



# ➤ Host genes shape the faecal microbiota of Holstein cows: evidence from sequence-based GWAS

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1 – GD Biotech – Gènes Diffusion, Lille, France

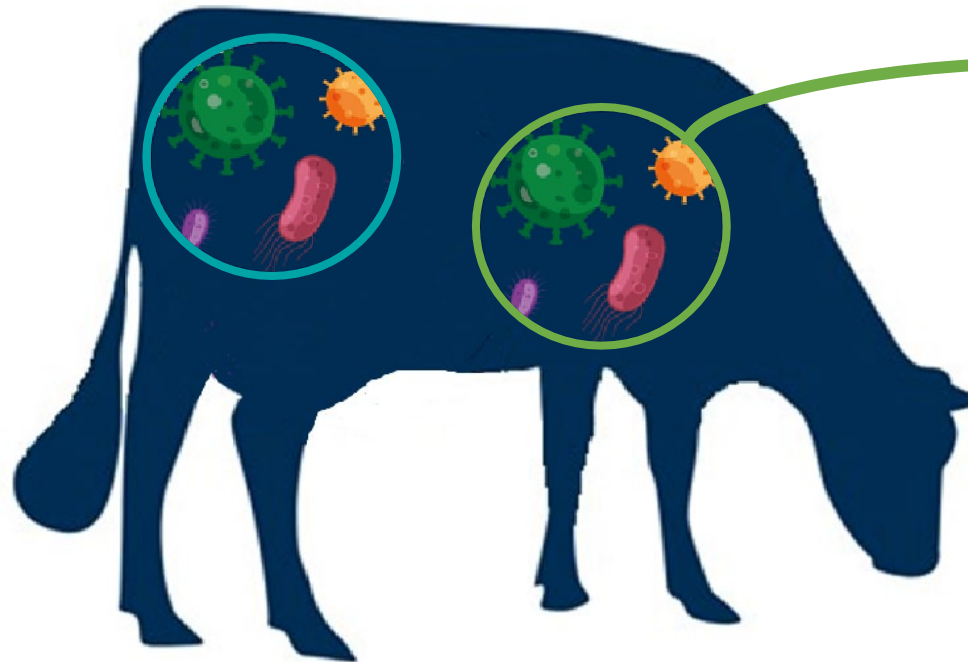
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# ➤ Why did we hypothesize that certain genes influence the faecal microbiota of dairy cows?

1 – Microbiota composition is heritable in Holstein cows.



## Rumen microbiota

- Low to moderate heritability estimates:  $h^2$  between 0.08 and 0.48 in Holstein cows

(Difford *et al.*, 2018; Saborío-Montero *et al.*, 2020; Zhang *et al.*, 2020)

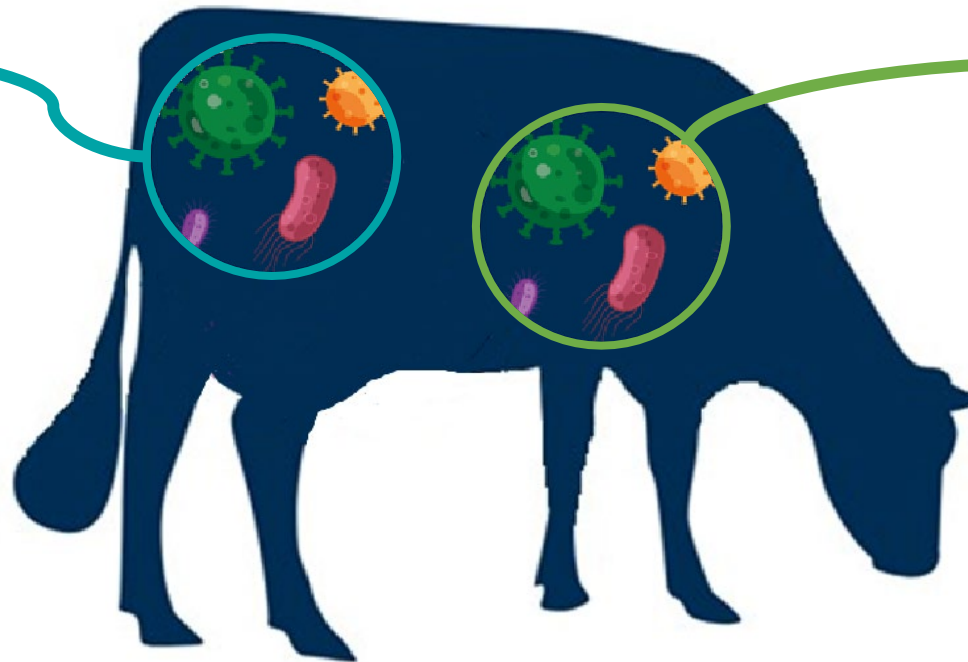
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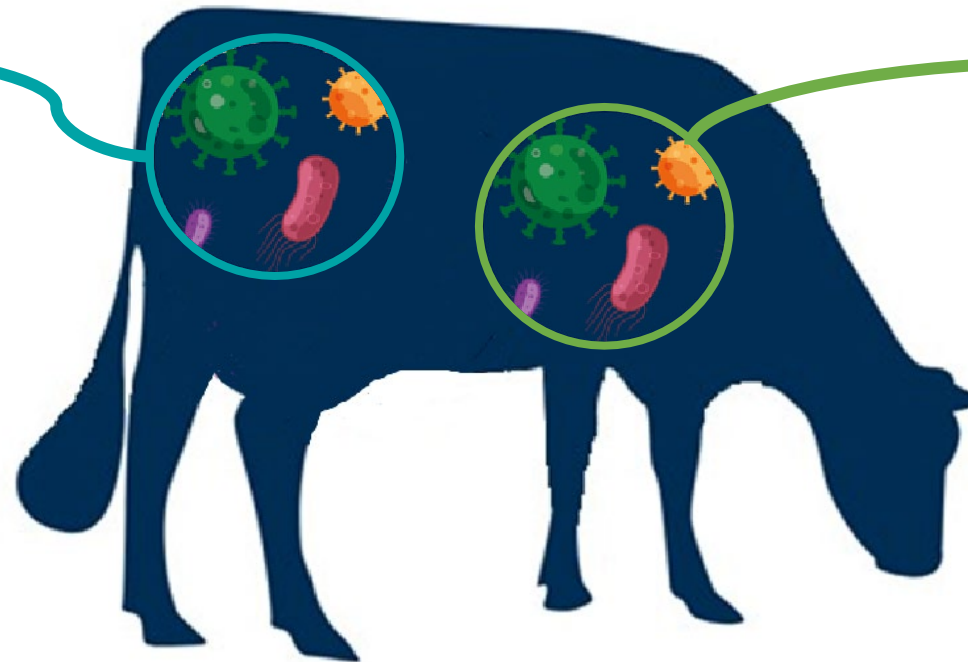
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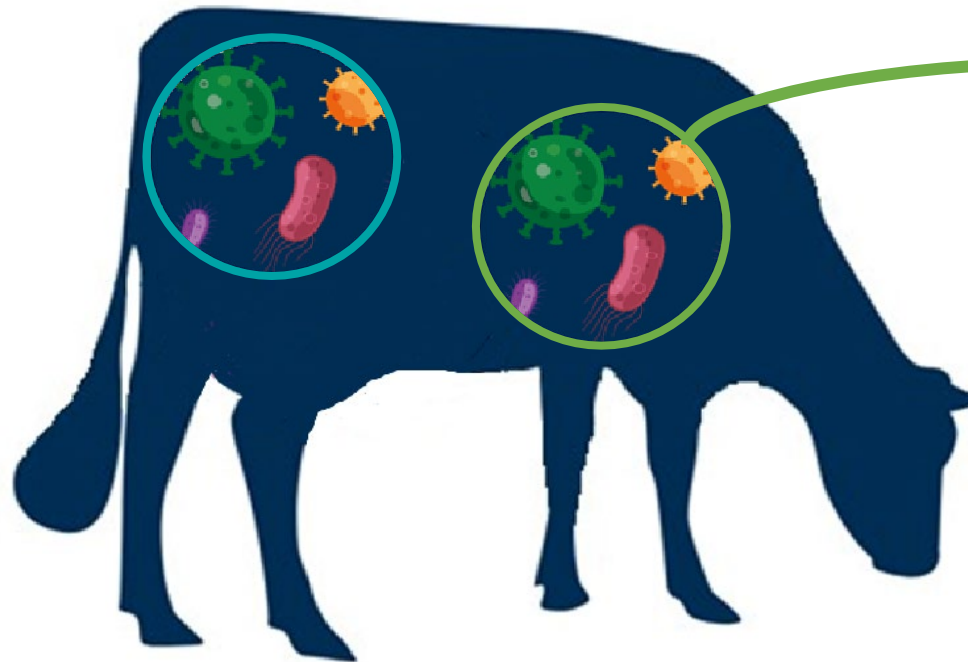
$h^2$ : 0.07 – 0.21  
(Grieneisen *et al.*, 2021)



