

Metagenomic and Gene Association Analysis in Ewe Fertility: A Comprehensive Study

Edgar L. Reinoso-Peláez^{1,2}

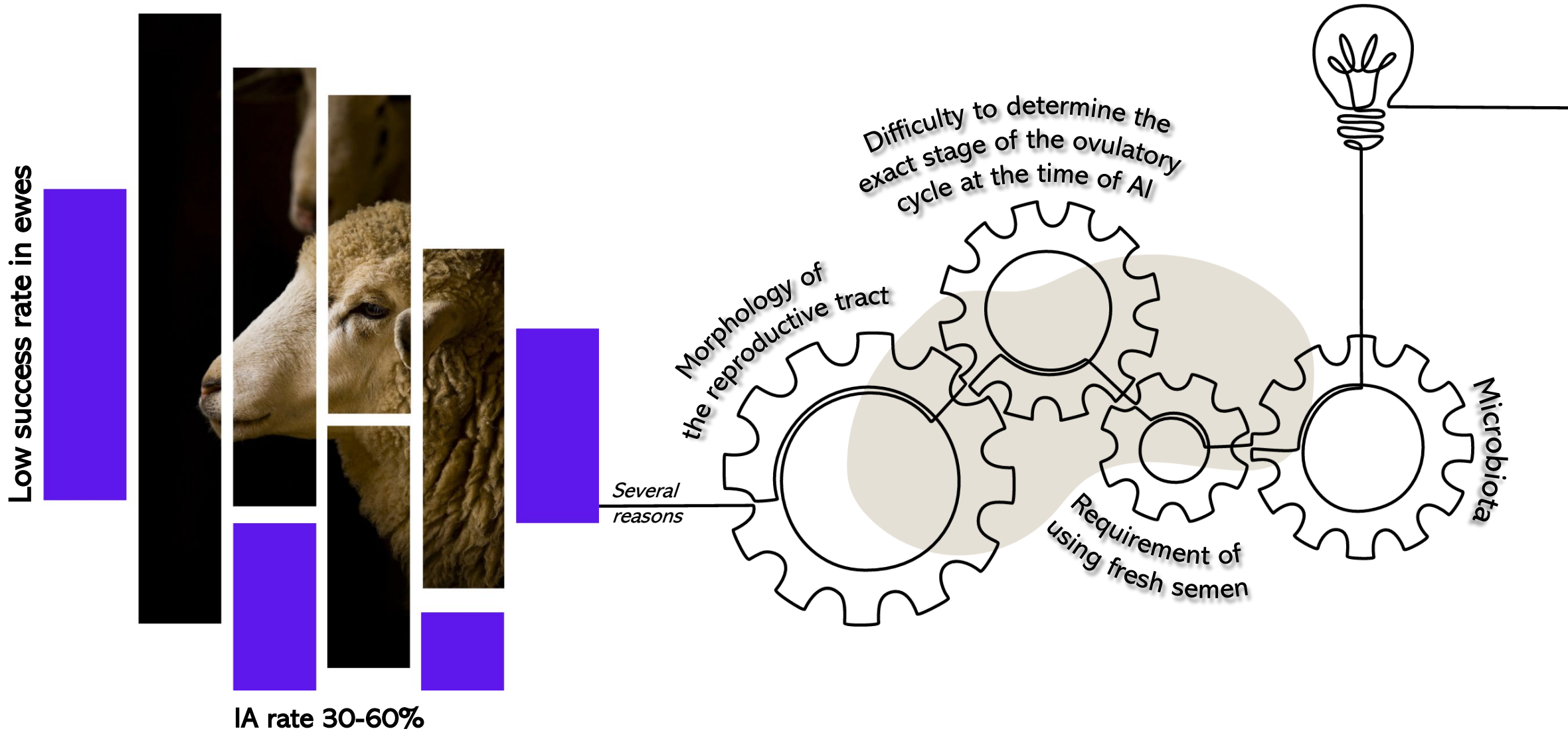
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¹ Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC), Madrid, España; ² Universidad politécnica de Madrid (UPM), Madrid, España; ³ Instituto de Investigaciones Mariñas (IIM-CSIC), Vigo, Spain

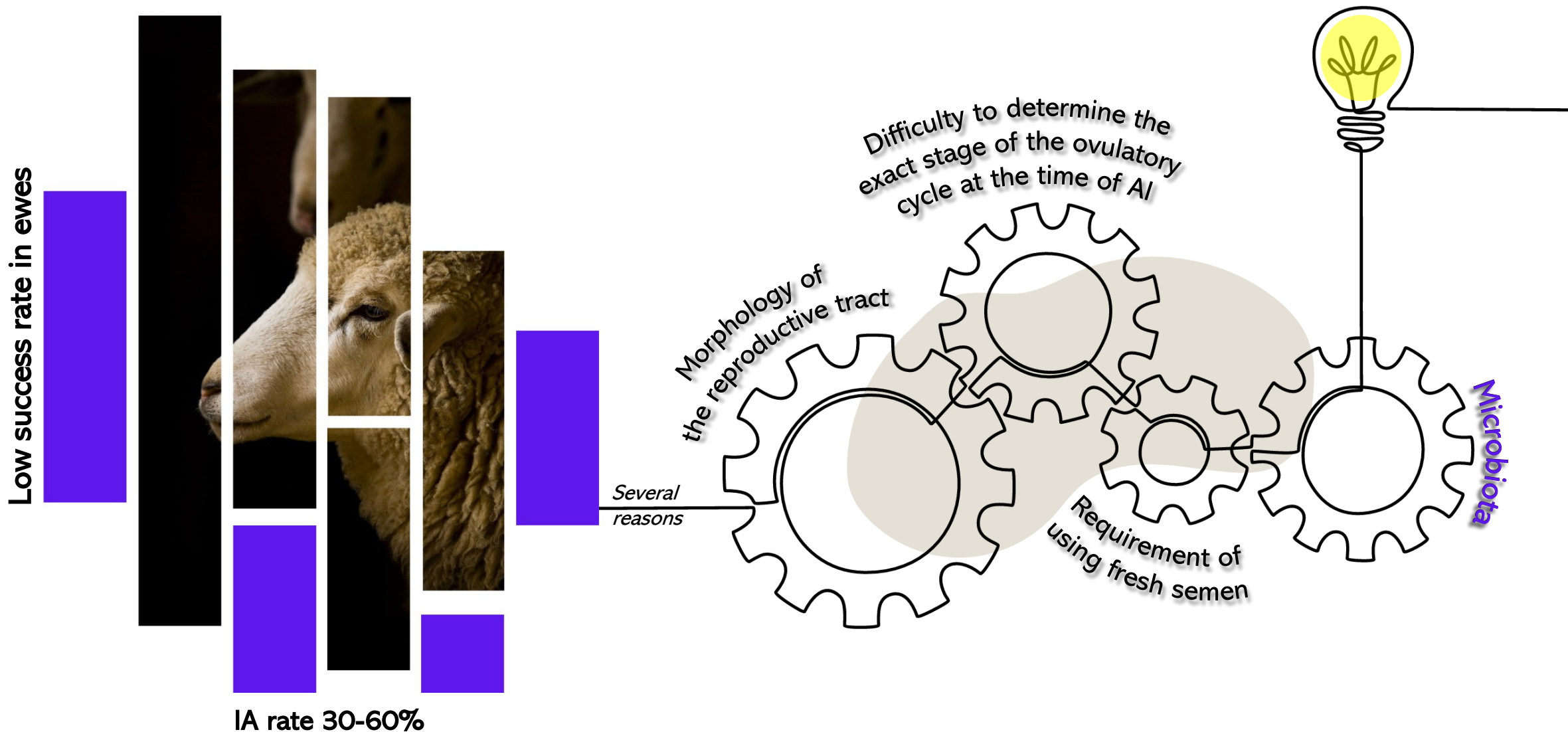
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The Challenge of Artificial Insemination



The Challenge of Artificial Insemination



Importance of Microbiota in Fertility



Many human studies
linking reproductive
microbiota to
fertility

EXAMPLES

- **Romero et al. 2014.** The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome*
- **Wee et al. 2018.** A retrospective pilot study to determine whether the reproductive tract microbiota differs between women with a history of infertility and fertile women. *Aust N Z J Obstet Gynaecol.*
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- **Murga Valderrama et al. 2023.** Exploring the microbiome of two uterine sites in cows. Scientific Reports .
- **Ault-Seay et al. 2023.** Importance of the female reproductive tract microbiome and its relationship with the uterine environment for health and productivity in cattle: A review. Frontiers in Animal Science 4:1111636..
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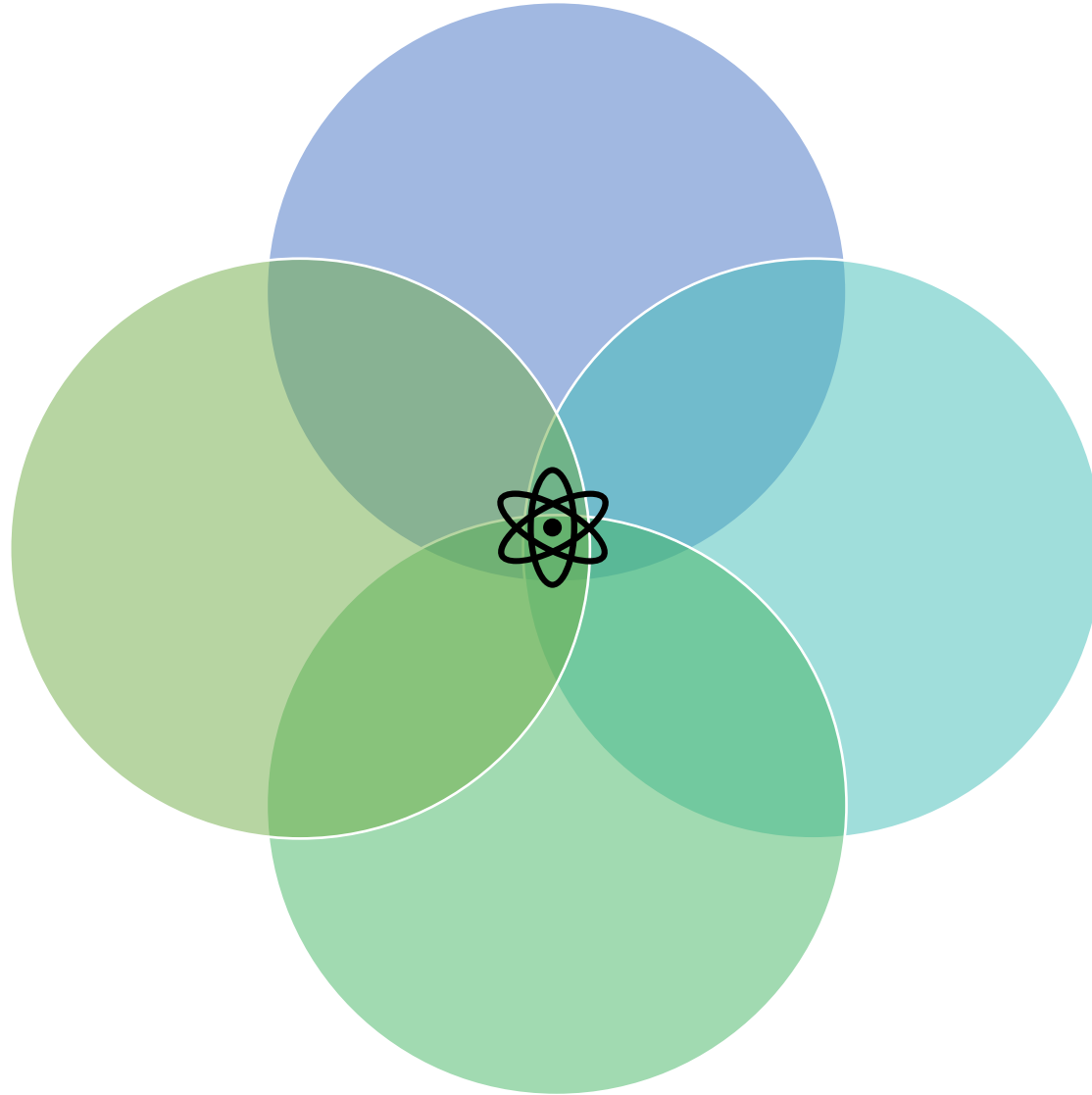
Increasing Yet Still Limited Research In sheep



EXAMPLES

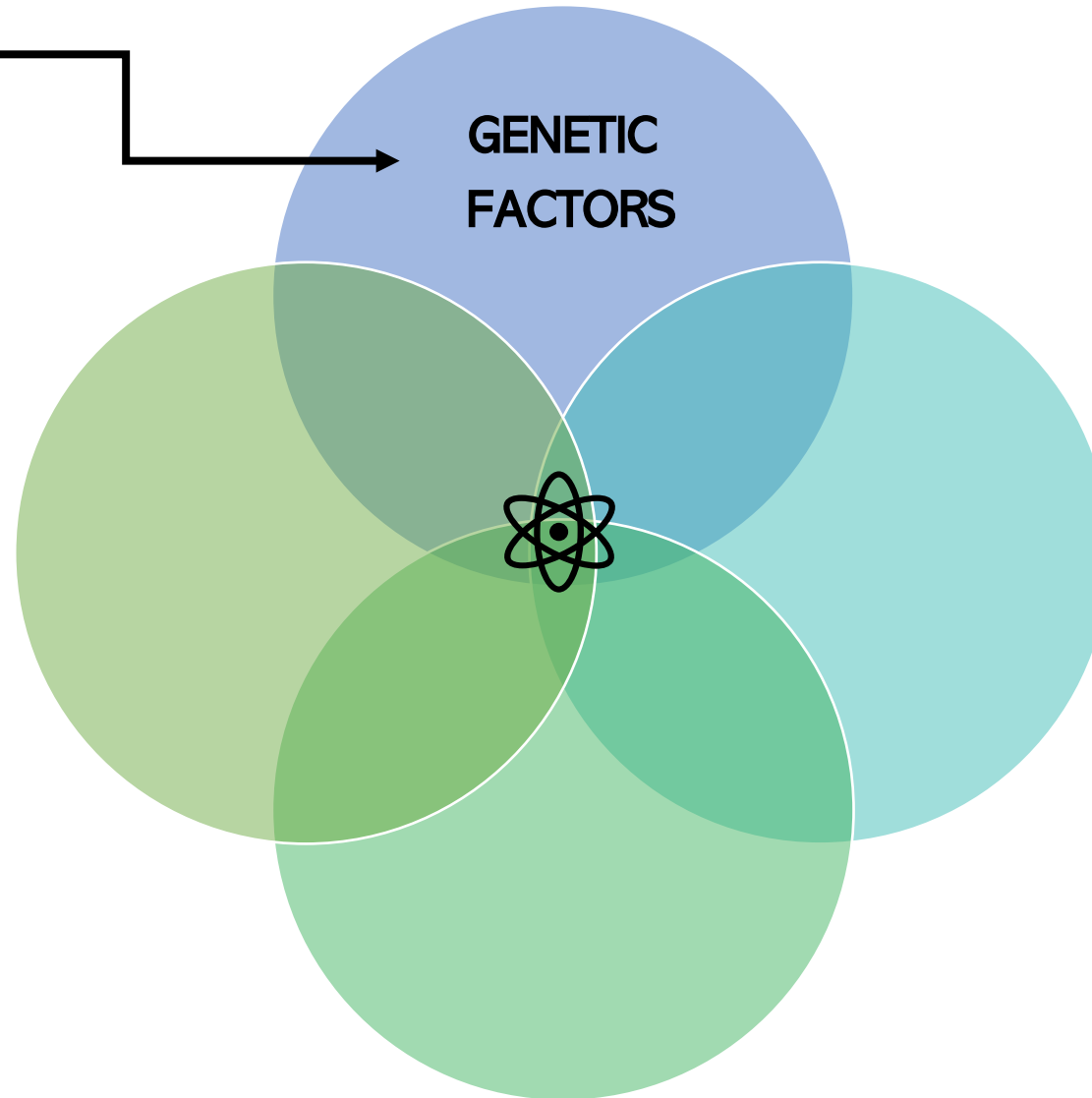
- **Serrano et al. 2020.** Influence of the ovine genital tract microbiota on the species artificial insemination outcome. A pilot study in commercial sheep farms. *High Throughput*
- **Reinoso-Peláez et al. 2023.** Impact of oestrus synchronization devices on ewes vaginal microbiota and artificial insemination outcome. *Front Microbiol*
- **Koester et al. 2021.** Ewe vaginal microbiota: associations with pregnancy outcome and changes during gestation. *Front Microbiol*
- **Barba et al. 2023.** Description of the vaginal microbiota in nulliparous ewes during natural mating and pregnancy: preliminary signs of the male preputial microbiota modulation. *Front Microbiol*

