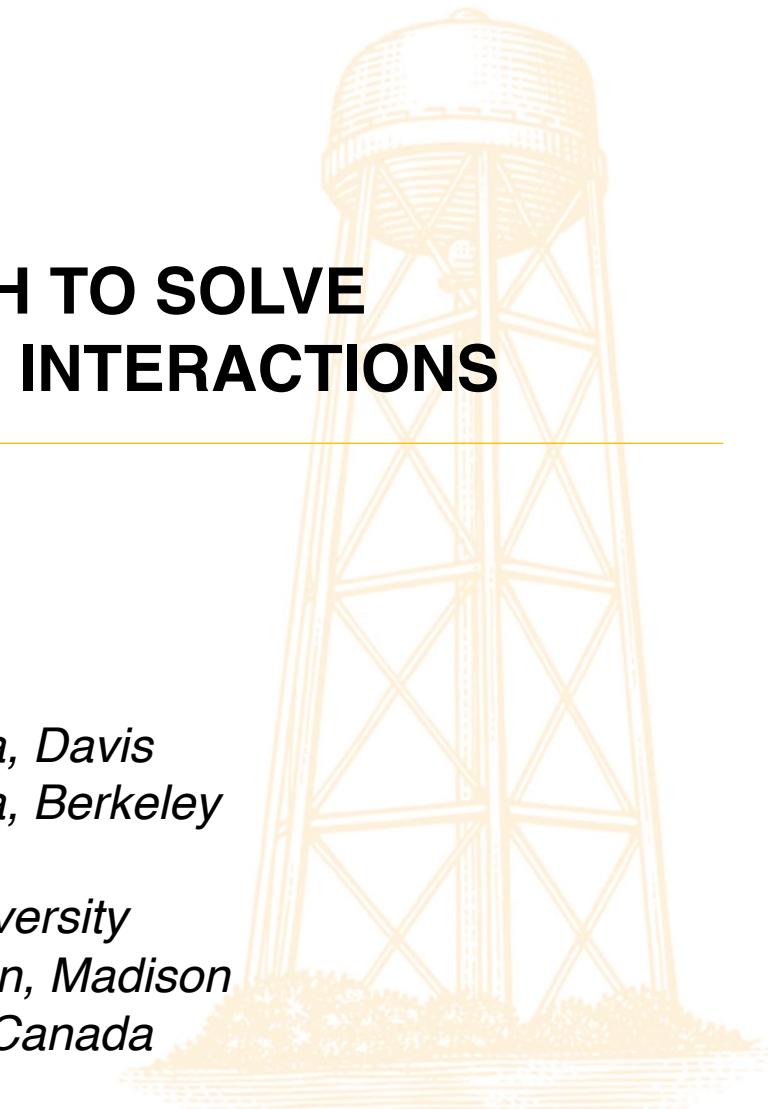


# A HOLISTIC MULTI-OMICS APPROACH TO SOLVE THE MECHANISM OF HOST-MICROBIOME INTERACTIONS

Hugo F. Monteiro, Ph.D.  
Postdoc, UC Davis



*University of California, Davis*  
*University of California, Berkeley*  
*University of Florida*  
*Washington State University*  
*University of Wisconsin, Madison*  
*University of Guelph, Canada*



# How to scale your microbiome analysis?

## BIOINFORMATICS APPLICATION NOTE

Vol. 28 no. 19 2012, pages 2520–2522  
doi:10.1093/bioinformatics/bts480

*Genome analysis*

Advance Access publication August 20, 2012

### **Snakemake—a scalable bioinformatics workflow engine**

Johannes Köster<sup>1,2,\*</sup> and Sven Rahmann<sup>1</sup>

<sup>1</sup>Genome Informatics, Institute of Human Genetics, University of Duisburg-Essen and <sup>2</sup>Paediatric Oncology, University Childrens Hospital, 45147 Essen, Germany

Associate Editor: Alfonso Valencia

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#### **ABSTRACT**

**Summary:** Snakemake is a workflow engine that provides a readable Python-based workflow definition language and a powerful execution environment that scales from single-core workstations to compute clusters without modifying the workflow. It is the first system to support the use of automatically inferred multiple named wildcards (or variables) in input and output filenames.