$h^2$ : 0.32 – 0.57  
(Camarinha-Silva *et al.*, 2017)

# ➤ Why did we hypothesize that certain genes influence the faecal microbiota of dairy cows?

2 – Some genomic regions already associated with microbiota composition



## Rumen microbiota

- Variants significantly associated with the abundances of some taxa
- No consensus

(Li *et al.*, 2019; Martinez Boggio *et al.*, 2022)

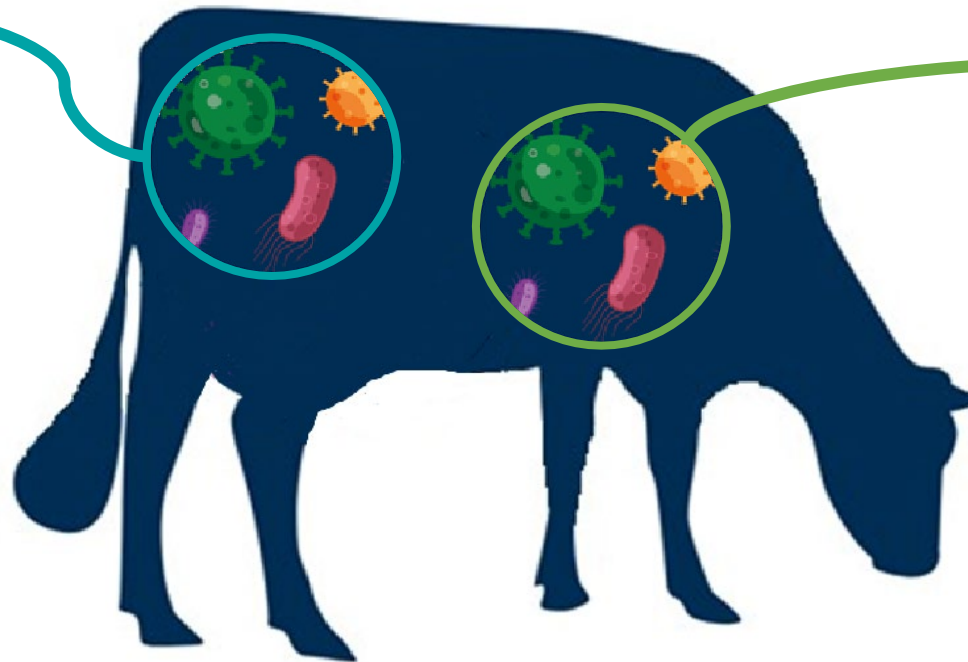
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## Faecal microbiota

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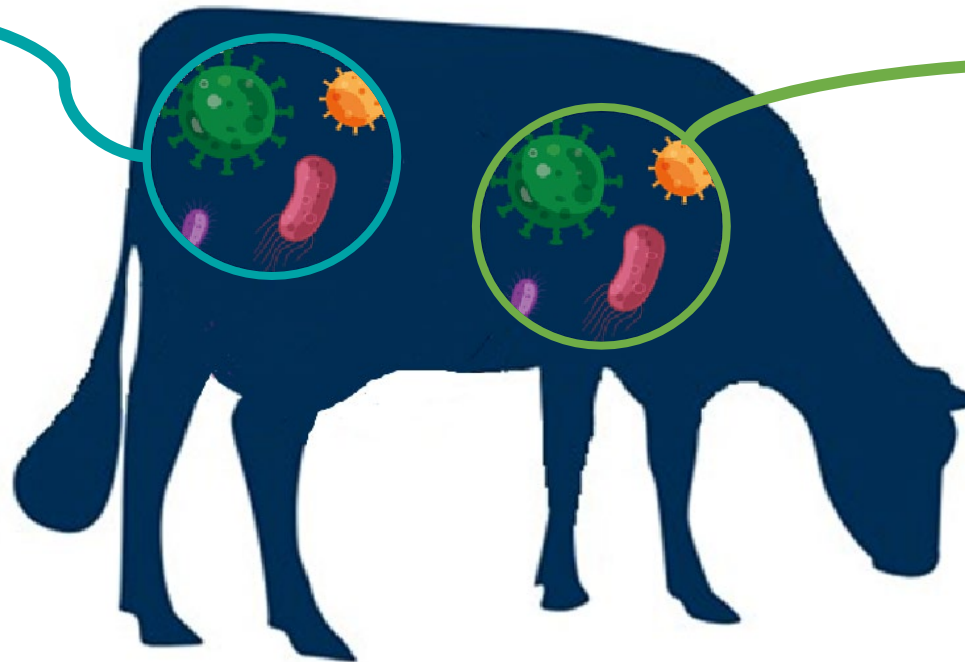
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(Davenport *et al.*, 2015)



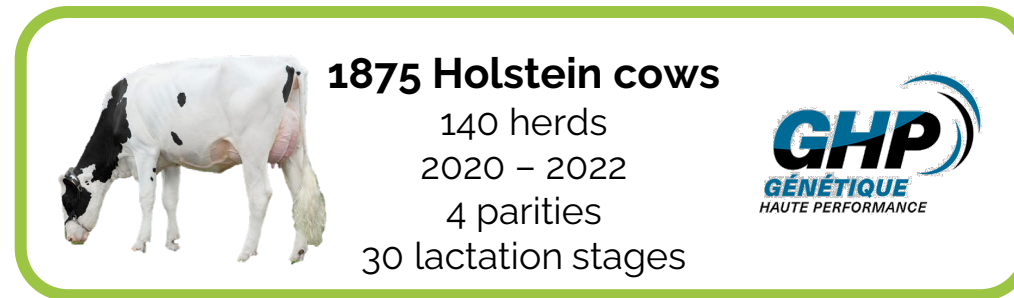
(Crespo-Piazuelo *et al.*, 2019)

➤ Are there some genomic **variants**  
**or genes** associated with the  
composition of **faecal microbiota**  
in **dairy cows**?