Microbiome: complex, interconnected systems



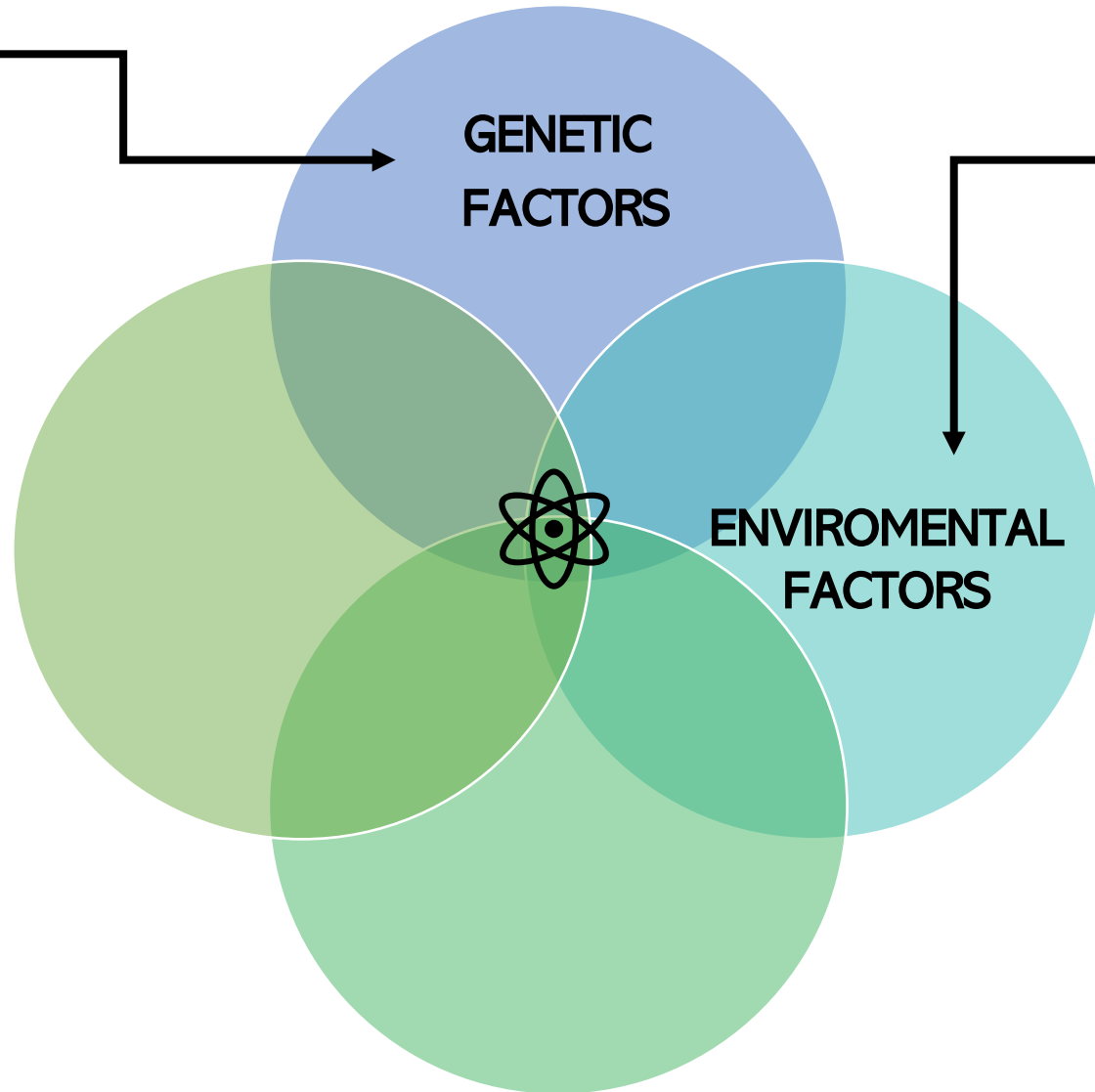
Microbiome: complex, interconnected systems

- Influence of the host



Microbiome: complex, interconnected systems

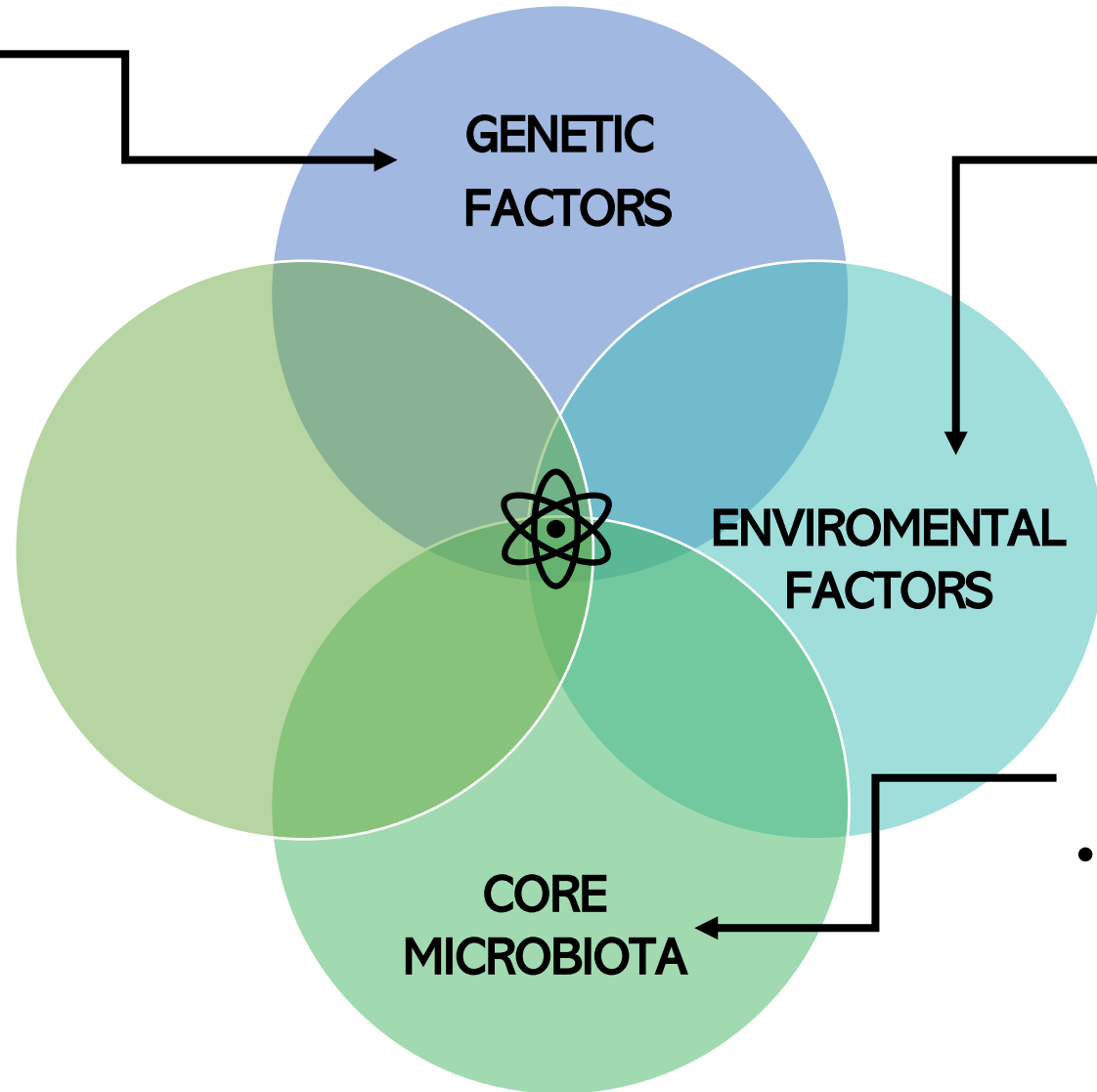
- Influence of the host



- Influence of external factors, example: diet, management, age.

Microbiome: complex, interconnected systems

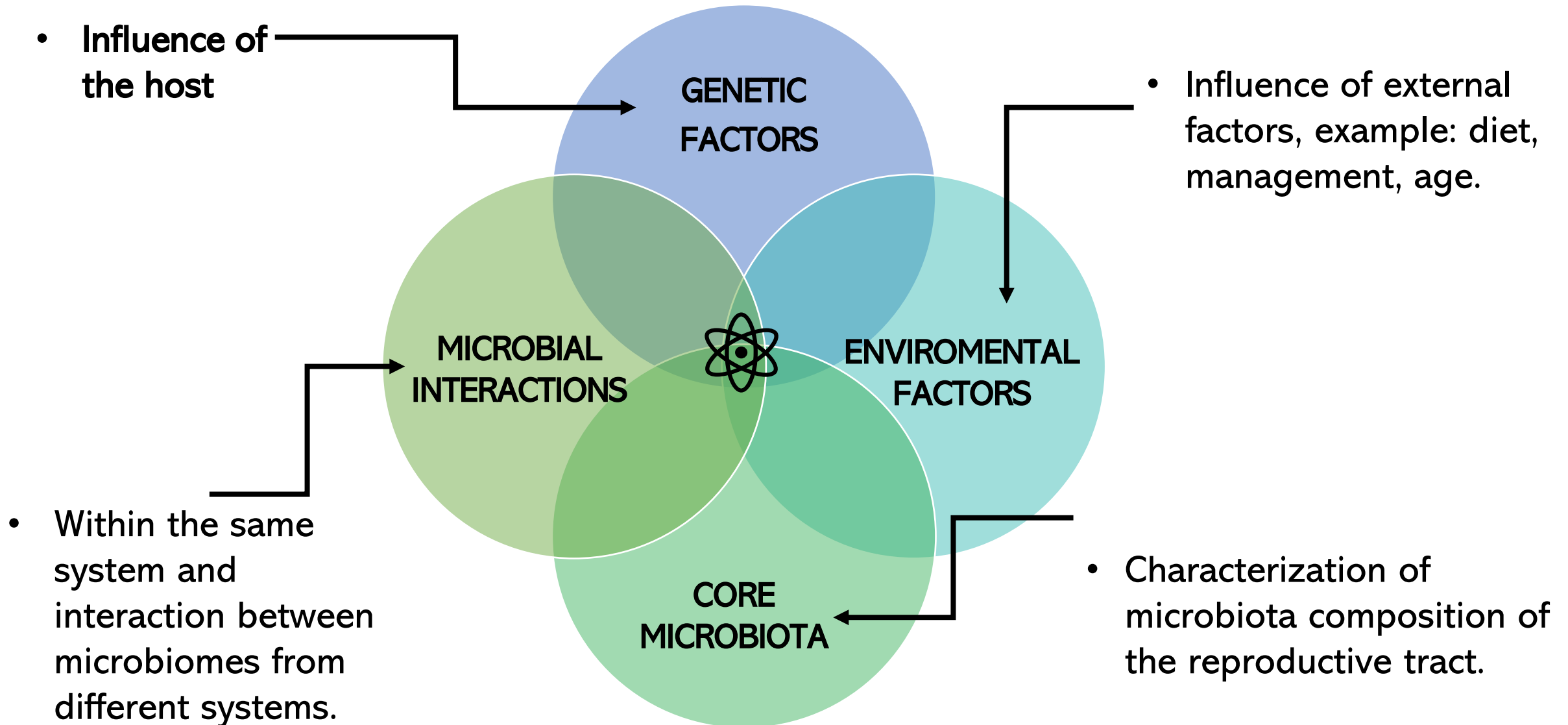
- Influence of the host



- Influence of external factors, example: diet, management, age.

- Characterization of microbiota composition of the reproductive tract.

