



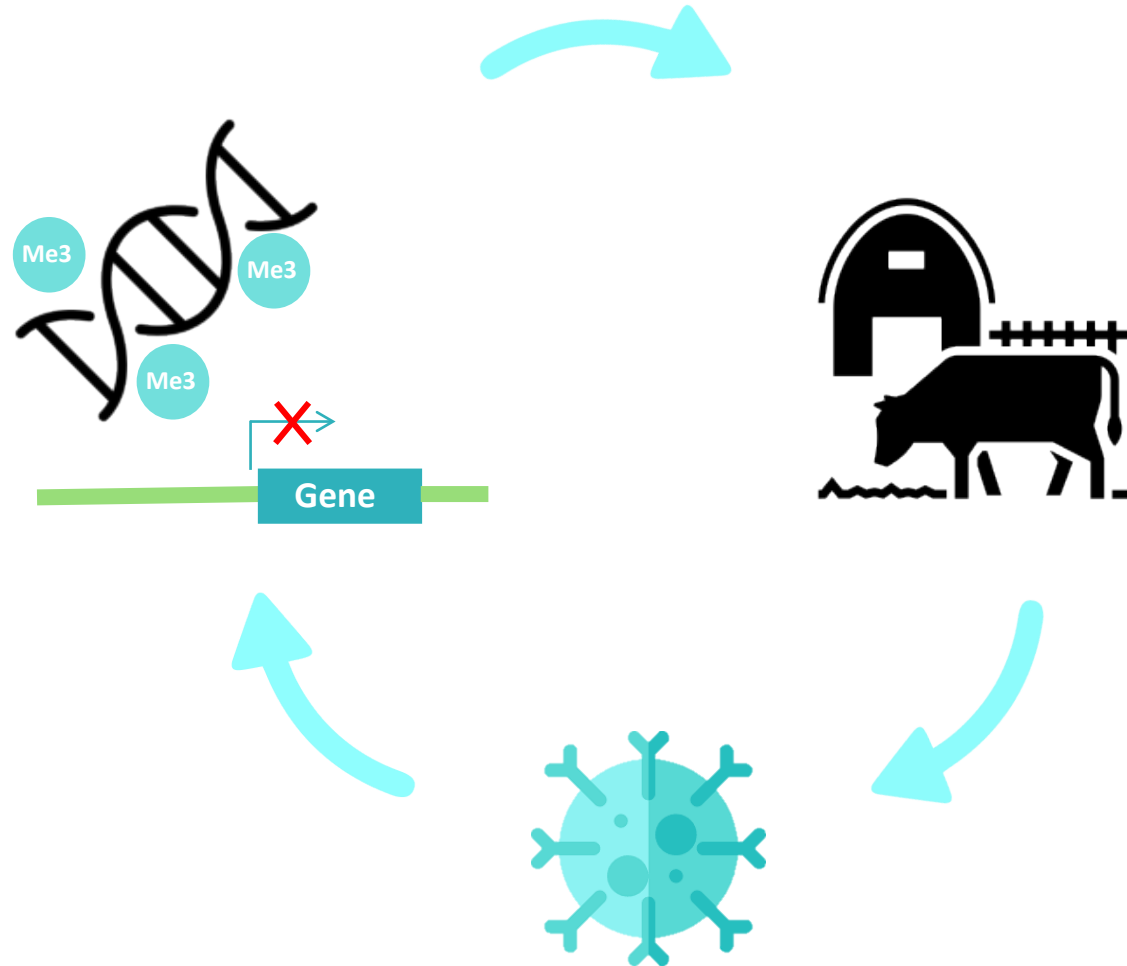
DNA-methylome analysis of milk somatic cells upon subclinical mastitis in Holstein cattle

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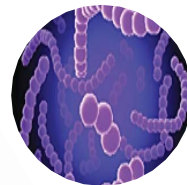
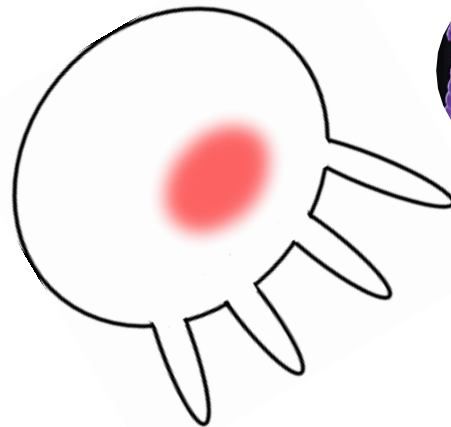
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Hypothesis

Is SCM associated with specific somatic cells methylation signatures?