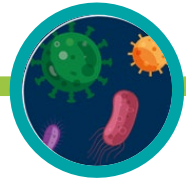




## ➤ Material & Methods



Faecal sampling



Genotyping & Imputation

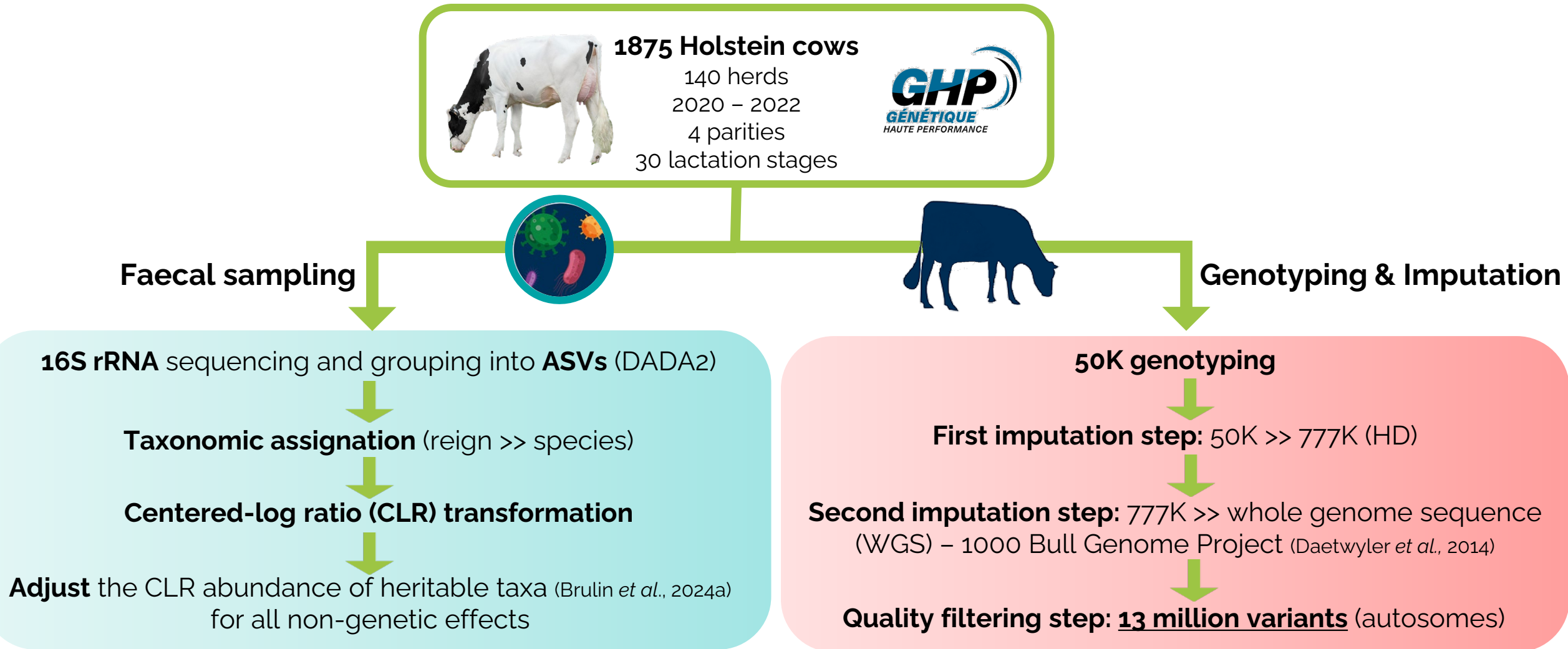
**16S rRNA** sequencing and grouping into **ASVs** (DADA2)

**Taxonomic assignation** (reign >> species)

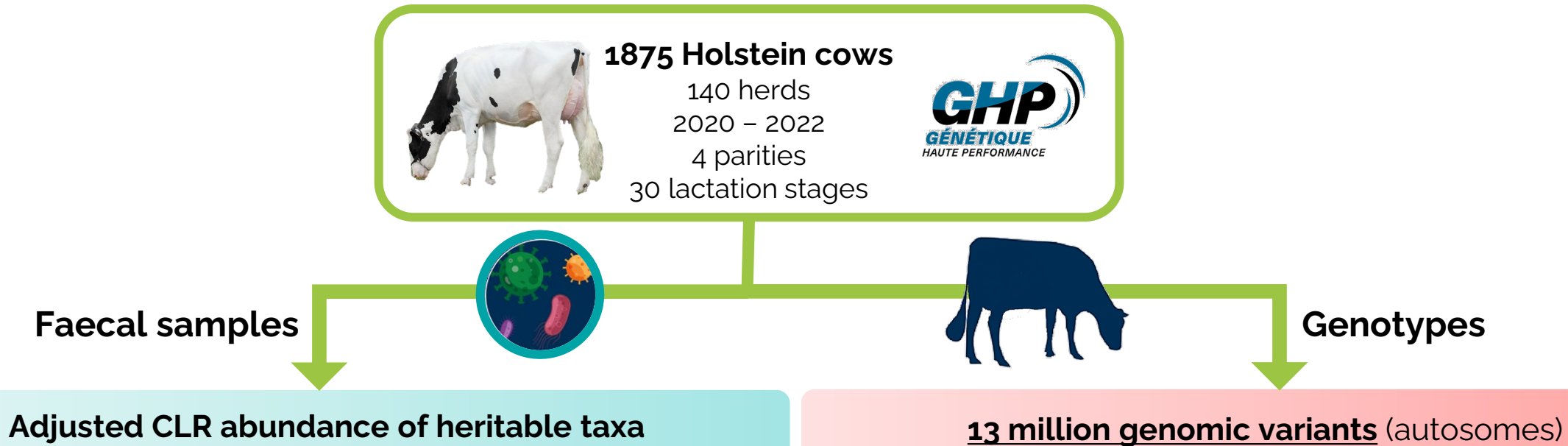
**Centered-log ratio (CLR) transformation**

**Adjust** the CLR abundance of heritable taxa (Brulin *et al.*, 2024a) for all non-genetic effects

## ➤ Material & Methods



## ➤ Material & Methods



1

### Sequence-based Genome-Wide Association Study (GWAS)

$$y = 1\mu + xb + g + e$$

$y$ : vector of adjusted phenotype ;

$1$ : a vector of 1s;

$\mu$ : the overall mean ;