Microbiome: complex, interconnected systems



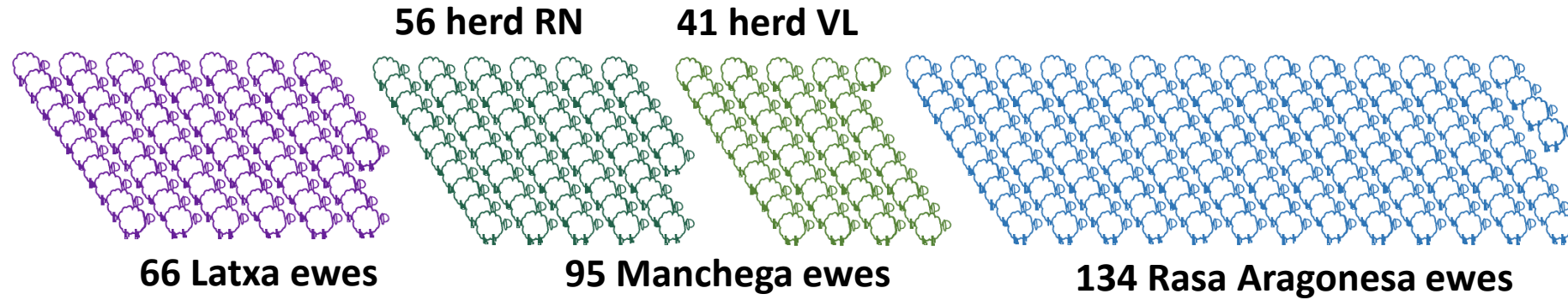
Objectives

Investigate the influence of vaginal microbiota and its genes on sheep fertility

- Describe the core microbiota and gene content of the ewe vaginal tract
- Determine the effect of breed and herd on the vaginal microbiota
- Determine the associations between the abundance of microbial communities and pregnancy outcomes

Sampling and Study Design

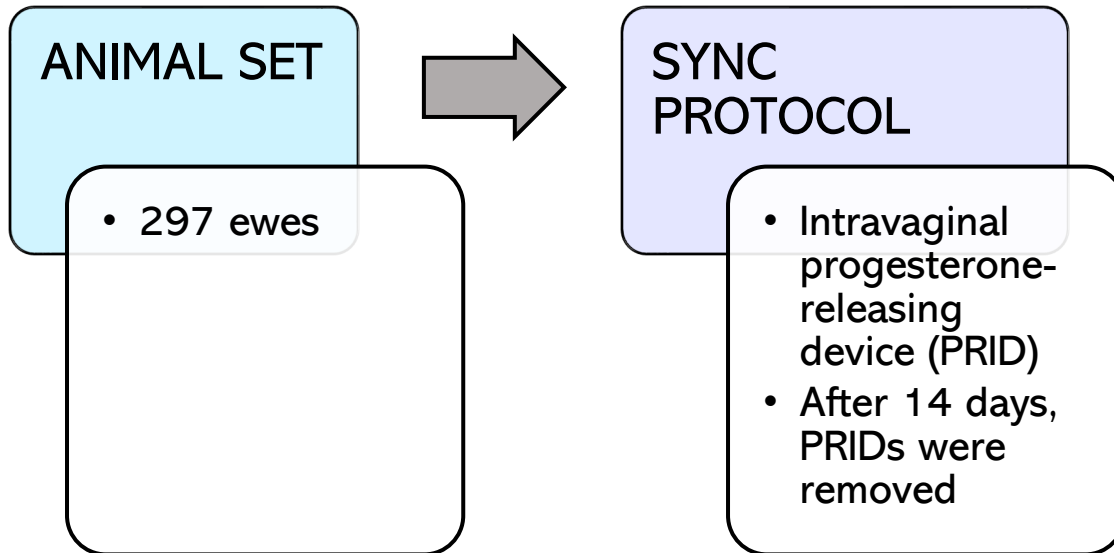
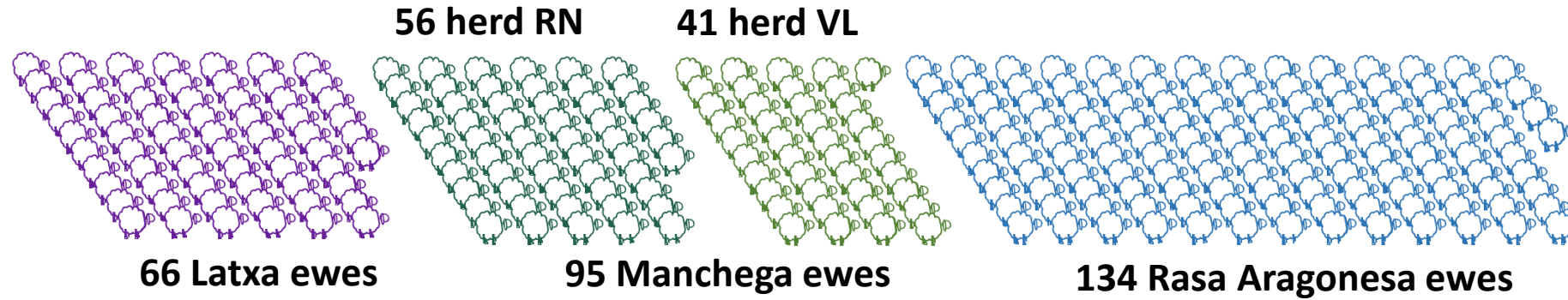
Sampling and Study Design



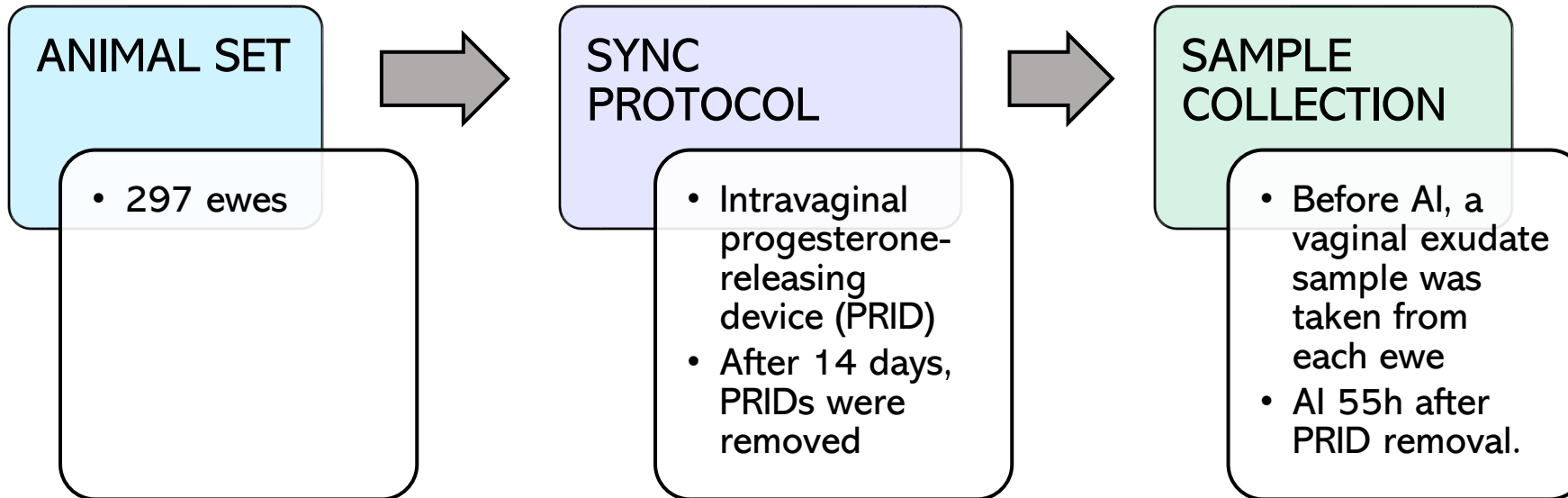
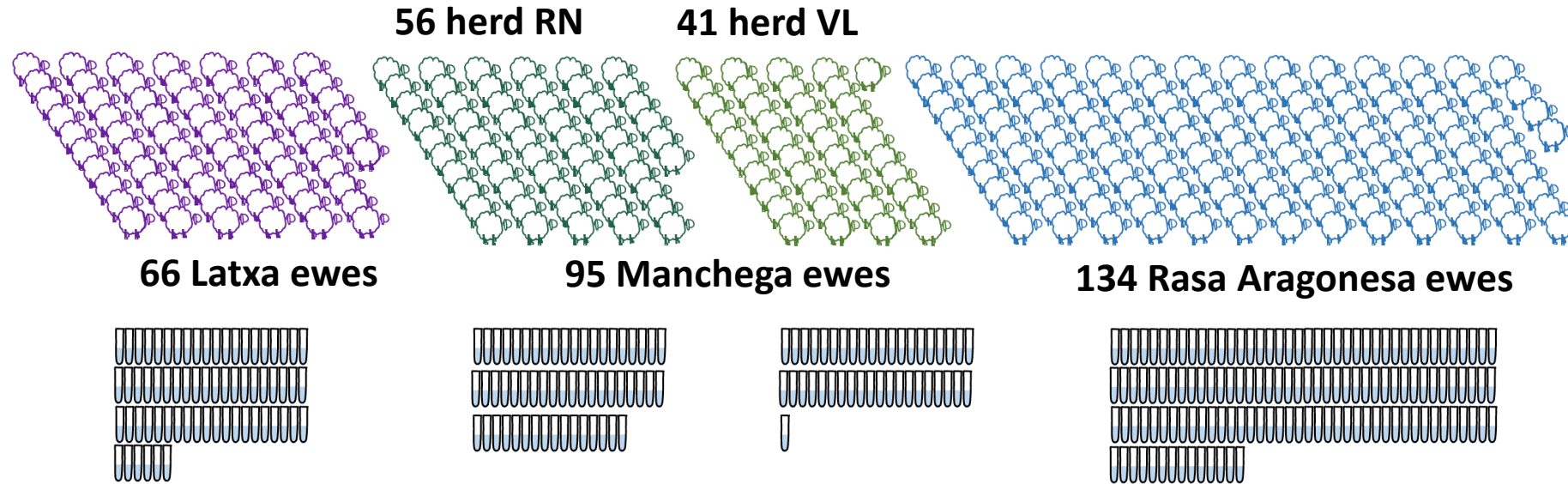
ANIMAL SET

- 297 ewes

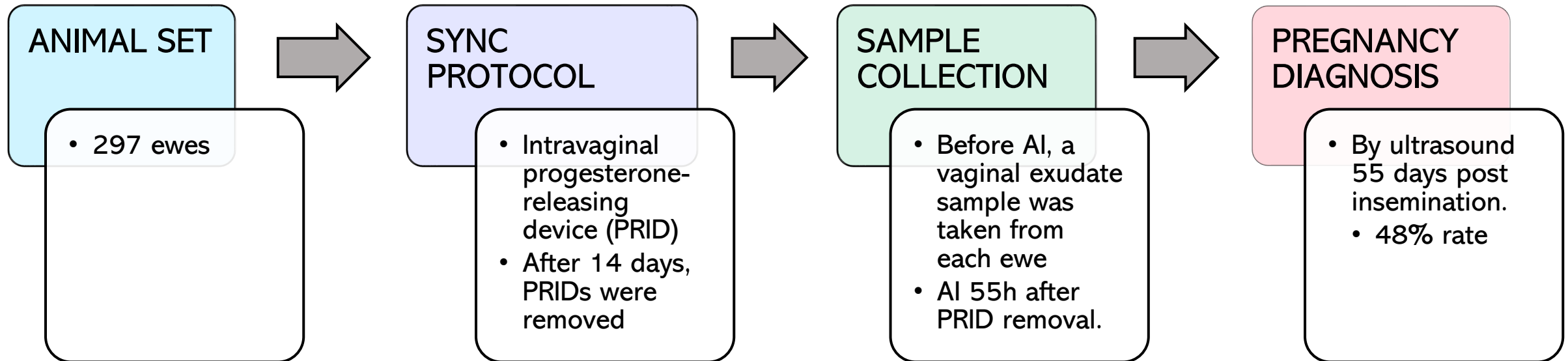
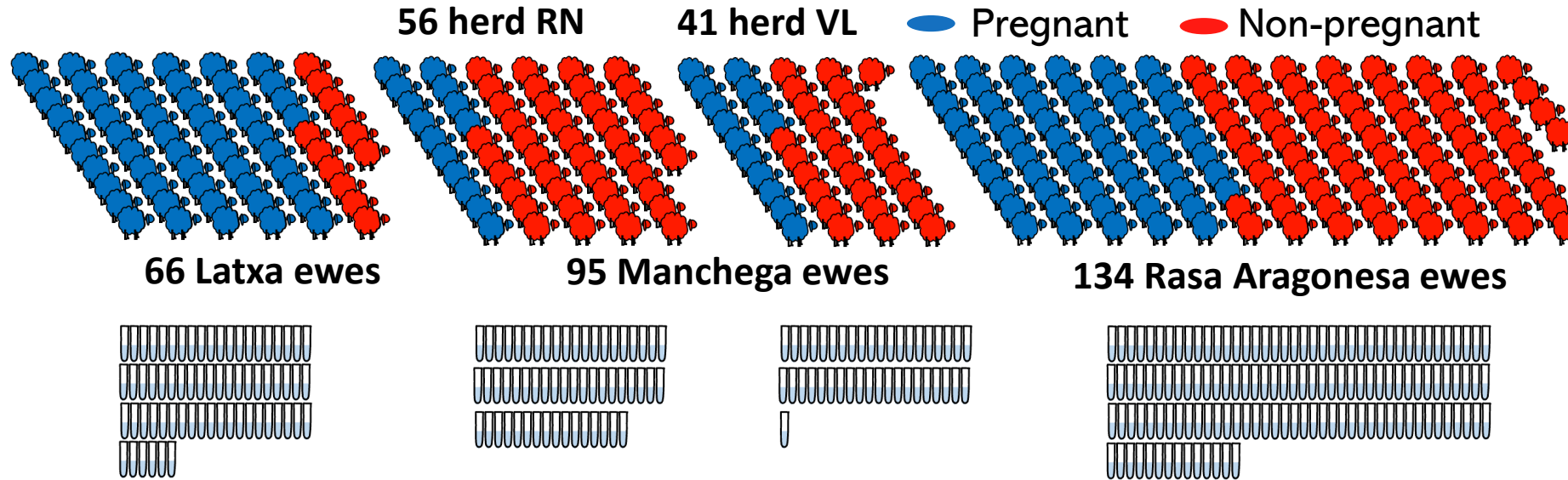
Sampling and Study Design



