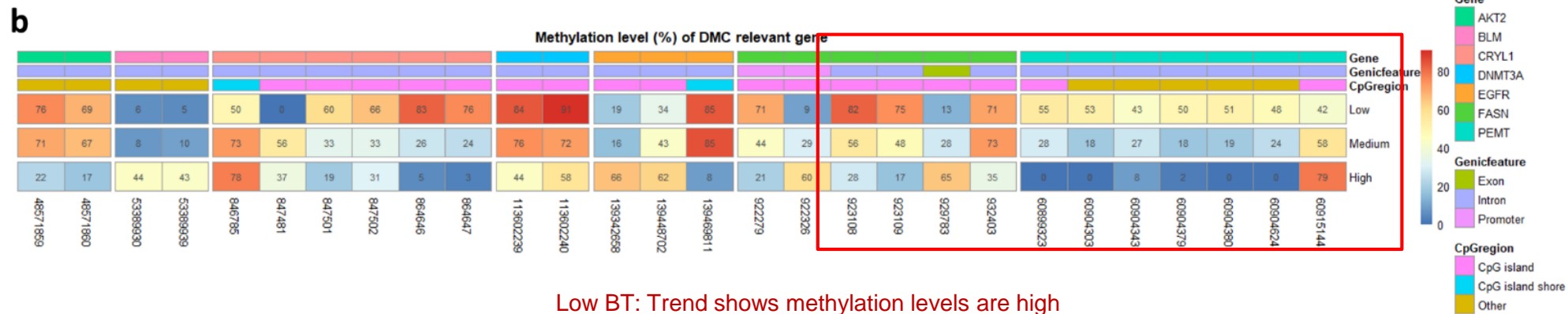
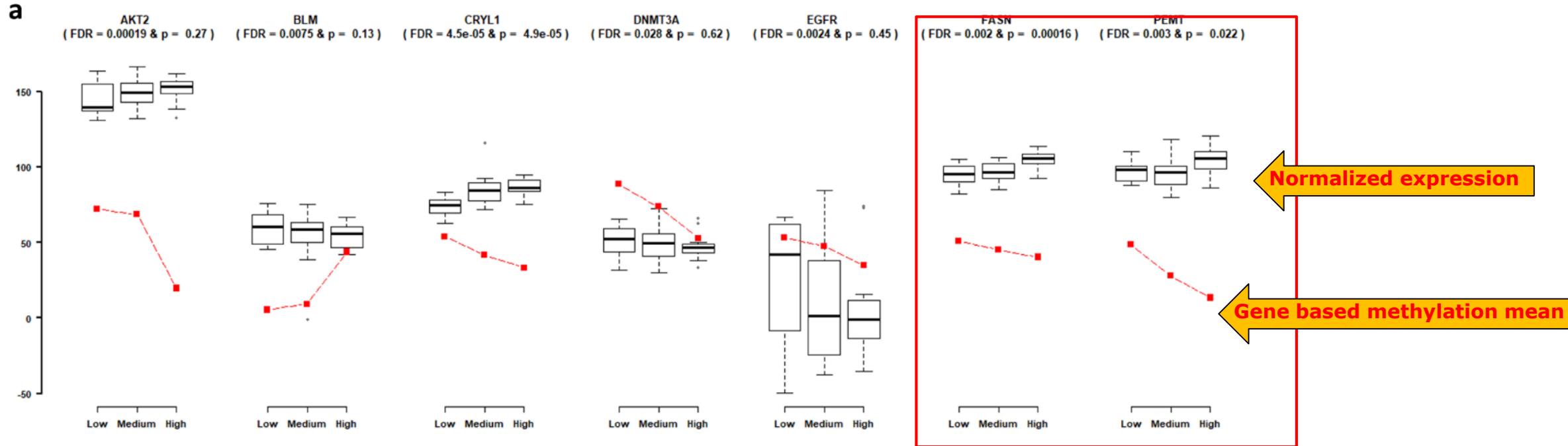
# Results – Pathways in hyper / hypo-methylation categories

- Only the upregulated genes (*AKT2*, *FBP1* and *FASN*) were presented in the hypo-methylated category
- Two most significant pathways were **AMPK signaling** (ssc04152) and **insulin signaling** (ssc04910)