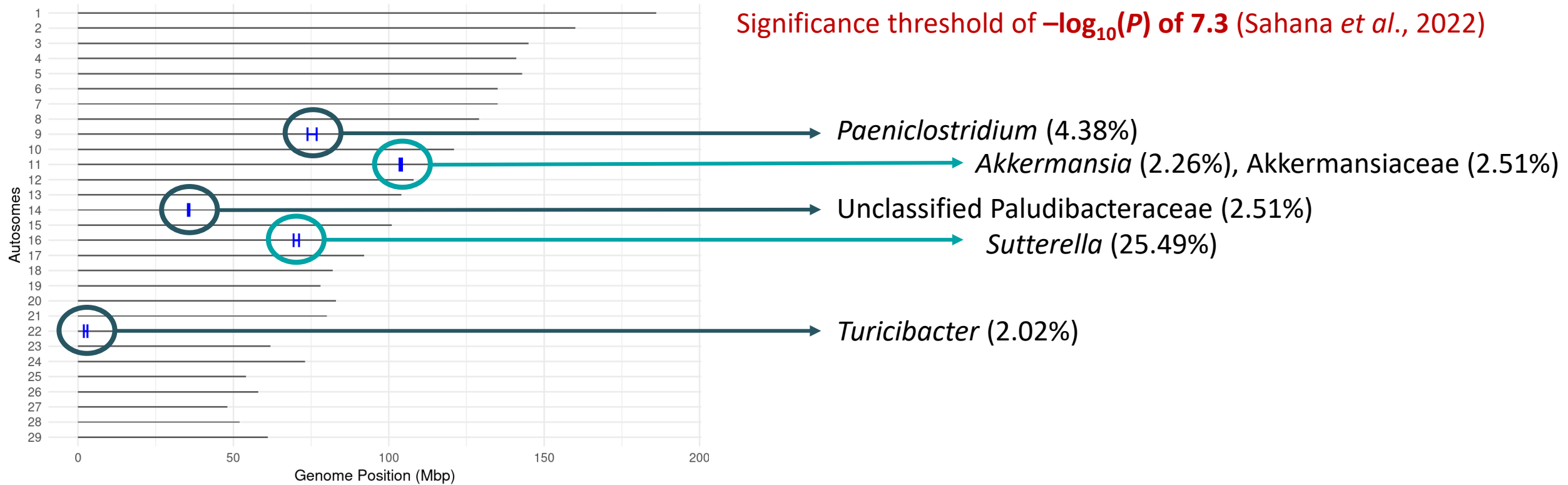
$b$ : the additive fixed effect of the variant tested;

$x$ : vector of imputed allele dosages for the tested variant;

$g \sim N(0, G\sigma_g^2)$ , vector of polygenic effects [with  $G$  the genomic relationship matrix and  $\sigma_g^2$  the polygenic variance];

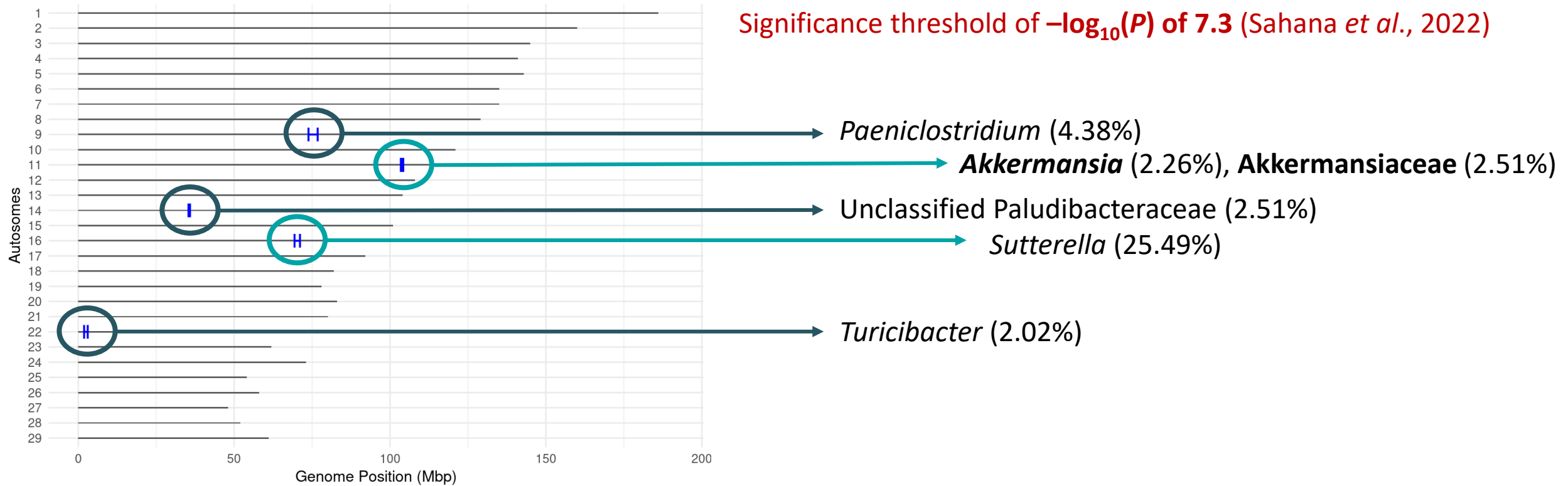
$e \sim N(0, I\sigma_e^2)$ : vector of random residual effects [with  $I$  the identity matrix and  $\sigma_e^2$  the residual variance].

## ➤ Results & Discussion: GWAS



Positions of the significant QTL (blue segments) on the *Bos taurus* autosomes

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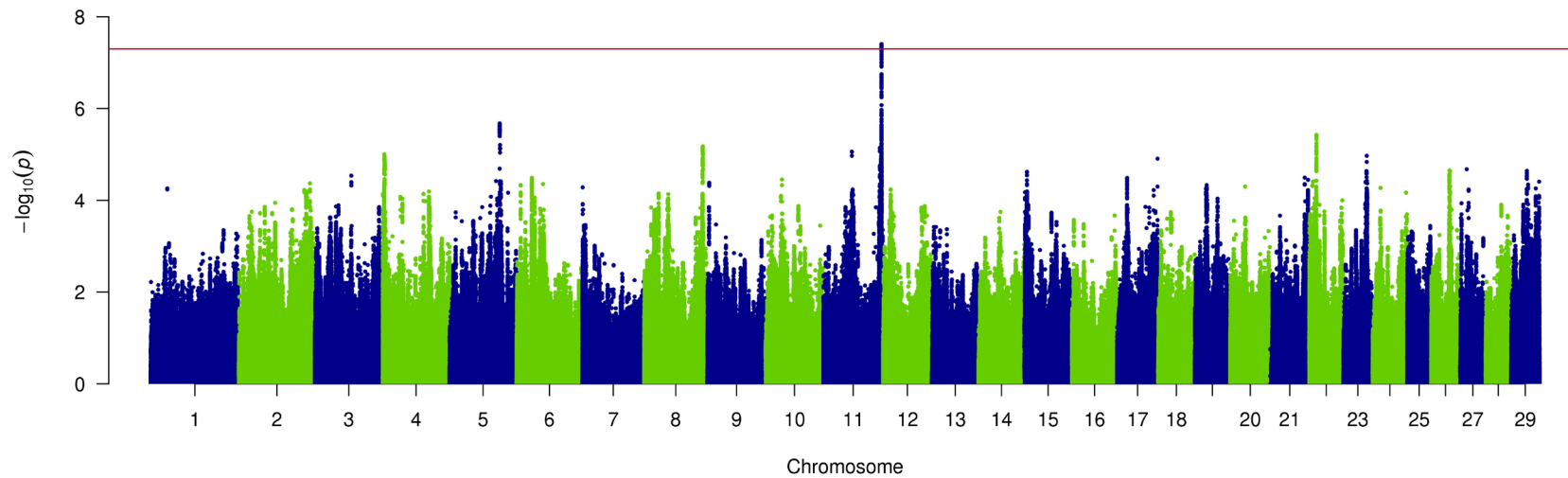