Sampling and Study Design



Sampling and Study Design



DNA Extraction

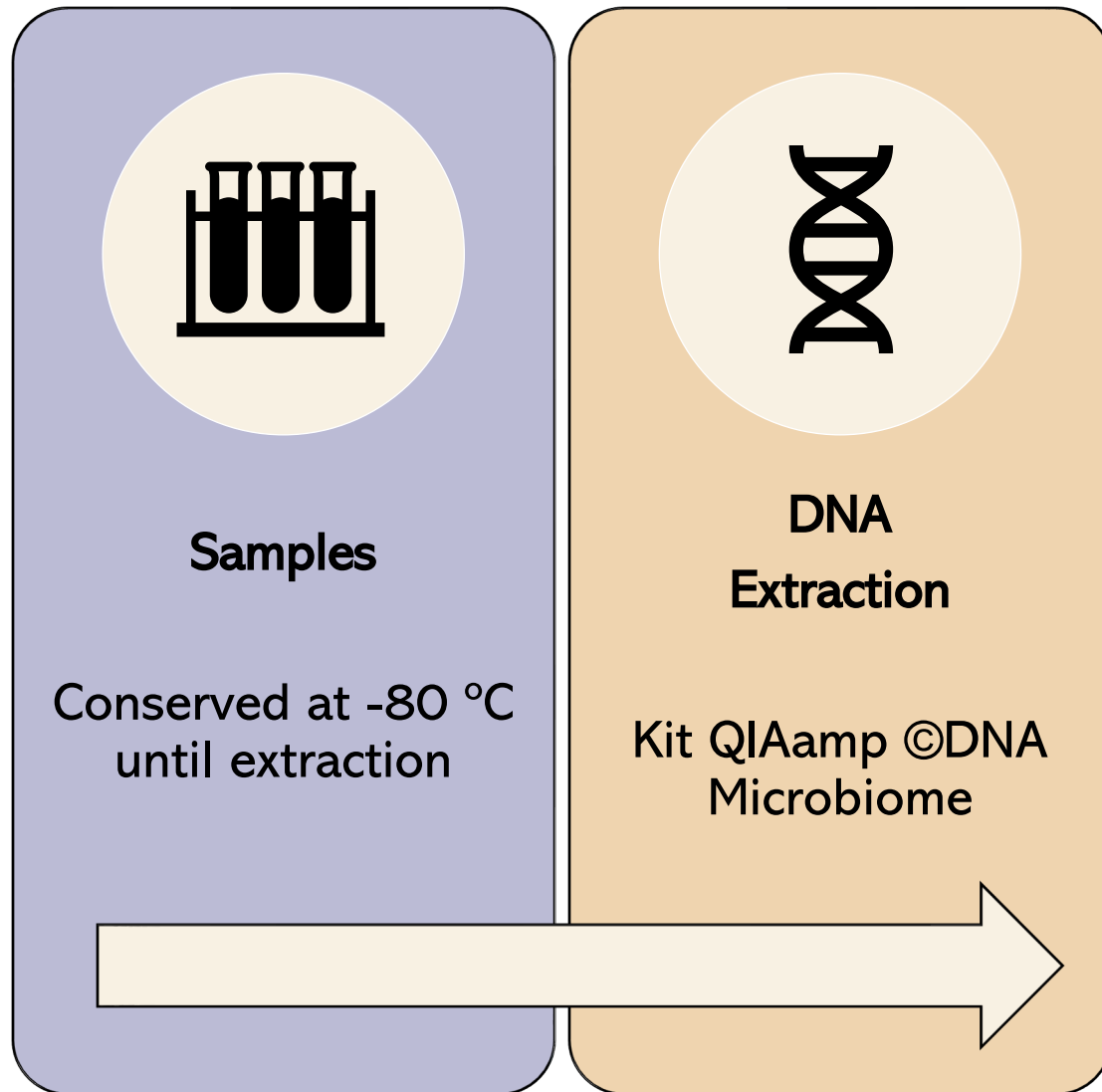
DNA Extraction



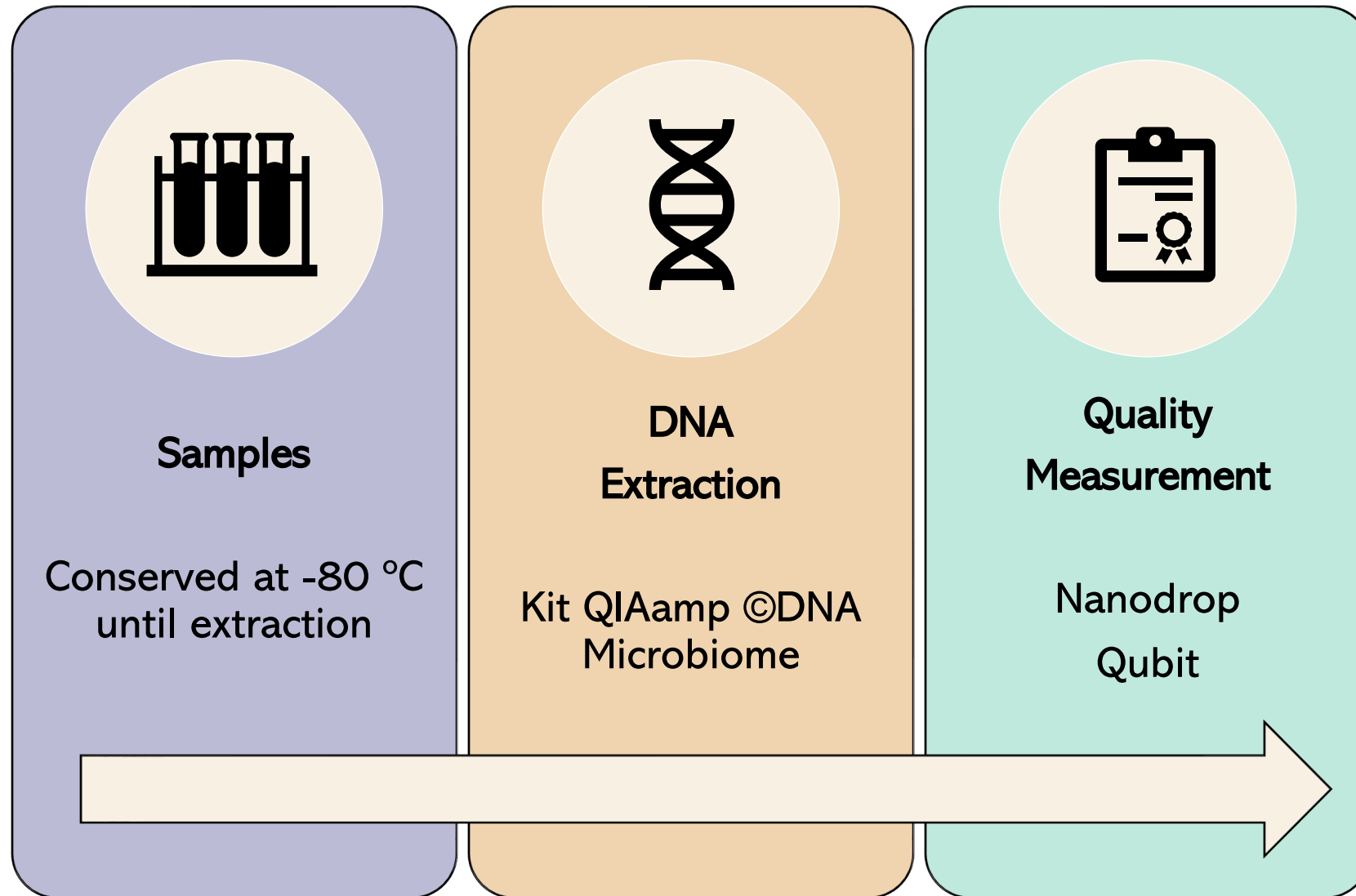
Samples

Conserved at -80°C
until extraction

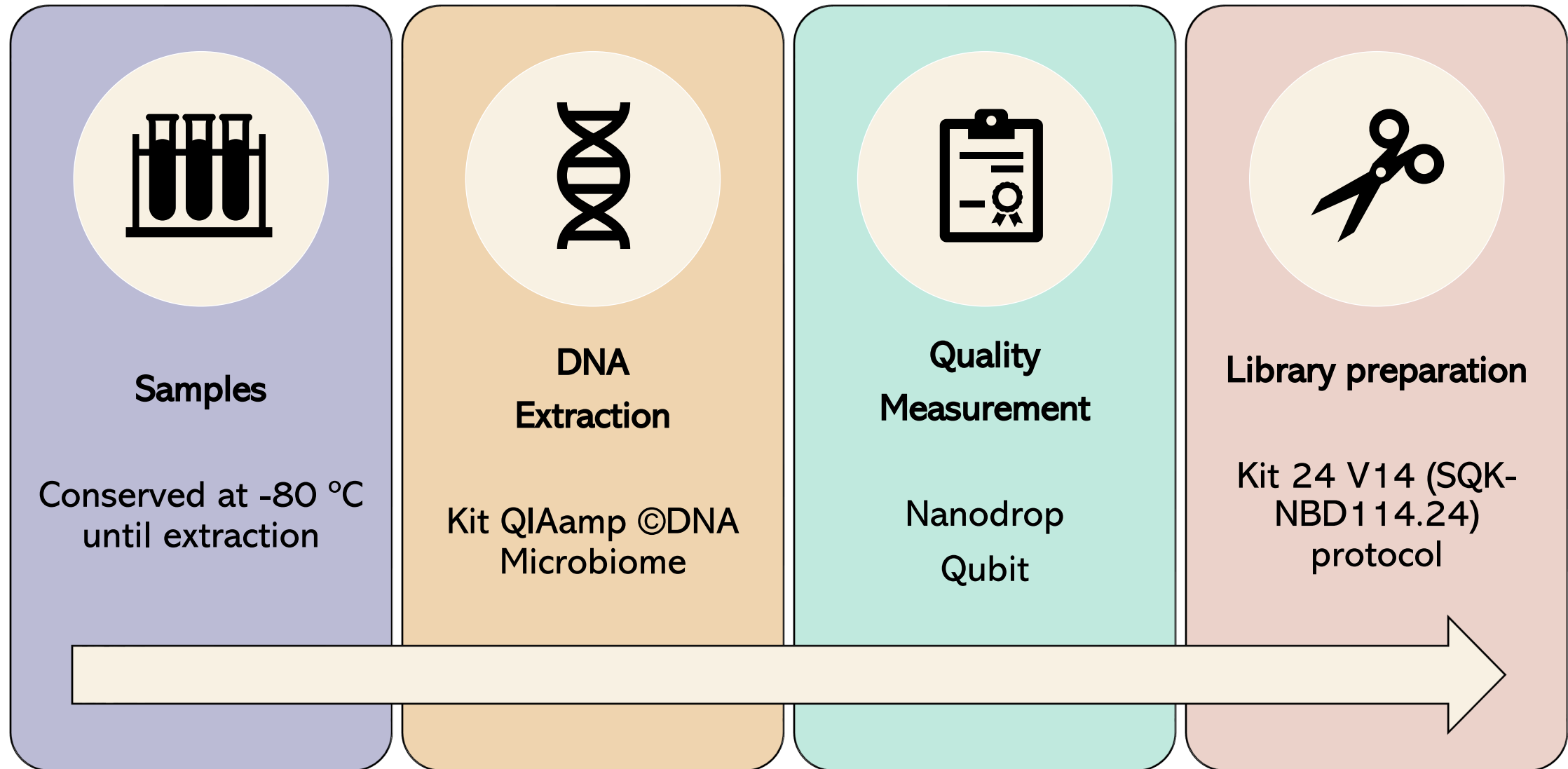
DNA Extraction



DNA Extraction



DNA Extraction



Sequencing

Sequencing



Nanopore technique

- GridION by Oxford Nanopore Technologies

Sequencing



Nanopore technique

- GridION by Oxford Nanopore Technologies



Whole-genome sequencing

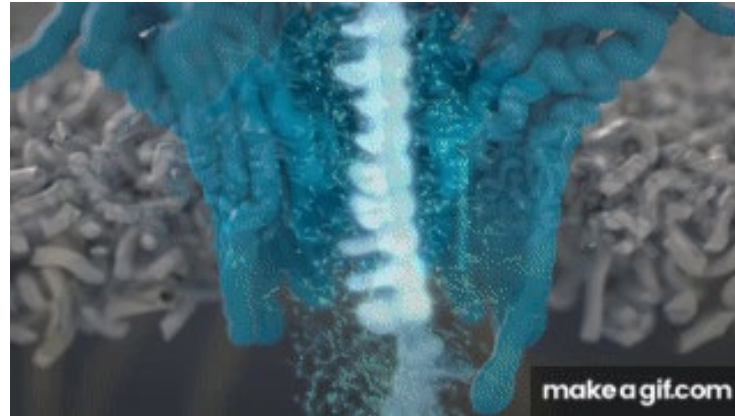
- Adaptive sampling to reject host reads

Sequencing



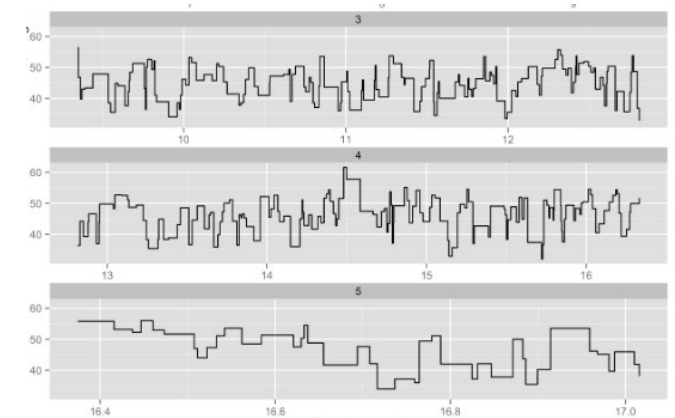
Nanopore technique

- GridION by Oxford Nanopore Technologies



Whole-genome sequencing

- Adaptive sampling to reject host reads



Fast5 files

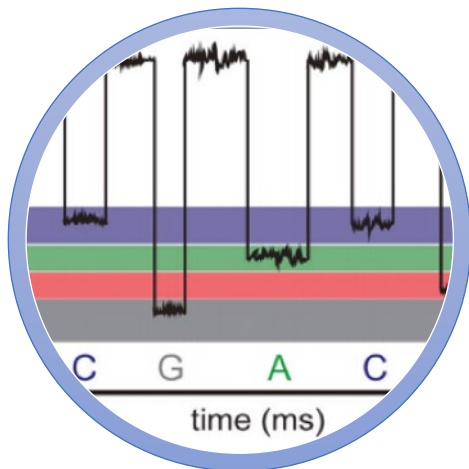
- Storing electrical signals

Bioinformatics

Basecalling

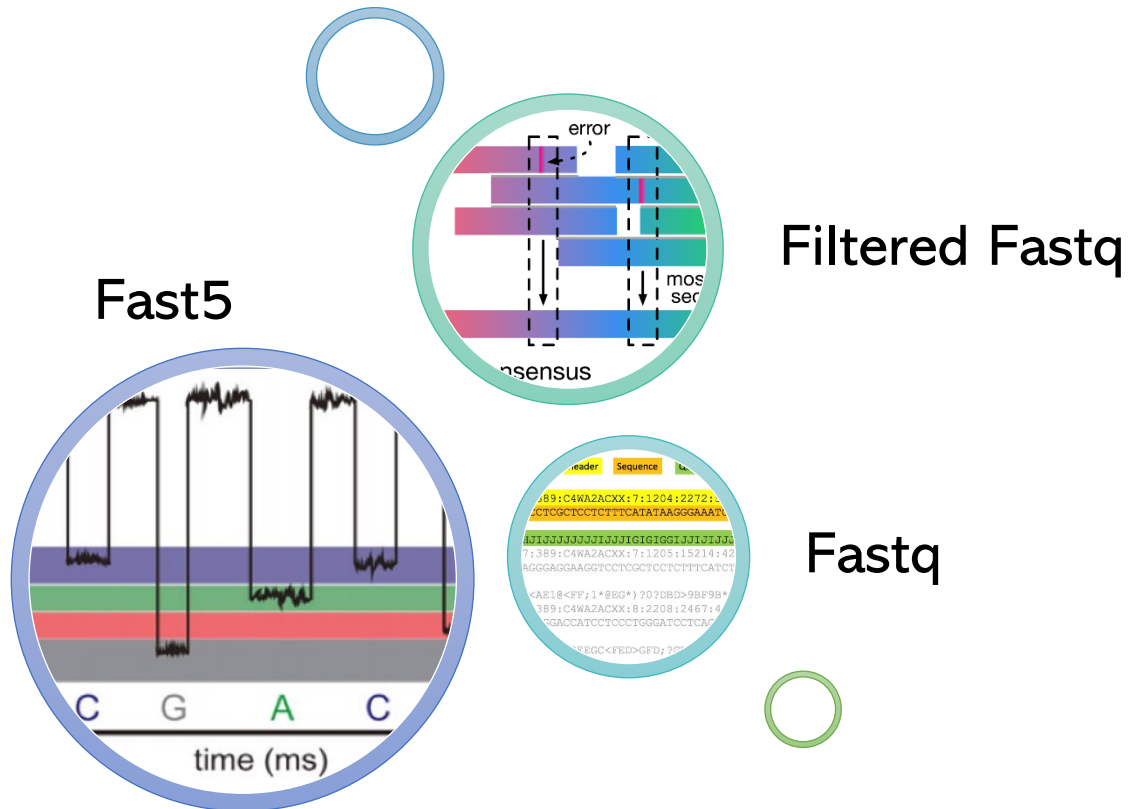
- SUP accuracy (Seq. quality > 10) Guppy software