**Availability:** <http://snakemake.googlecode.com>.

**Contact:** [johannes.koester@uni-due.de](mailto:johannes.koester@uni-due.de)

Received on May 14, 2012; revised on June 28, 2012; accepted on July 28, 2012

custom server processes running on the cluster nodes. Finally, Snakemake is the first system to support file name inference with multiple named wildcards in rules.

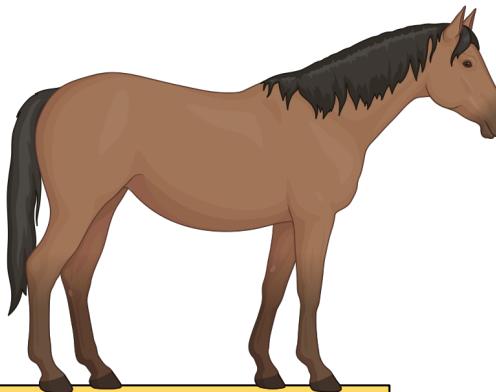
#### **2 SNAKEMAKE LANGUAGE**

A workflow is defined in a ‘Snakefile’ through a domain-specific language that is close to standard Python syntax. It consists of rules that denote how to create output files from input files. The workflow is implied by dependencies between the rules that arise from one rule needing an output file of another as an input file. A rule definition specifies (i) a name, (ii) any number of input

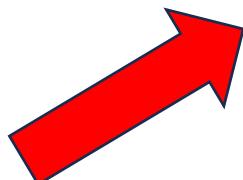
## SOME COMPLEX HOST-MICROBIOME INTERACTION STUDIES:



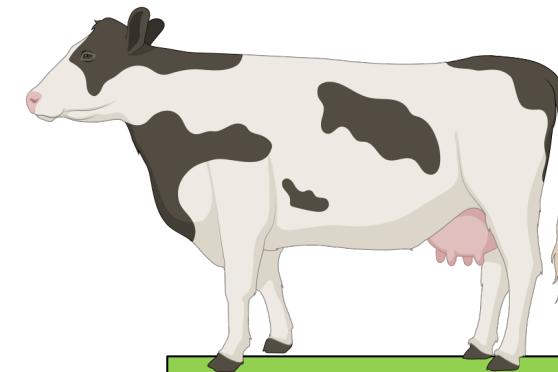
Pouya Dini  
UC Davis



**Host-Pathogen** Interactions  
in **LOW BIOMASS** tissue



**PLACENTA**

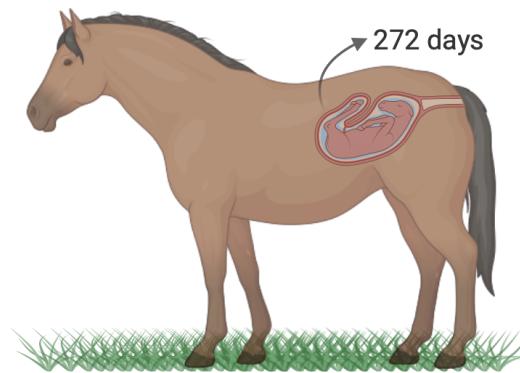


Fabio Lima  
UC Davis

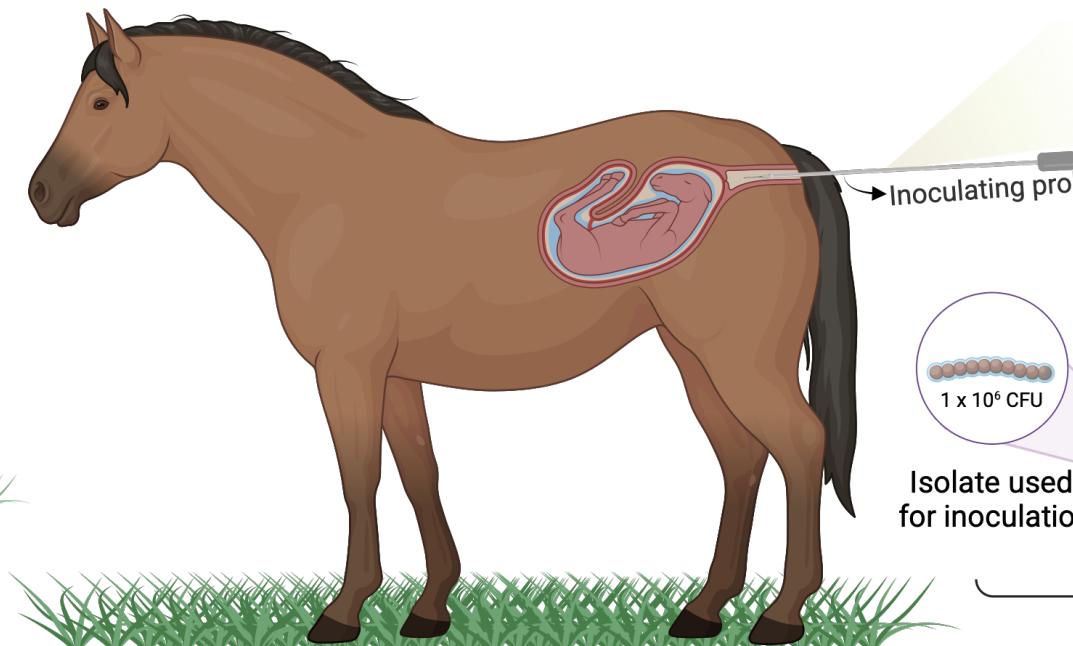
**Host-Microbiome** Interactions  
in **HIGH BIOMASS** sample

**RUMEN**

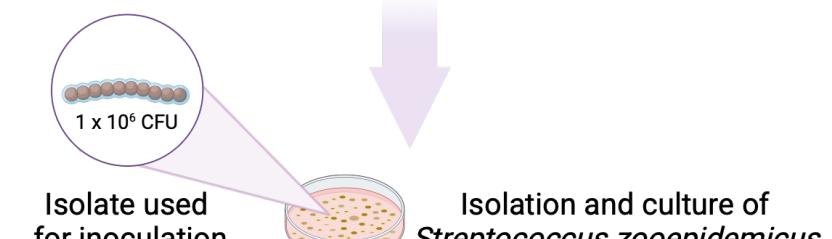
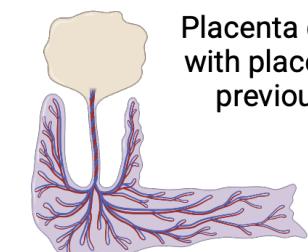
**Host-Pathogen Interactions  
In LOW BIOMASS tissue**