Positions of the significant QTL (blue segments) on the *Bos taurus* autosomes



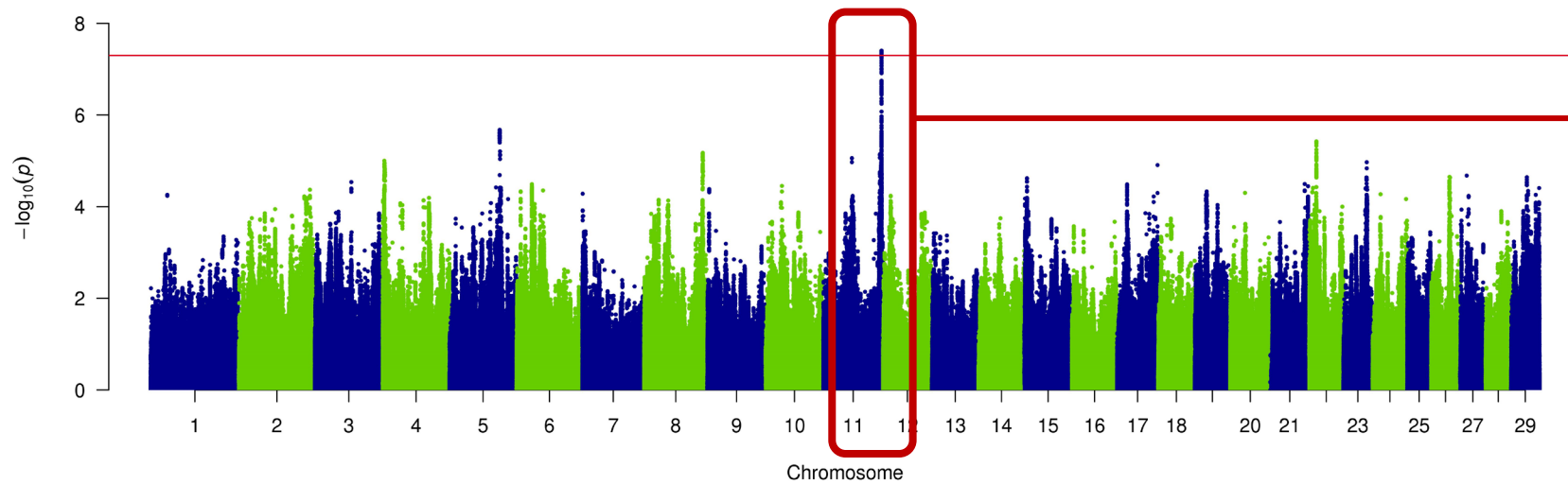
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Manhattan plot with the  $-\log_{10}(P)$  values plotted against the variant on *Bos taurus* autosomes for the abundance of *Akkermansia*



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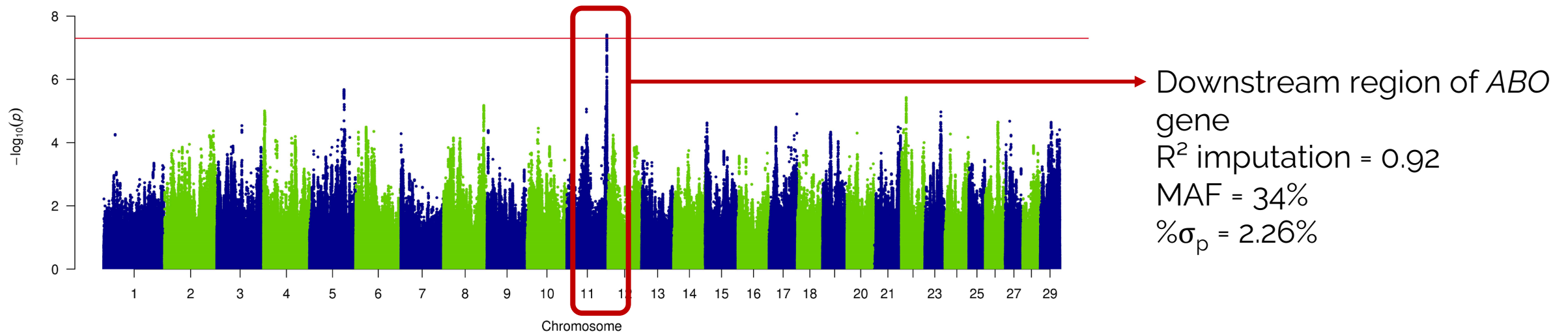
Manhattan plot with the  $-\log_{10}(P)$  values plotted against the variant on *Bos taurus* autosomes for the abundance of *Akkermansia*



Downstream region of *ABO* gene  
 $R^2$  imputation = 0.92  
MAF = 34%  
 $\% \sigma_p = 2.26\%$

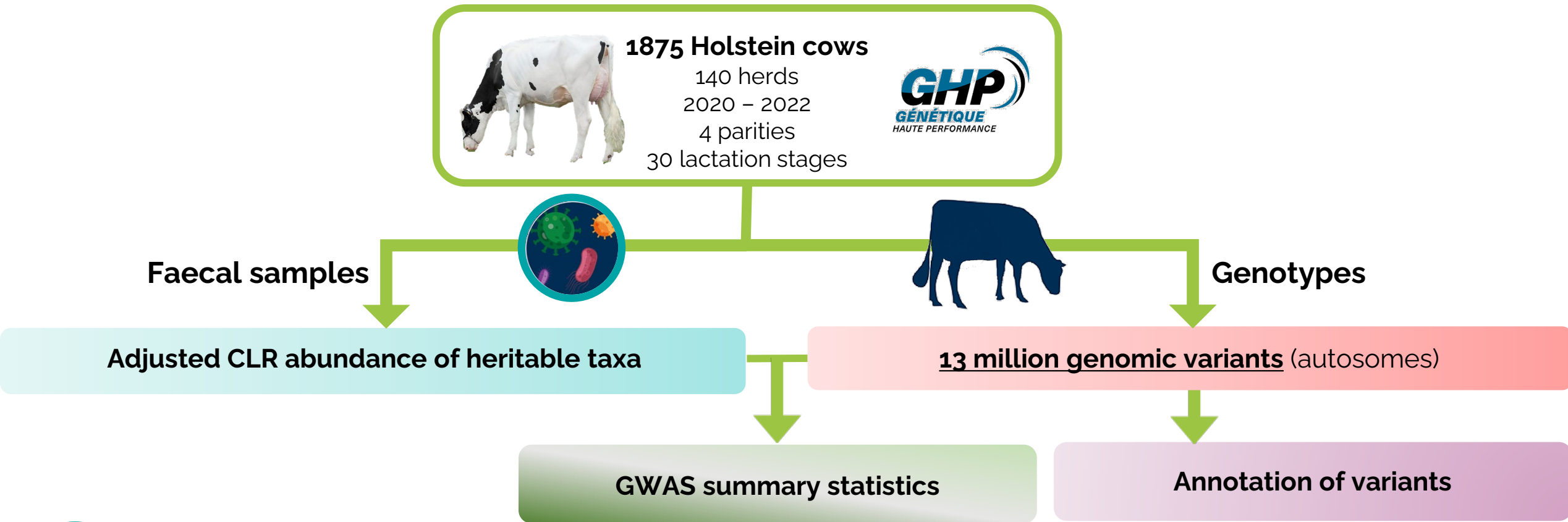
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Manhattan plot with the  $-\log_{10}(P)$  values plotted against the variant on *Bos taurus* autosomes for the abundance of *Akkermansia*