Fast5



Fastq





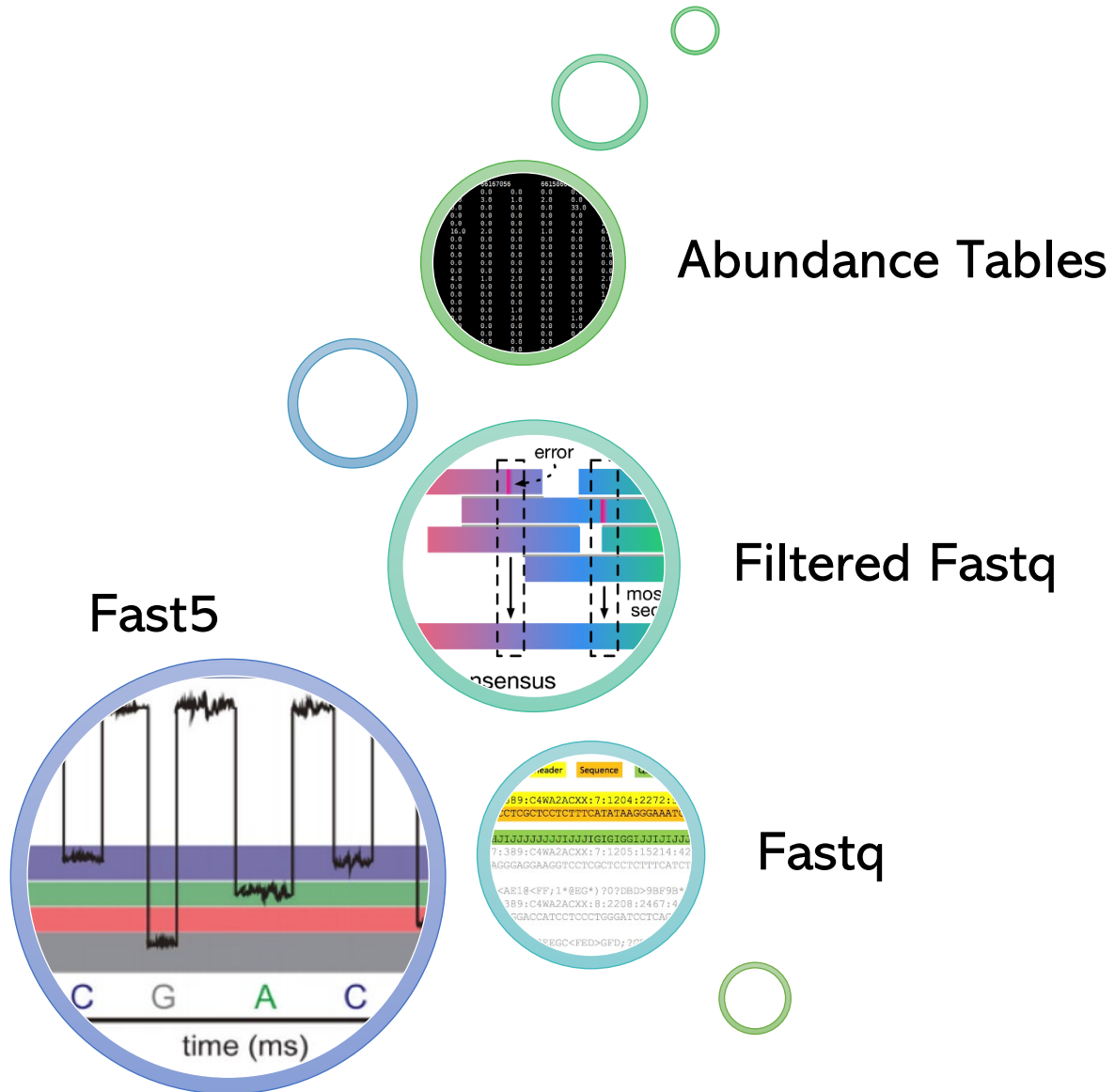
Basecalling

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Filtering

- Remove host seq. by Minimap2 software
- Remove short (< 200 bp) seq. by Cutadapt software

Bioinformatics



Basecalling

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Mapping with SqueezeMeta pipeline

- At phylum and genus Level
- COG: Clusters of Orthologous Genes
- KEGG: Kyoto Encyclopedia of Genes and Genomes

Statistical analysis

Statistical analysis

Abundance
tables

Genus, Phylum, COG, KO

Statistical analysis

Abundance
tables

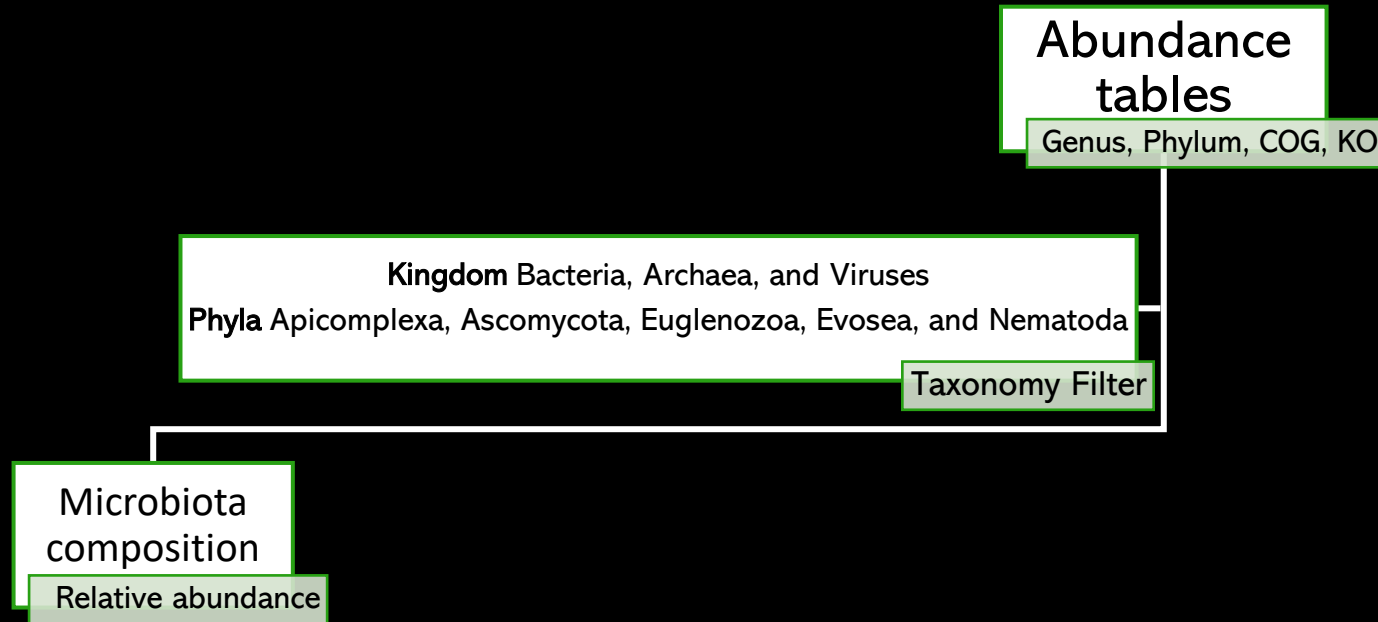
Genus, Phylum, COG, KO

Kingdom Bacteria, Archaea, and Viruses

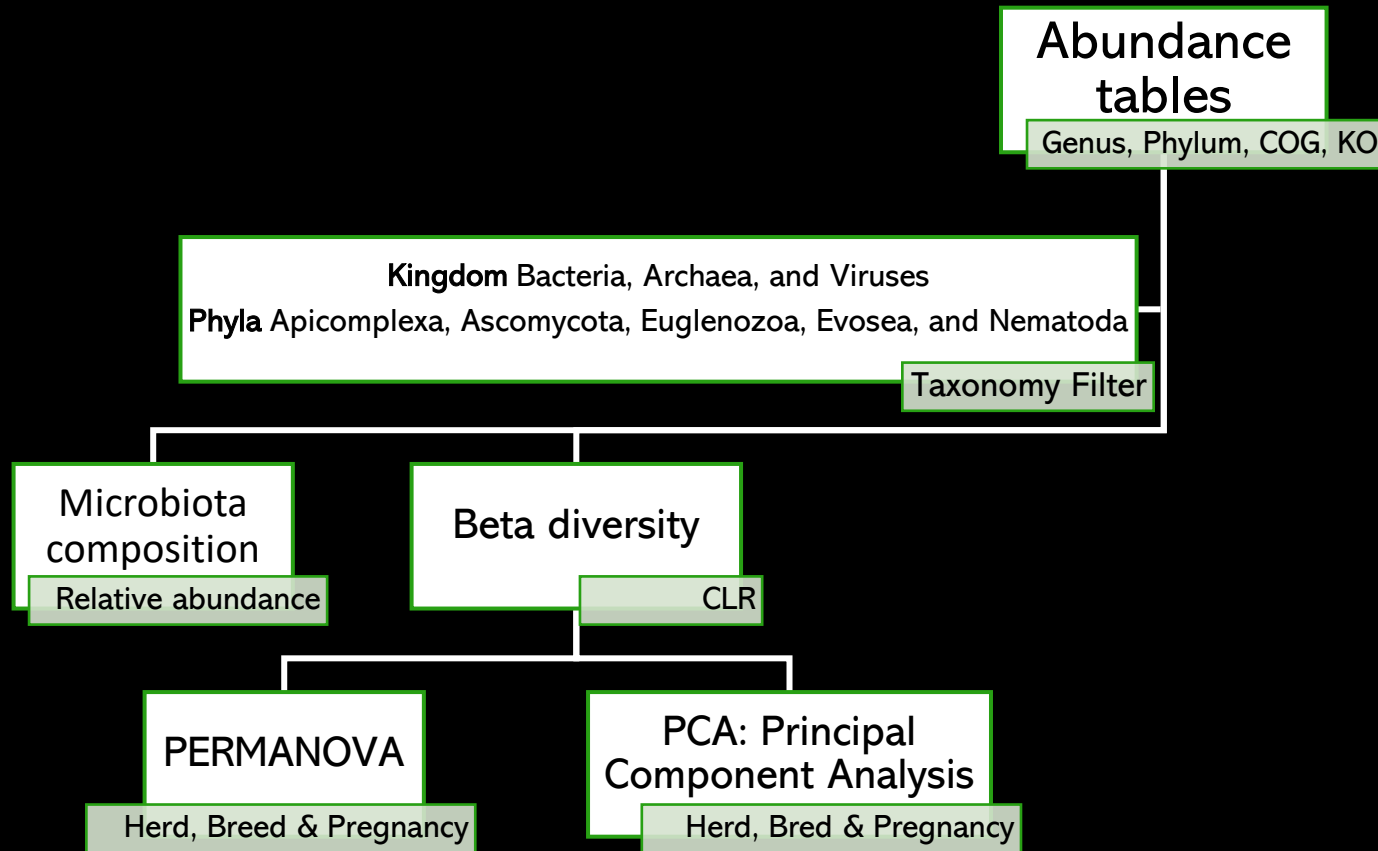
Phyla Apicomplexa, Ascomycota, Euglenozoa, Evosea, and Nematoda