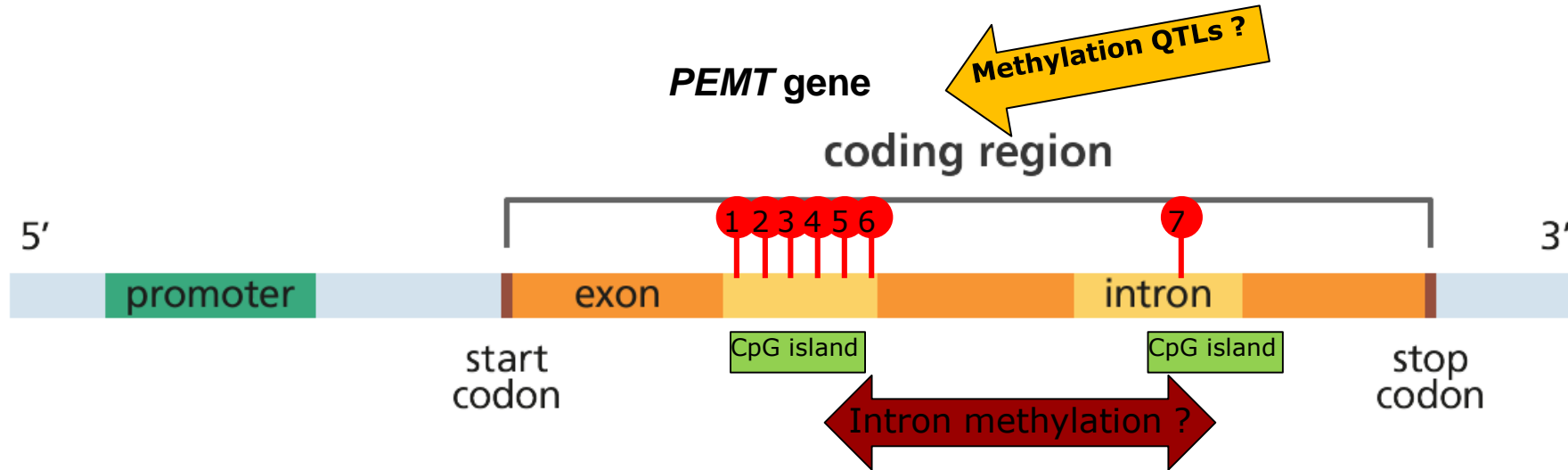


# Integrative Epigenomics: Normalized gene expression vs. methylation level



# Future direction: Methylated candidate genes have genetic variation – SNPs / methylQTLs -- > Epimutations

Gene	Methylation	logFC	DMC	Gene structure	CpG	Illumina 60K Procine chip name	SNP	<sup>h</sup> MAF
<i>AKT2</i>	-52.51	0.67	2	Intron <sup>2</sup>				
<i>BLM</i>	38.41	0.54	2	Intron <sup>2</sup>		ALGA0041544, ALGA0041552	A/G, A/G	0.24, 0.28
<i>CRYL1</i>	-20.40	0.31	6	Intron <sup>6</sup>	CpG <sup>5</sup> & Shore <sup>1</sup>			
<i>DNMT3A</i>	-35.92	-0.42	2	Intron <sup>2</sup>	CpG <sup>2</sup>			
<i>EGFR</i>	-18.59	-1.90	3	Intron <sup>3</sup>	CpG <sup>2</sup> & Shore <sup>1</sup>	ALGA0055330, ALGA0055337	T/G, A/G	0.08, 0.03
<i>FASN</i>	-16.94	0.46	4	Exon <sup>1</sup> & Intron <sup>3</sup>	CpG <sup>4</sup>			
<i>PEMT</i>	-35.20	0.79	7	Intron <sup>7</sup>	CpG <sup>2</sup>			





# Discussion

- This is the first study to report Genome-wide DNA methylation profiles of boar taint in pigs using NGS (RRBS) methods
- Gene based weighted methylation levels are useful for the investigations of gene methylation and the comparisons of gene expression
- Identified candidate genes *AKT2*, *BLM*, *CRYL1*, *DNMT3A*, *EGFR*, *FASN* and *PEMT*
- Integrative analysis of gene expressions with methylation levels in different genic features (e.g., promoter, exon, intron regions)
- Potential analysis of methylation quantitative trait locus (QTLs) and epimutations
- Variation in genes involved in epigenetic processes influencing phenotypic outcome might offer new insights into understanding biological variation and epigenetic processes of BT in pigs
- Impacts genomic prediction methods that may include epigenetic data



# An Epigenome-Wide DNA Methylation Map of Testis in Pigs for Study of Complex Traits

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Anders Strathe

Postdoc – quant.genetics,  
genomic selection,GWAS



Ruta Skinkte

Research assistant –  
Experiments / Molecular bio



Markus Drag

PhD candidate –  
Experiments, transcriptomics  
& Bioinformatics Work



Xiao Wang

PhD candidate – Epigenetics &  
Bioinformatics Work

# Funding and /or collaborations



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