Prototheca spp.



Strep. agalactiae

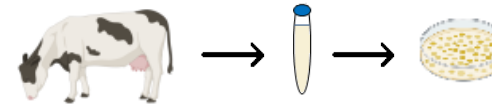
T0

Herd's choice



- One herd in Veneto region
- Holstein Friesian breed
- Prevalence for *Strep. agalactiae* and *Prototheca* spp. (IZSVe)

Milk sampling & analysis



- 188 animals (no clinical signs of mastitis or treated with antibiotics)
- Aseptic manual milking
- Bacteriological screening: **Sa+**, **P+**, **H**

15 days

T1

Groups definition

(DIM>90, parity ≥ 2)

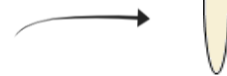
P+
(n=11)



Sa+
(n=12)



H
(n=15)



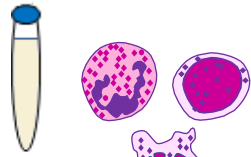
Milk analysis

- Milk composition, SCC and DSCC
- Flow cytometry
- Peptidomics
- Transcriptomics
- **Epigenomics**

MBD-seq and data analyses

DNA extraction

NucleoSpin® Tissue



Covaris® S220

Sequencing

Illumina Hiseq X, 150 PE



TruSeq® DNA Nano



agritech

National Center for Technology in Agriculture

Data analyses

FASTQC

Bowtie 2 (ARS-UCD1.2)