Taxonomy Filter

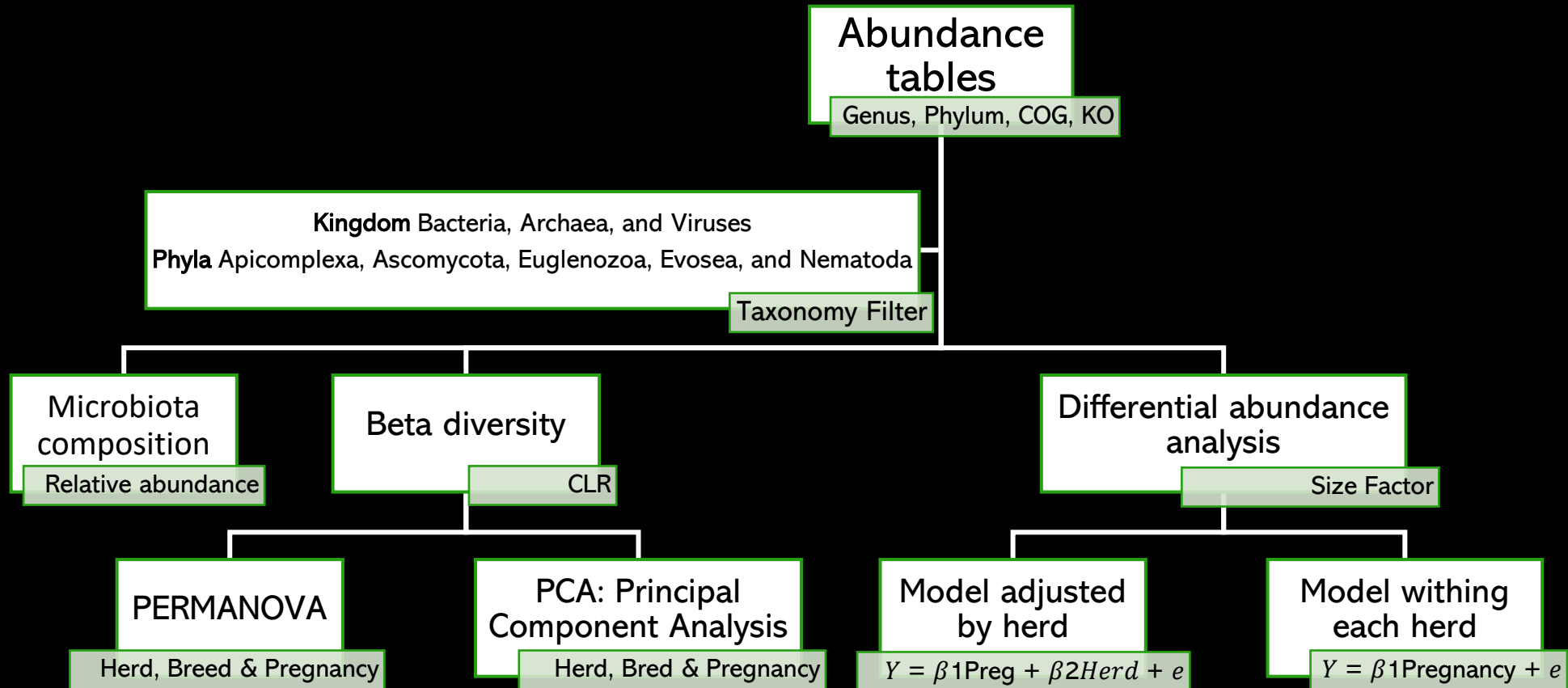
Statistical analysis



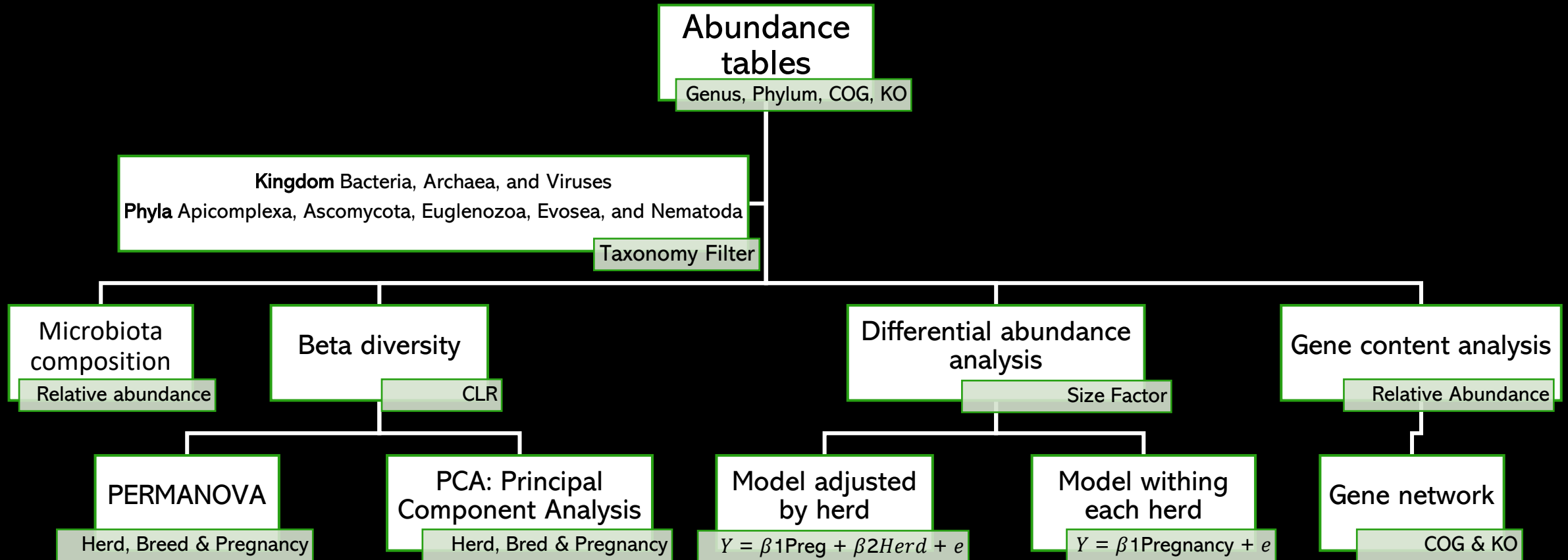
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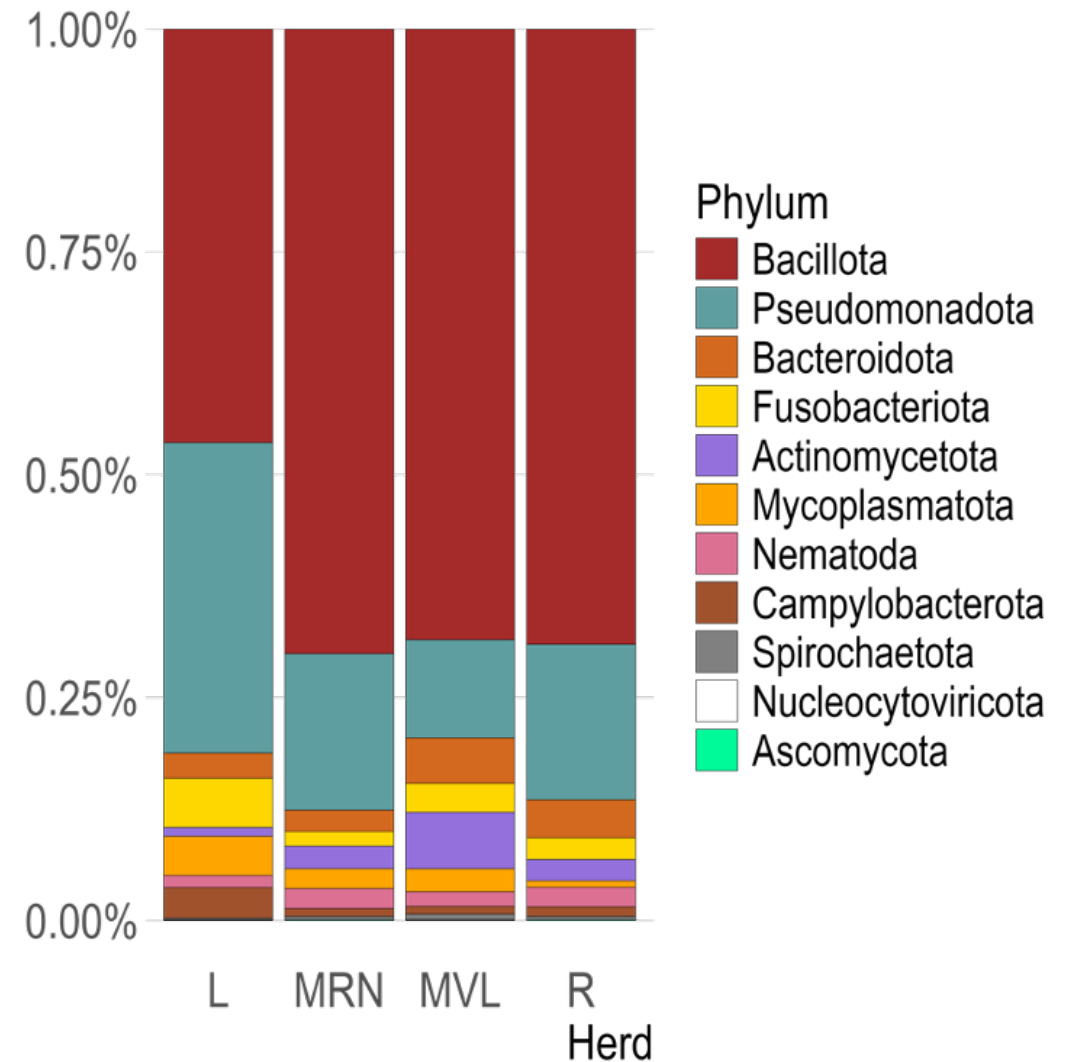
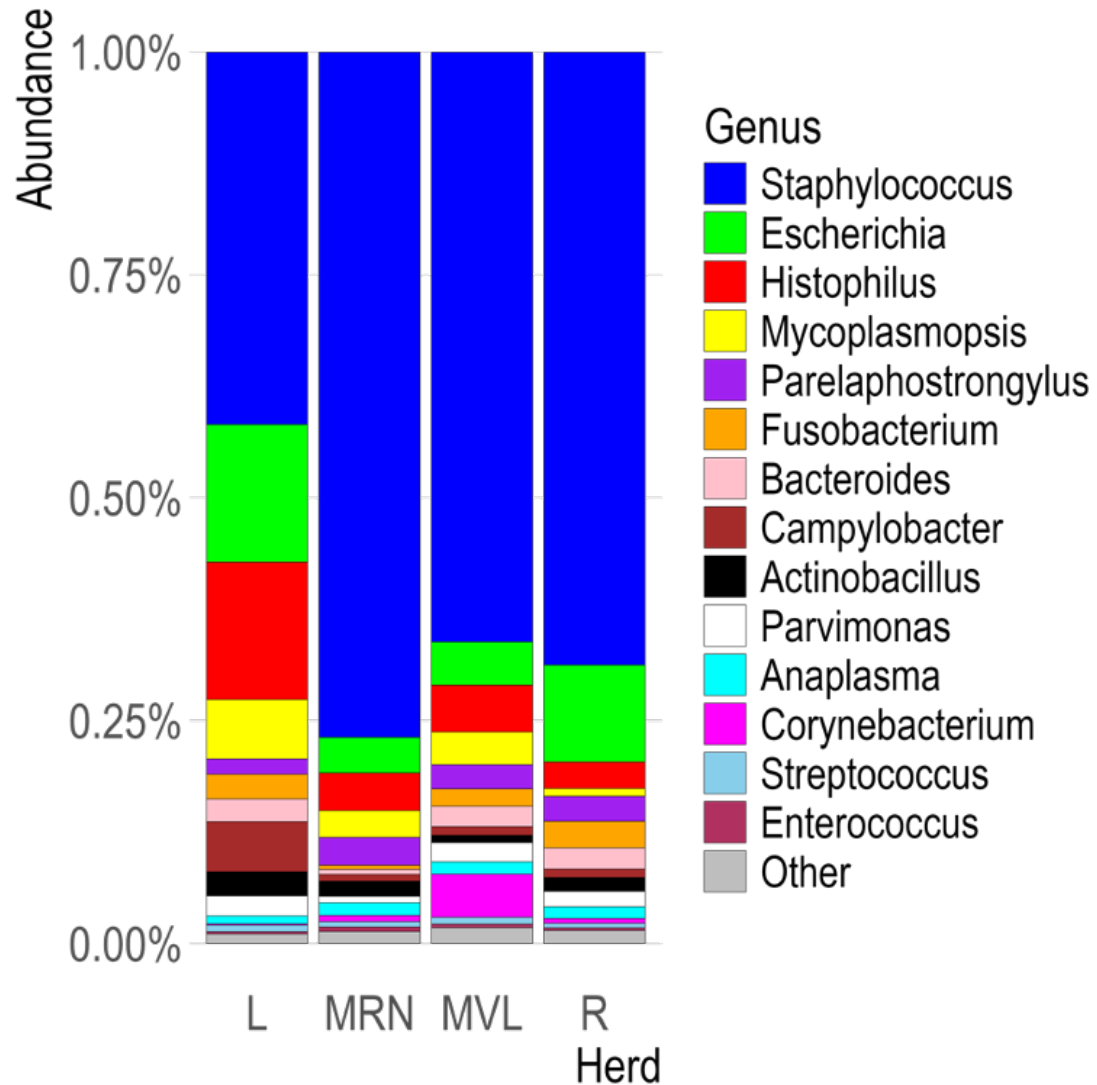


Statistical analysis

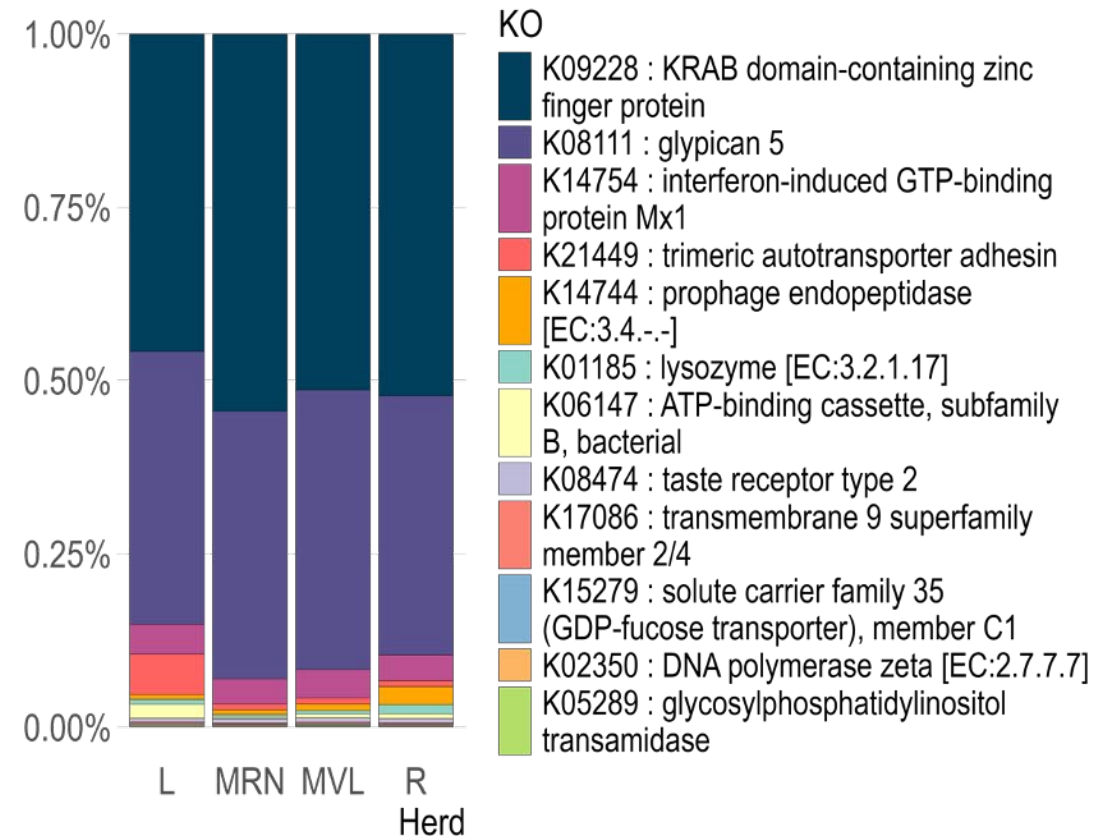
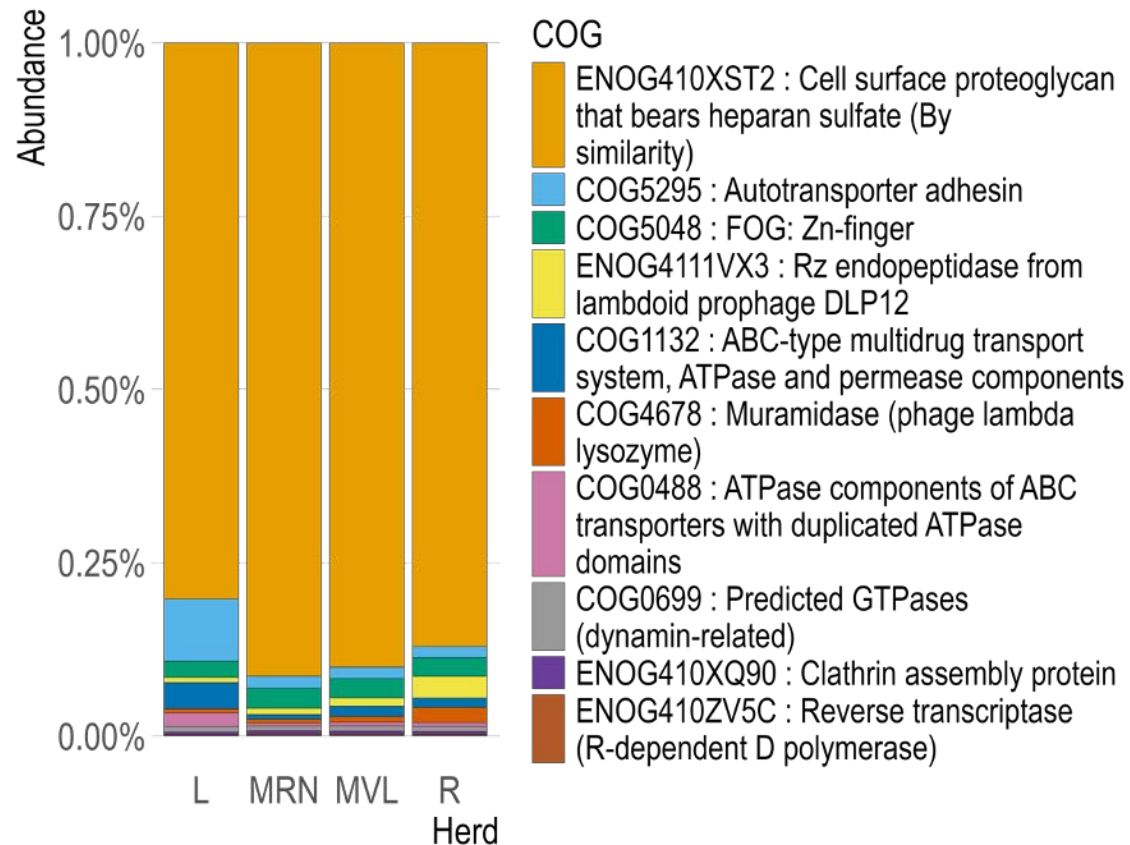