- *ABO* gene was also found significantly associated with the **gut microbiota composition** in human (Lopera-Maya *et al.*, 2022) and in pig (Yang *et al.*, 2022)
- *ABO*, involved in the **blood group** determination, is highly expressed in the **digestive tract of cattle**
- **First time** associated with *Akkermansia* genus ( $h^2 = 0.21$ ), a generally beneficial gut bacterium

## ➤ Material & Methods

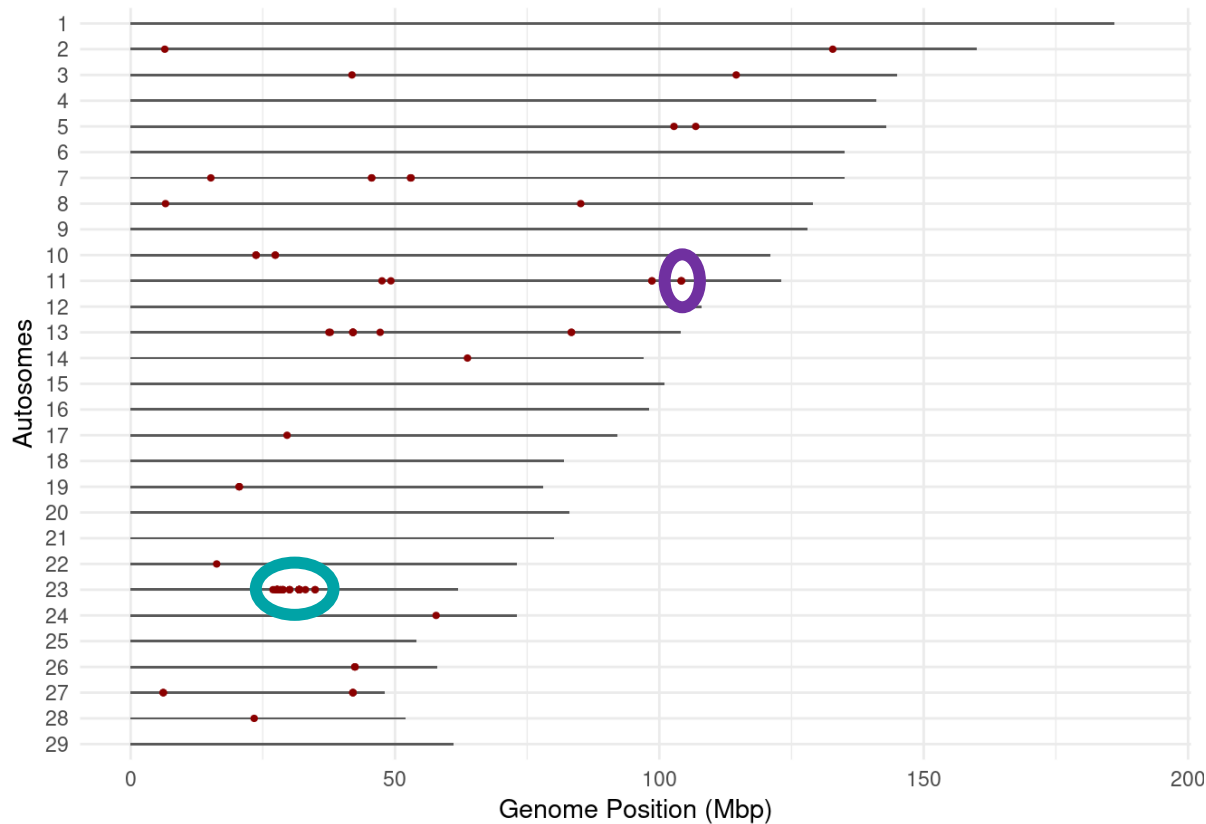


2

## Gene-based Association Study

➔ Compute p-values for each gene based on the GWAS summary statistics and linkage disequilibrium information to find **genes that are the most likely** to be associated with the taxa abundances

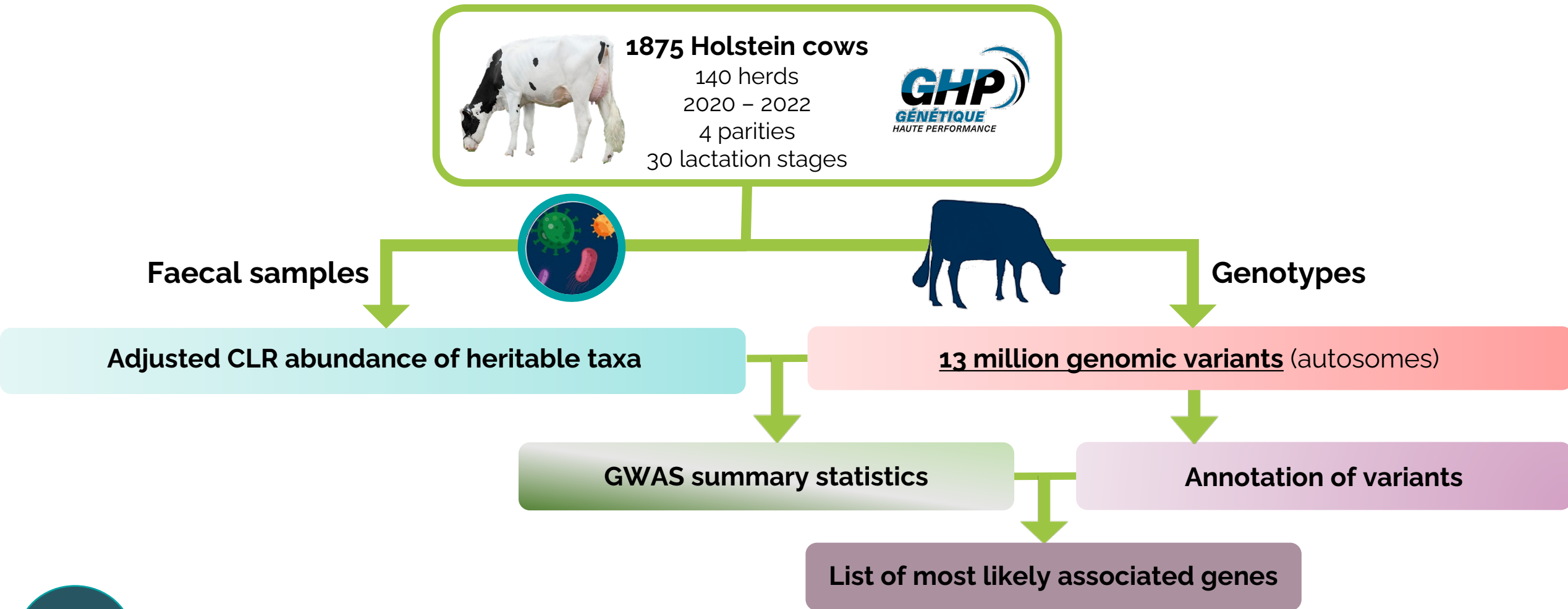
## ➤ Results & Discussion: Gene-based association study