MACS2

DiffBind R package

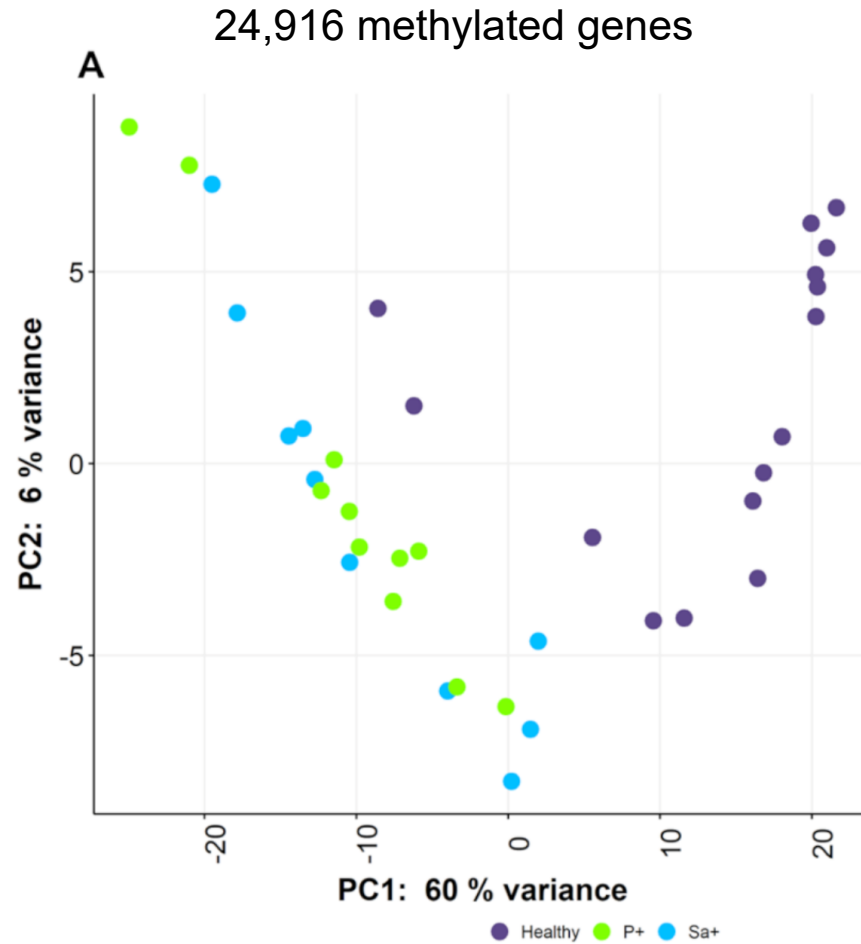
DeSeq2 R package

Homer

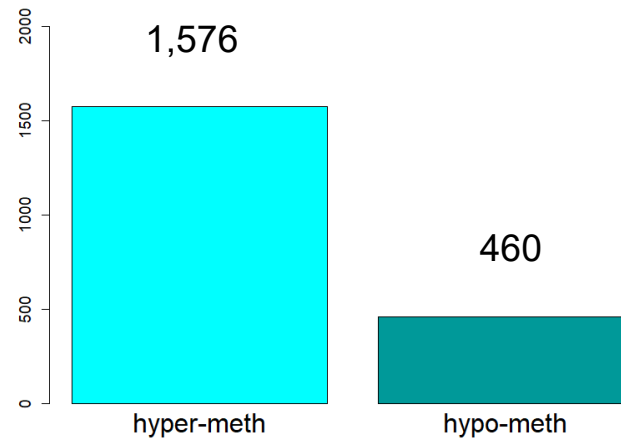
Cluego, Pathifier

MBD
MethylMiner™ Methylated DNA Enrichment Kit



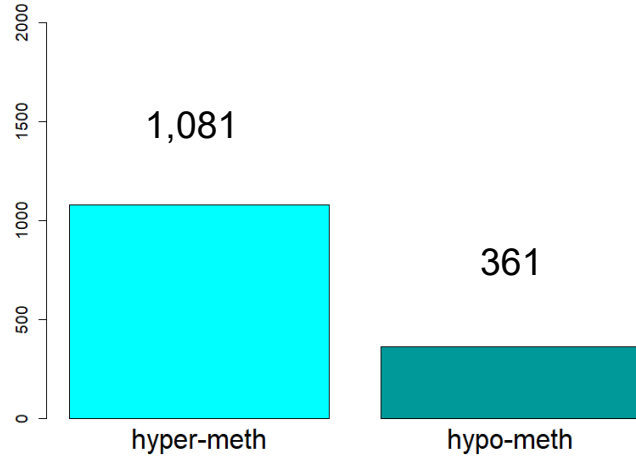


MAST vs H



IL17REL (log₂FC=1.3, promoter)

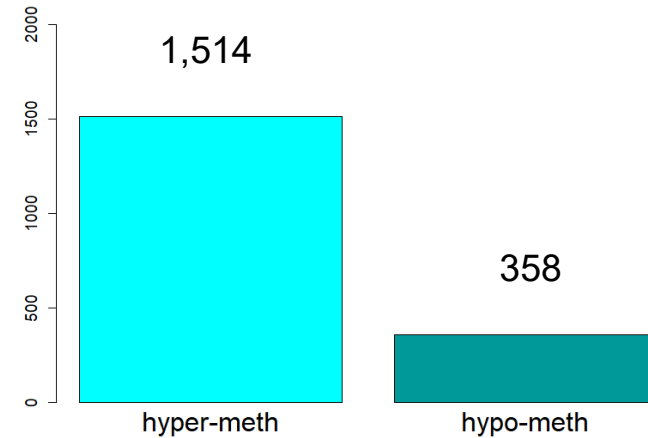
Sa+ vs H



IL-10RB (log2FC=-3.2, promoter)

CIITA (log2FC=-5.7, first intron)