Results

Results: Microbiota composition

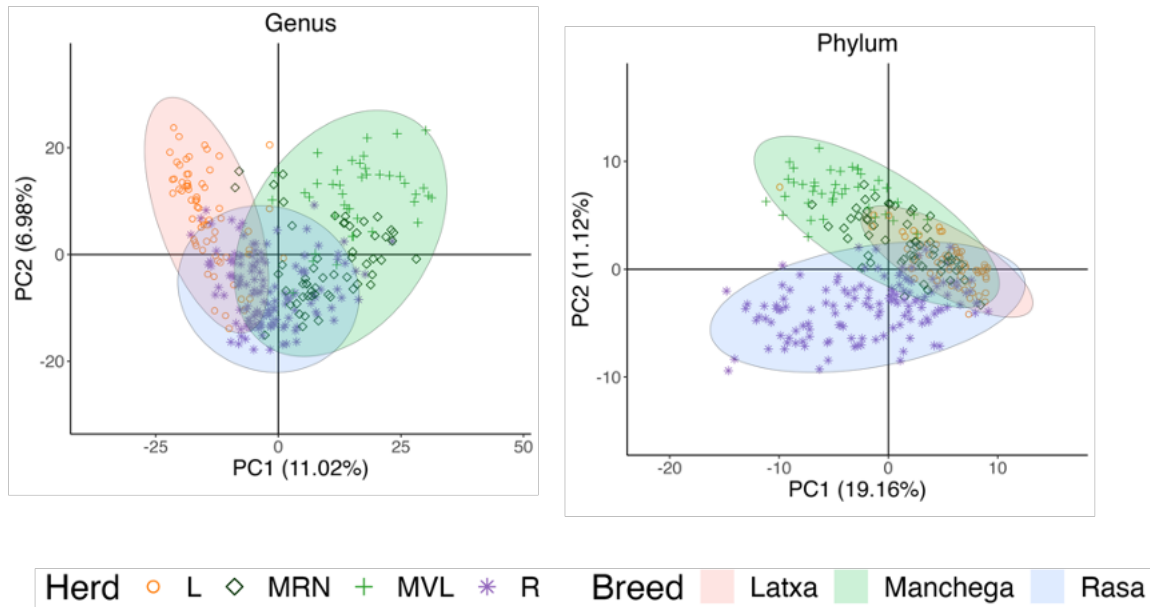


Results: Gene composition



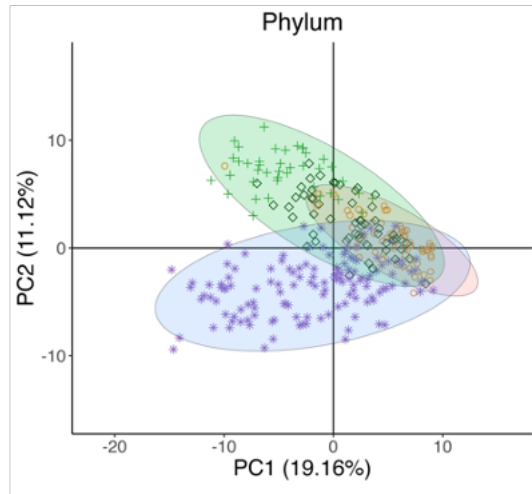
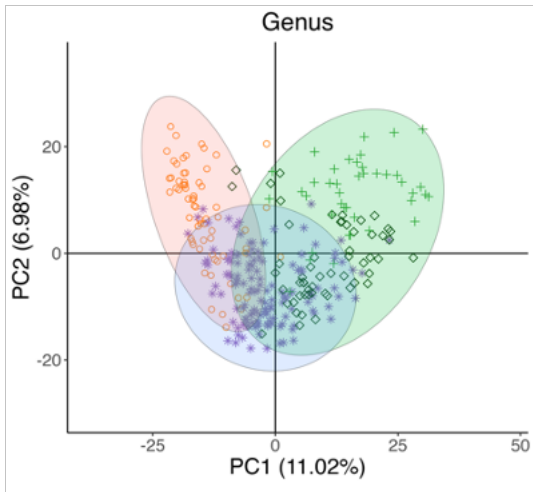
The COGs focus on cell surface interactions, multidrug transport, and enzymatic activity. The KOs are mainly involved in immune response, membrane transport, and cellular adhesion

Results: Beta diversity



Differences in the microbiota composition were mainly detected at breed and herd level

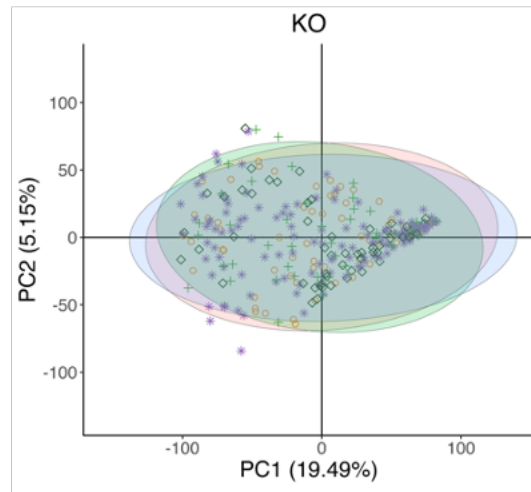
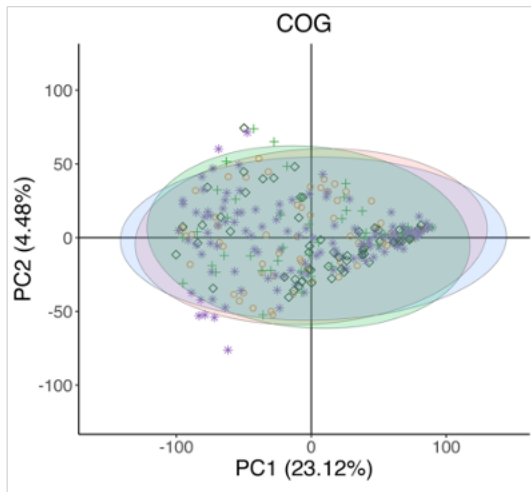
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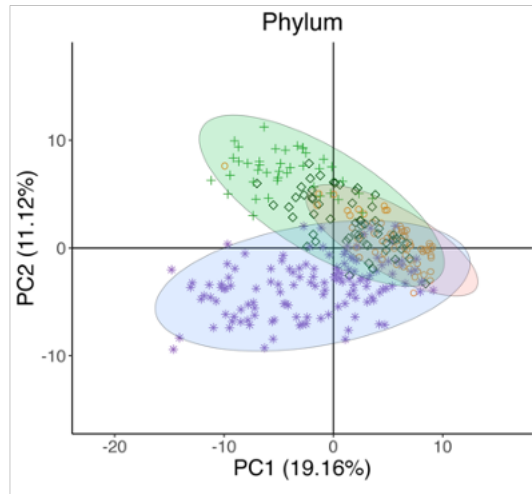
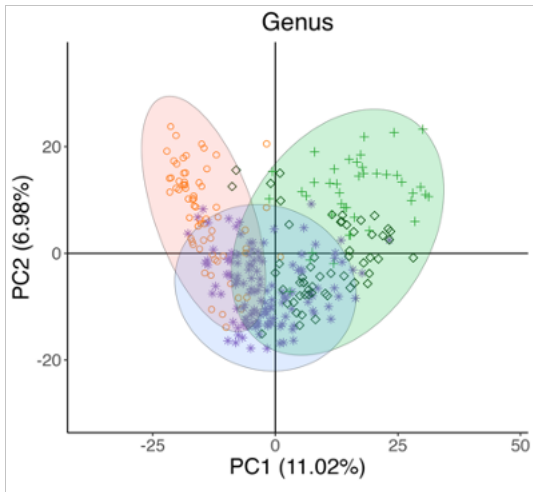
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No differential patterns were observed in the genes (COG and KO)

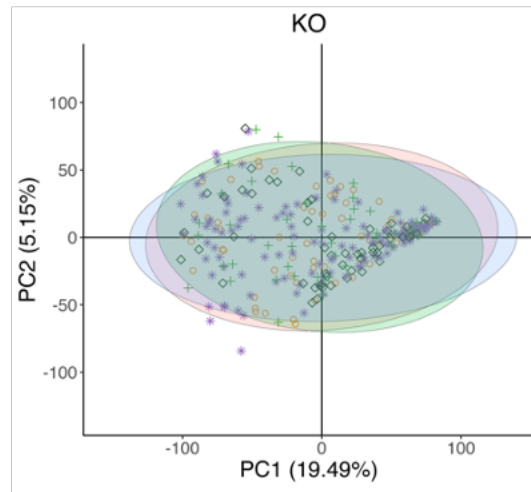
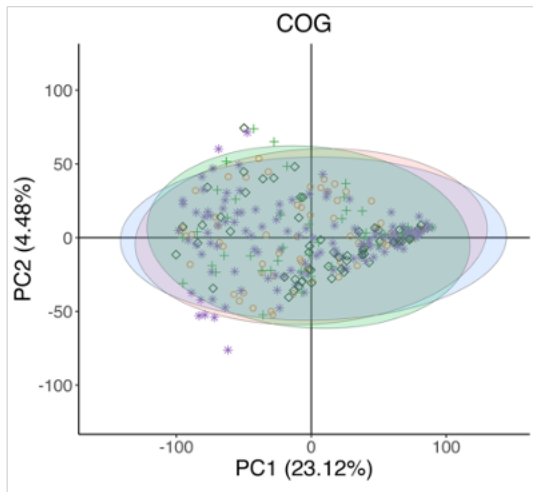
Herd ○ L ◇ MRN + MVL * R Breed Latxa Manchega Rasa



Results: Beta diversity



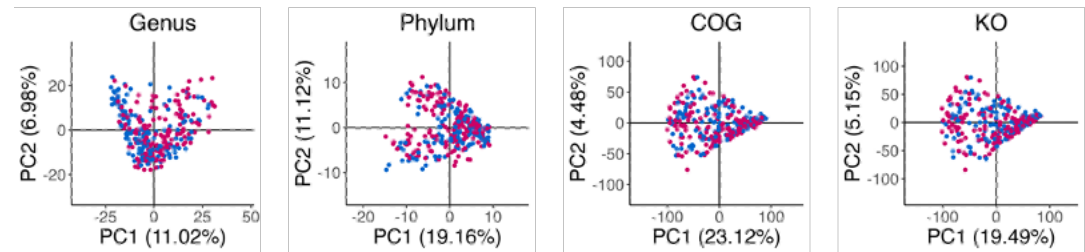
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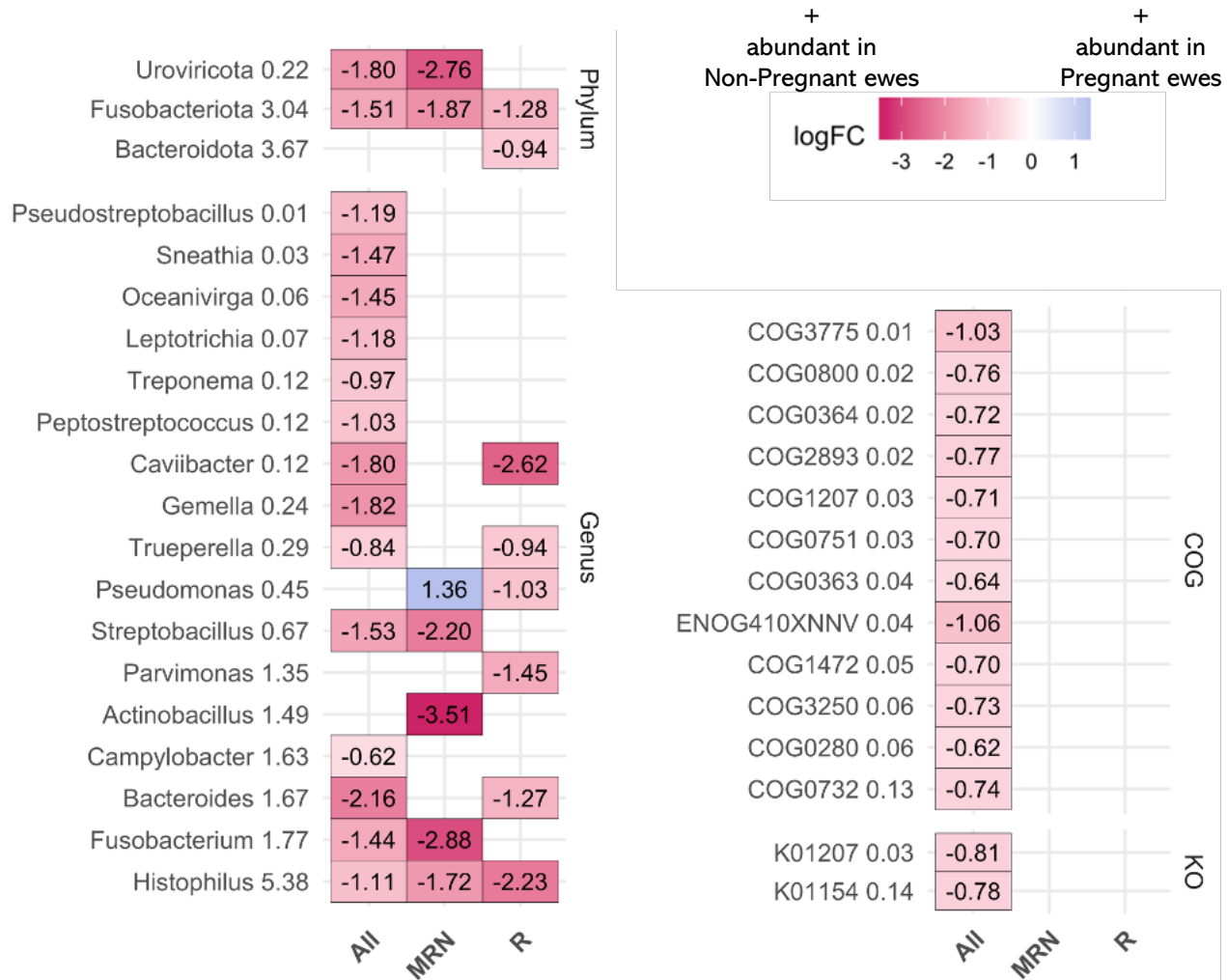
No differential patterns were observed in the genes (COG and KO)