Positions of the most likely associated genes (red dots) on the *Bos taurus* autosomes

- **92 candidate genes** on 17 autosomes:
  - 8 genes ⇔ 5 ASVs
  - 60 genes ⇔ 8 genera
  - 63 genes ⇔ 8 families
  - 2 genes ⇔ one phylum
- **41 genes linked with >1 taxon**
  - **From same taxonomic group:** ex. *Akkermansia* & Akkermansiaceae with *ABO* gene on BTA11
  - **From different taxonomic groups:** explain the strong genetic correlations between some taxa (Brulin *et al.*, 2024a)
- Many genes on BTA23: around the **Major Histocompatibility Complex** (MHC) whose polymorphisms have been associated with the microbiota composition in various species (Bolnick *et al.*, 2014; Khan *et al.*, 2019; Derakhshani *et al.*, 2018)

## ➤ Material & Methods



3

### Gene ontology (GO) enrichment analysis



EAAP 2024 – Session 55

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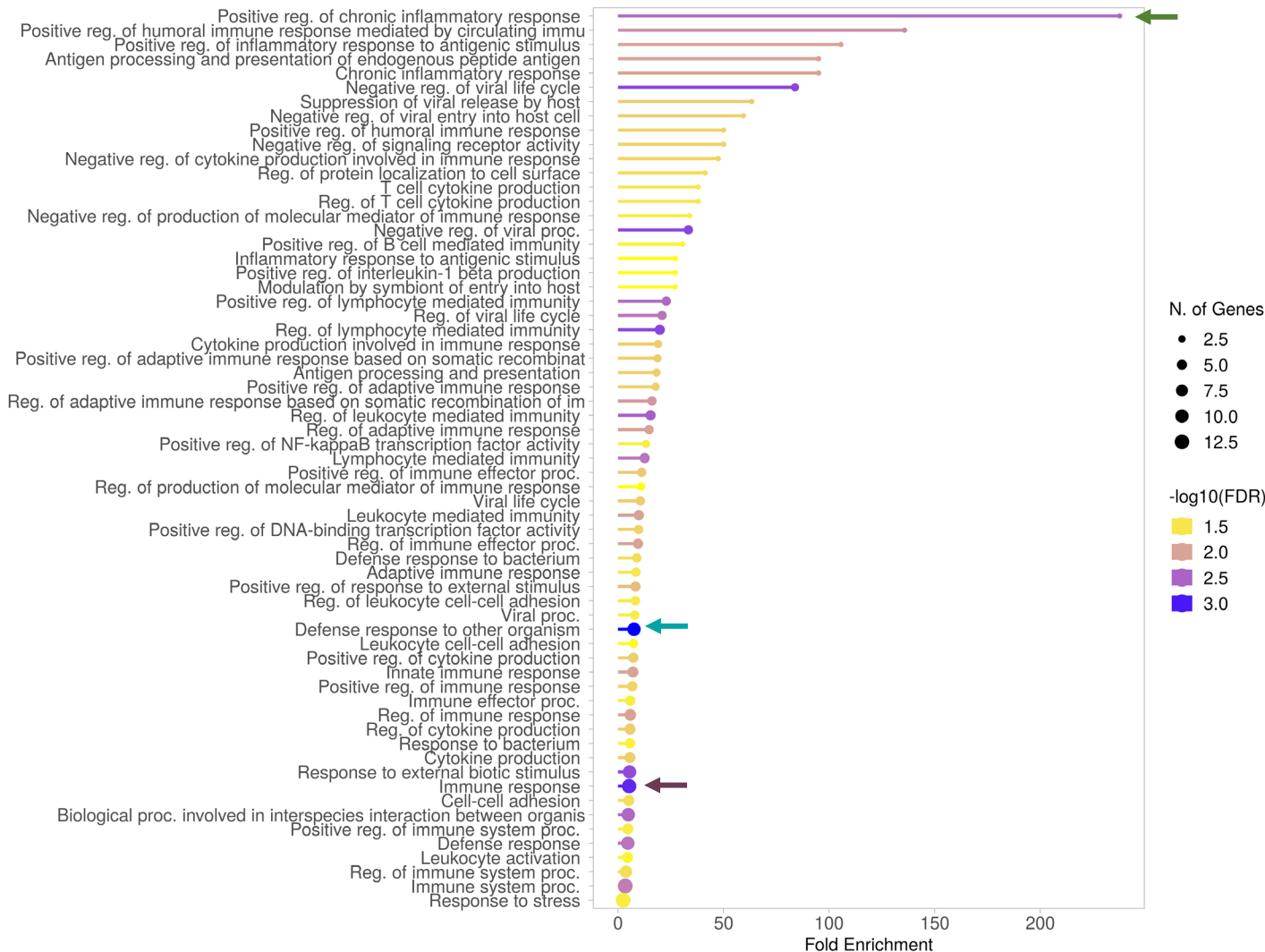
➔ Use the GO system of classification (biological processes, cellular components, molecular functions) to interpret gene sets

Performed with ShinyGO software (Ge et al., 2020)



# ➤ Results & Discussion: Gene ontology enrichment analysis

Barplots showing the significant fold enrichment of GO biological processes using the genes significantly associated with the faecal taxa abundances



- **63 GO terms significantly enriched**
- Most significant pathway:
  - *Defense response to other organism*
  - *Immune response*
- Most enriched pathway:
  - *Positive regulation of chronic inflammatory response*
- Warning! : a lot of genes on BTA23 in the MHC complex that could increase the risk of false positive genes and therefore pathways
- Conclusions: A lot of pathways associated with the host's **immune response**, in line with Fan *et al.*, (2021)'s study in sucking cattle (faecal microbiota composition). Need additional works to validate this observation.

## ➤ Conclusion & Perspectives

- Association between **Holstein cows' genomic regions** and the **faecal microbiota** composition
- **Identification of some genes** that are more likely to be associated with taxa abundances (ex. *ABO* gene)
- Role of genetics underlying the **host's immune response** on the faecal microbiota of dairy cattle?
- Perspective of using **genomic information to select** dairy cattle for a faecal microbiota that promotes improved performance (Brulin *et al.*, 2024b, Monteiro *et al.*, 2022) or health status (Zhang *et al.*, 2019).