P+ vs H

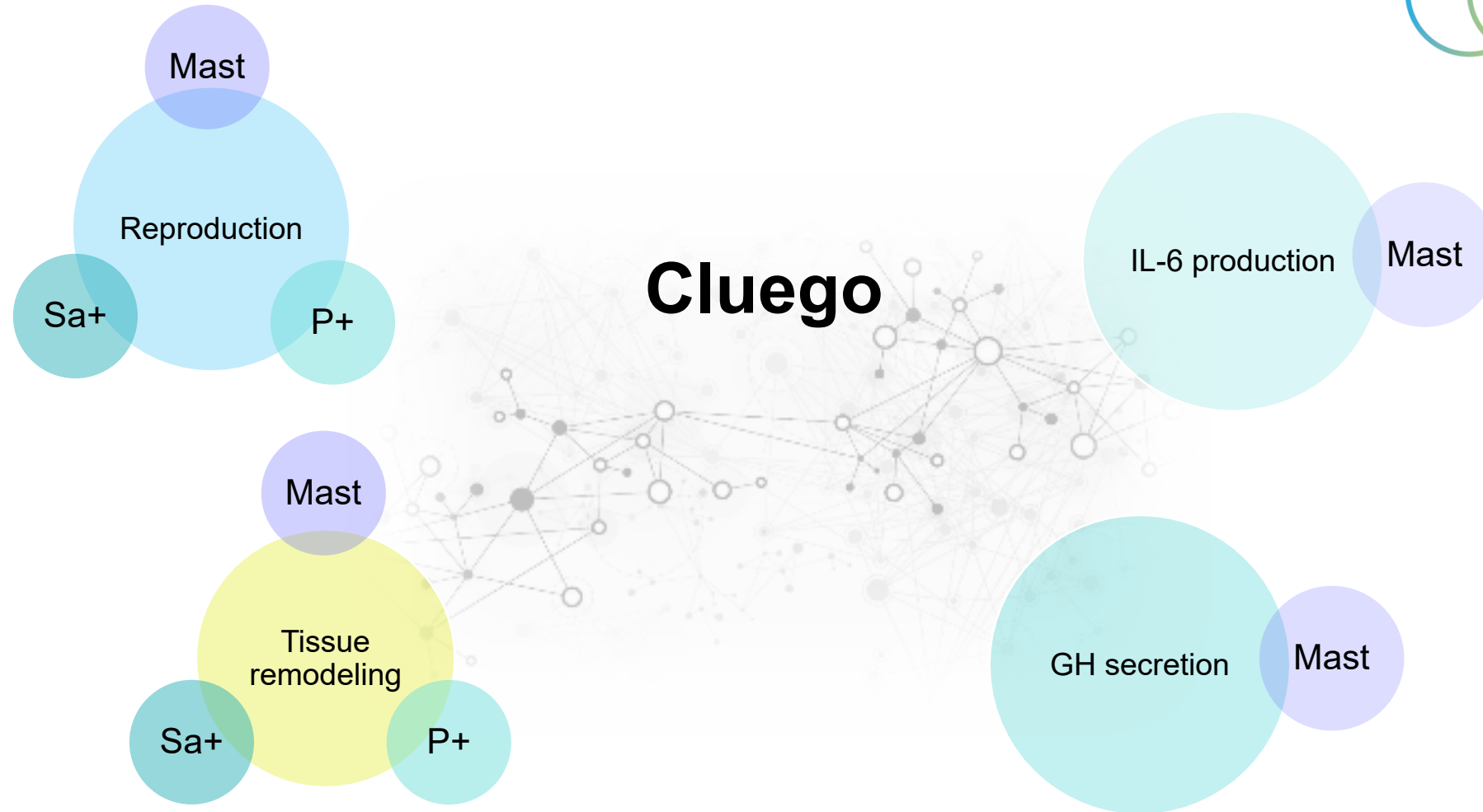


ADAM11 (log2FC=-5.6, promoter)