No differential patterns were observed in the PCA for pregnancy, although significant differences were found in the PERMANOVA (P value = 0.001)



Pregnancy • Negative • Positive

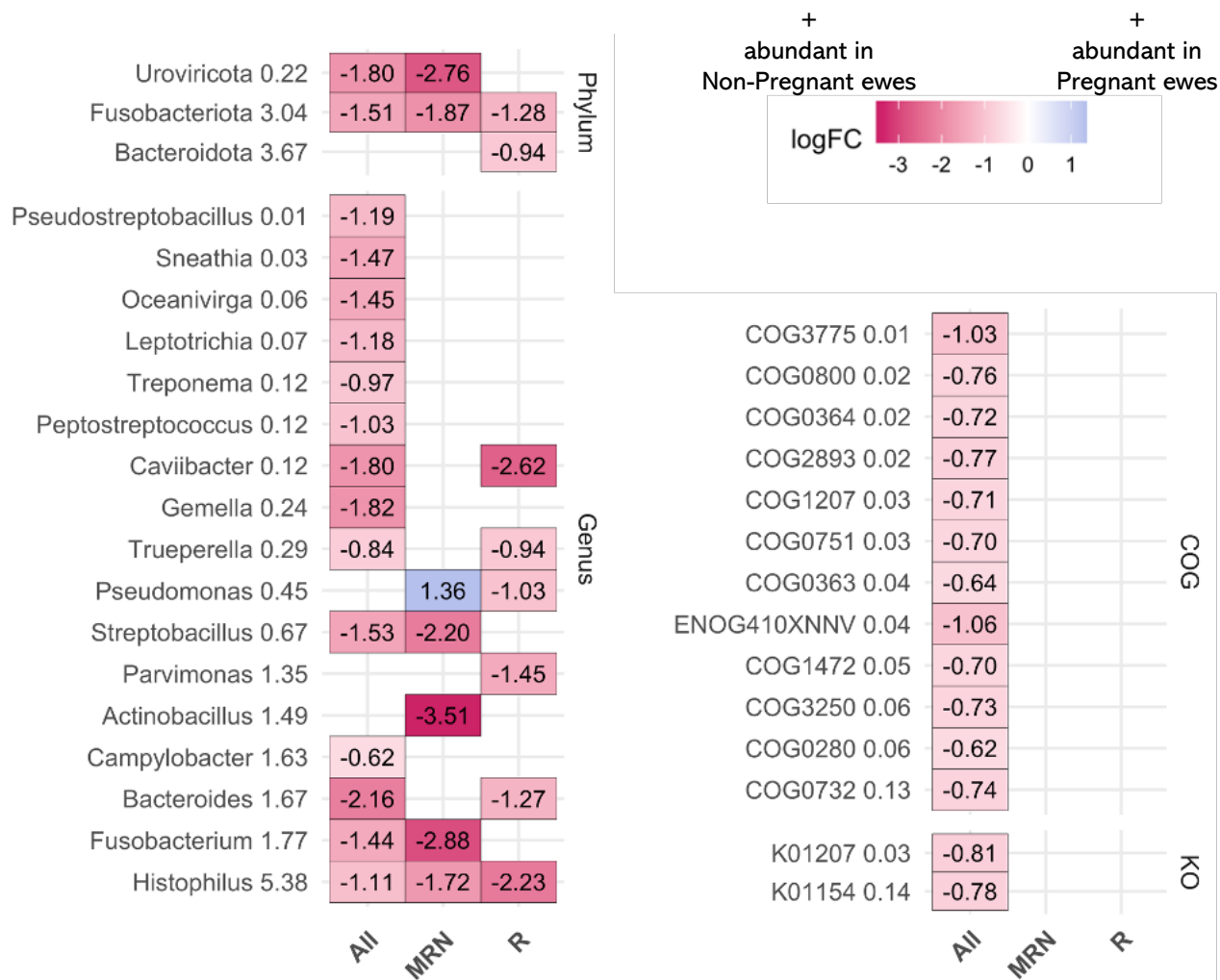
Results: Differential analysis



Adjusted
P-value > 0.05

3 Phyla, 17 Genera, 12 COG,
2 KO

Results: Differential analysis

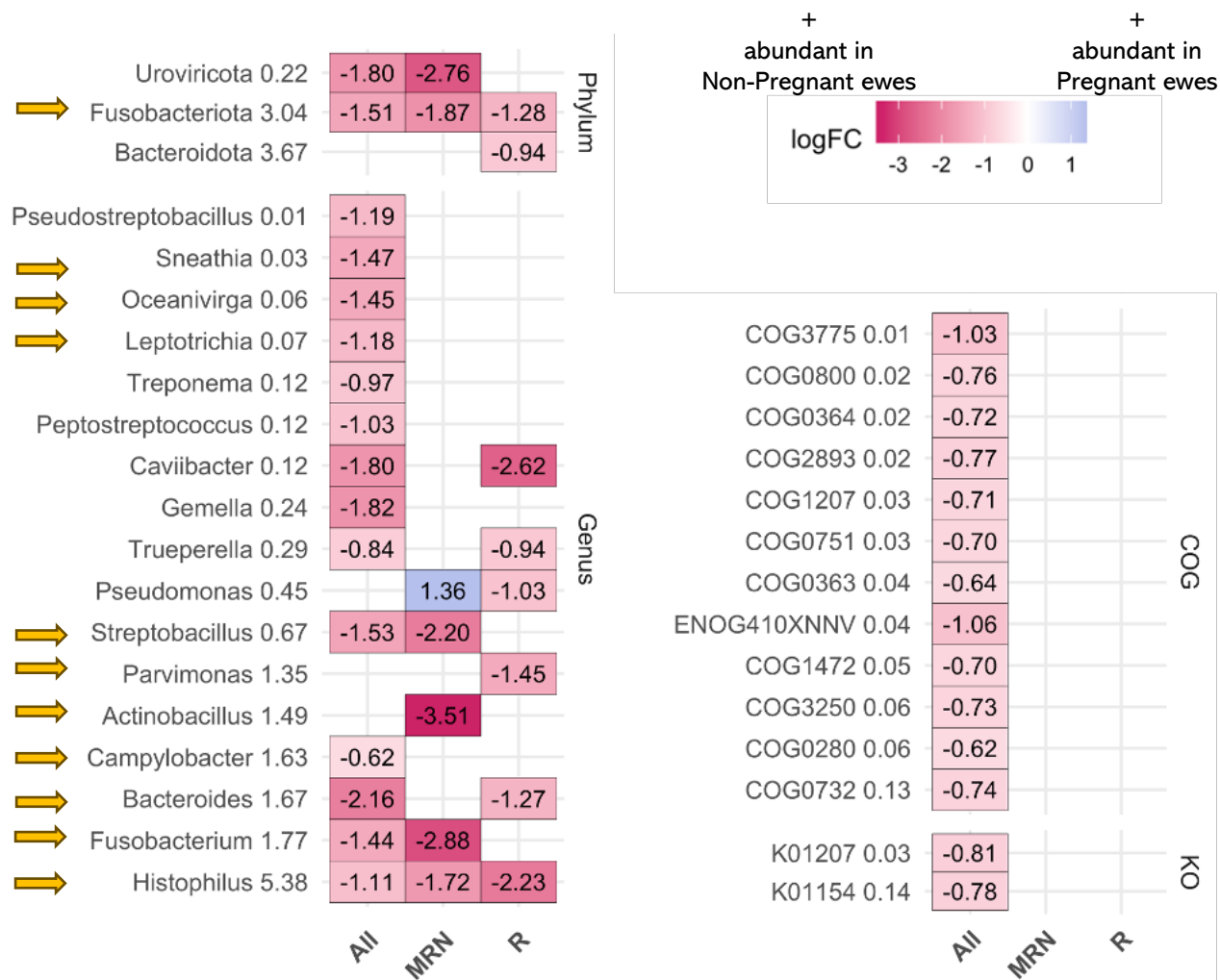


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Most of significantly
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ewes

Results: Differential analysis



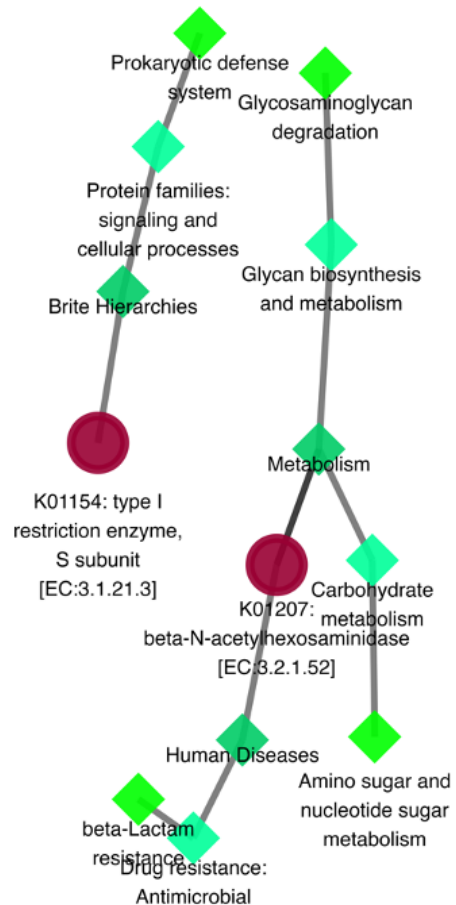
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Some taxa have also been
reported in other studies
negatively associated with
fertility (Serrano et al. 2020,
Koester et al. 2021, and
Reinoso et al. 2023, 2024)

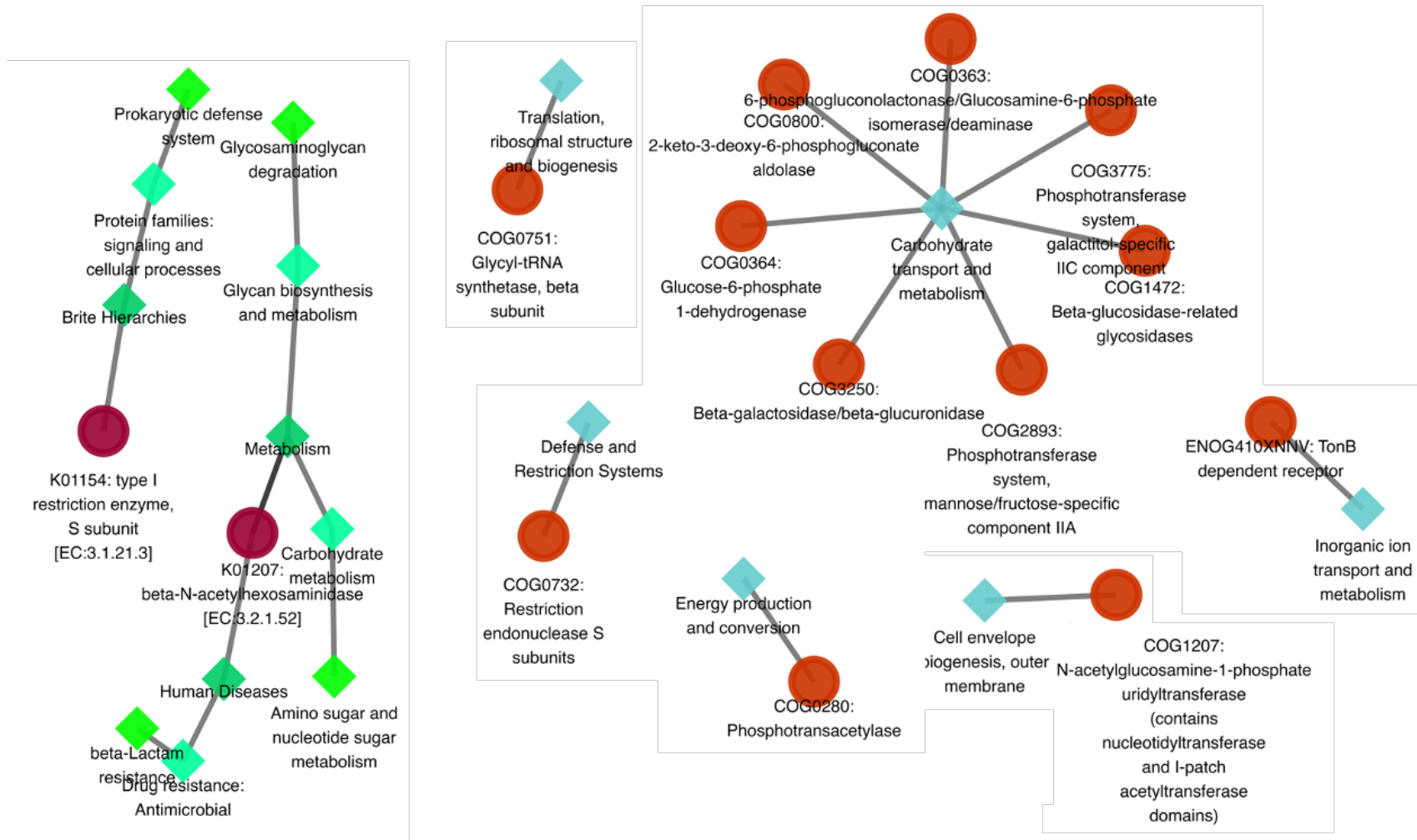
Results: Genes pathway network



The KOs associated with pregnancy are mainly related to:

- ➡ Signaling and cellular processes,
- ➡ glycan synthesis and metabolism,
- ➡ and drug resistance: antimicrobial.

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- ➔ glycan synthesis and metabolism,
- ➔ and drug resistance: antimicrobial.

COGs are primarily related to:

- ➔ Carbohydrate transport,
- ➔ Ribosomal structure and biogenesis,
- ➔ Defence and restriction systems,
- ➔ Energy production,
- ➔ cell envelope,
- ➔ and inorganic ion transport

Conclusions

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*The core microbiota of vaginal tract was primarily composed of genera *Staphylococcus*, *Escherichia* and *Histophilus**

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*Key genera associated to non-pregnant ewes included *Sneathia*, *Oceanivirga*, *Leptotrichia*, *Streptobacillus*, *Parvimonas*, *Actinobacillus*, *Campylobacter*, *Bacteroides*, *Fusobacterium*, and *Histophilus*. While *Fusobacteriota* and *Bacteroidota* was notable at the phylum level.*

Conclusions



The core microbiota of vaginal tract was primarily composed of genera Staphylococcus, Escherichia and Histophilus

There was a high effect of breed (and herd) in the microbiome composition

Key genera associated to non-pregnant ewes included Sneathia, Oceanivirga, Leptotrichia, Streptobacillus, Parvimonas, Actinobacillus, Campylobacter, Bacteroides, Fusobacterium, and Histophilus. While Fusobacteriota and Bacteroidota was notable at the phylum level.

Genes associated to pregnancy failure were linked primarily to carbohydrate metabolism, defence mechanisms, transport and structural resilience.

Acknowledgements



ONT bursary



75th EAAP Scholarship



PROYECTO RTI2018-096487



Grazie mille



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