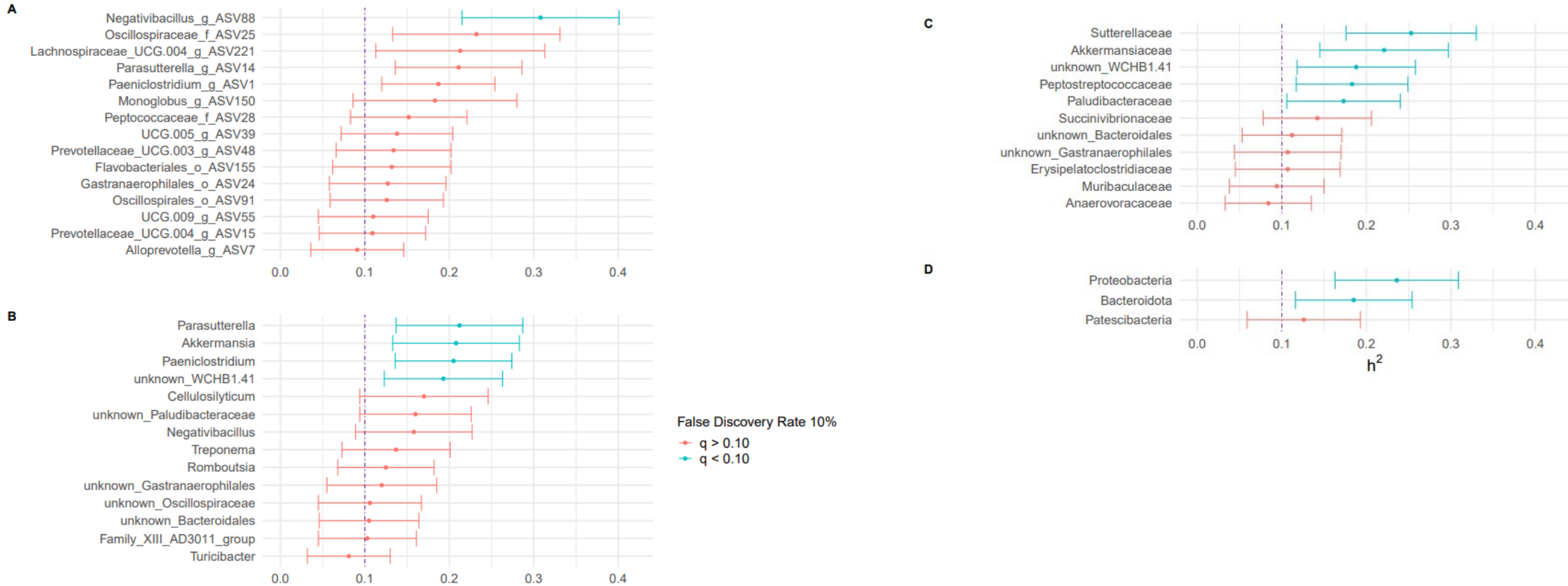






**Thank you for your attention!**

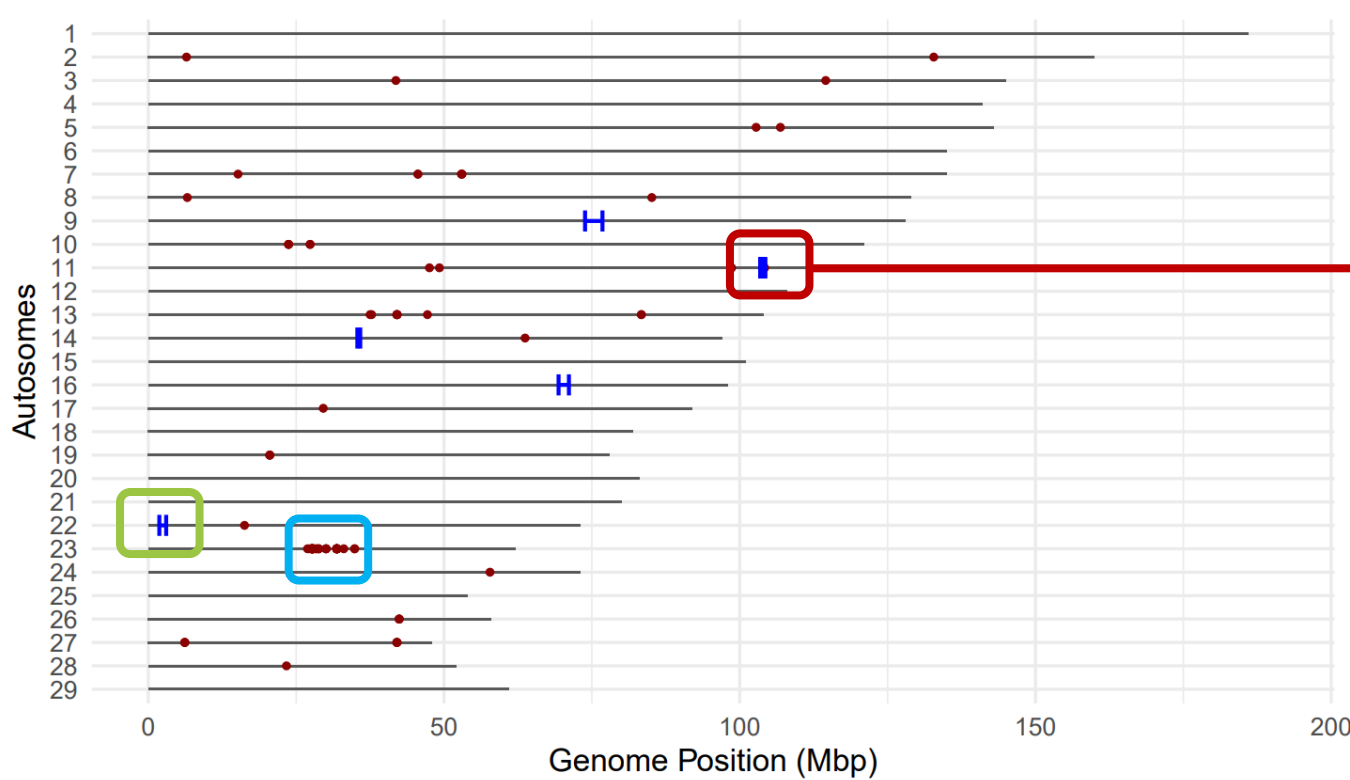
## ➤ Additional figures: Heritability estimates of faecal taxa



Barplots presenting the significant heritability estimates and the associated SE

A = ASVs; B = Genera; C = Families; D = Phyla

## ➤ Results & Discussion: Common results



Positions of the most likely associated genes (red dots) and significant QTL (blue segments) on the *Bos taurus* autosomes

Only QTL with significant candidate genes:

- **ABO**
- *U6*
- *DIPKIB*

Some significant genes outside QTL: not enough detection power to highlight small effect (**MHC**)

Some significant variants not associated with significant genes: mutation outside a gene and influencing the expression of a gene (**QTL on BTA22**)

These findings highlight the **complementarity** of the two analyses.