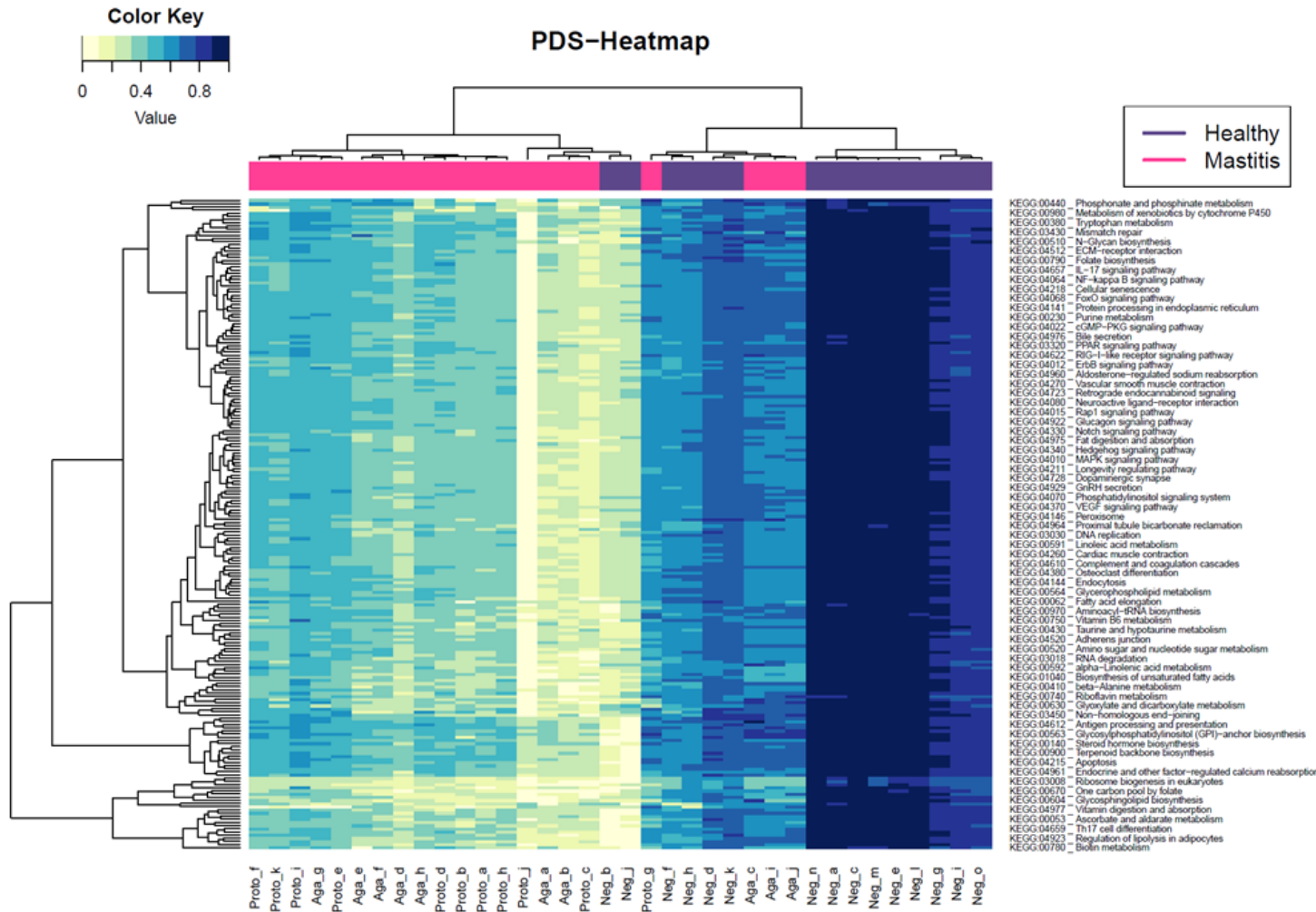
IFNAG (log2FC= -4.2, first exon)



4 DMR for Sa+ vs P+



Results- Functional analyses of SC methylome



Higher PDS in P+

- NF-kB signaling
- RIG-I-like signaling
- TLR signaling
- T cell receptor signaling
- Th17 cell differentiation
- IL-17 signaling





- ▶ Regulation of methylation profile of genes involved in **reproduction, tissue remodeling** and **immune response**
- ▶ No evident pathogen-specific signature
- ▶ **Genomic and epigenomic** information for directional application in dairy cattle breeding



Thank You!

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