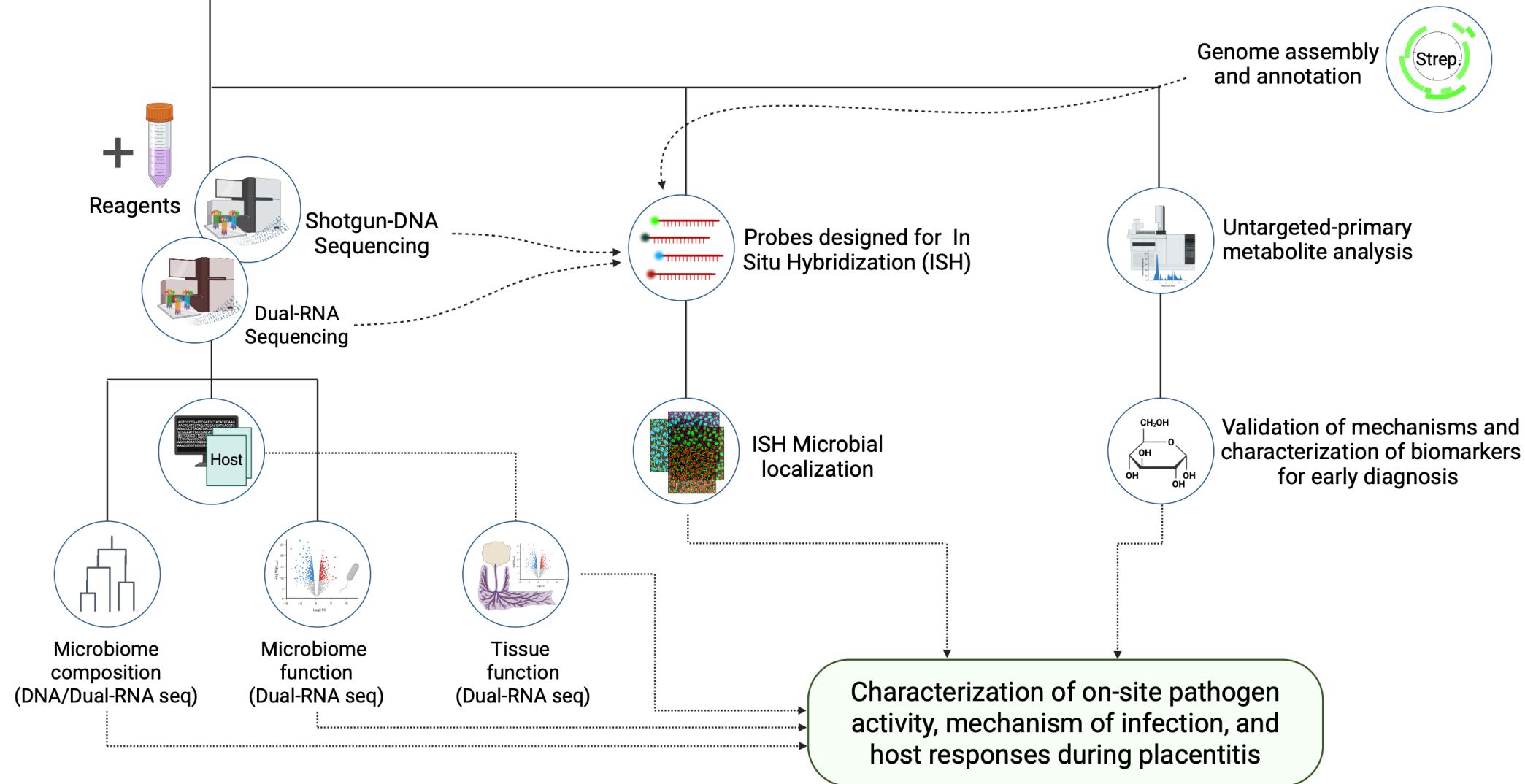


**NEGATIVE CONTROLS**  
 $n = 6$



**INDUCED PLACENTITIS**  
 $n = 6$



**LOW BIOMASS  
tissue SAMPLE**

# Some of the critical issues in low biomass tissues

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nature microbiology

Consensus Statement

<https://doi.org/10.1038/s41564-025-02035-2>

## Guidelines for preventing and reporting contamination in low-biomass microbiome studies

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Received: 9 December 2021

A list of authors and their affiliations appears at the end of the paper

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Accepted: 15 May 2025

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Published online: 20 June 2025

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 Check for updates

Numerous important environments harbour low levels of microbial biomass, including certain human tissues, the atmosphere, plant seeds, treated drinking water, hyper-arid soils and the deep subsurface, with some environments lacking resident microbes altogether. These low microbial biomass environments pose unique challenges for standard DNA-based sequencing approaches, as the inevitability of contamination from

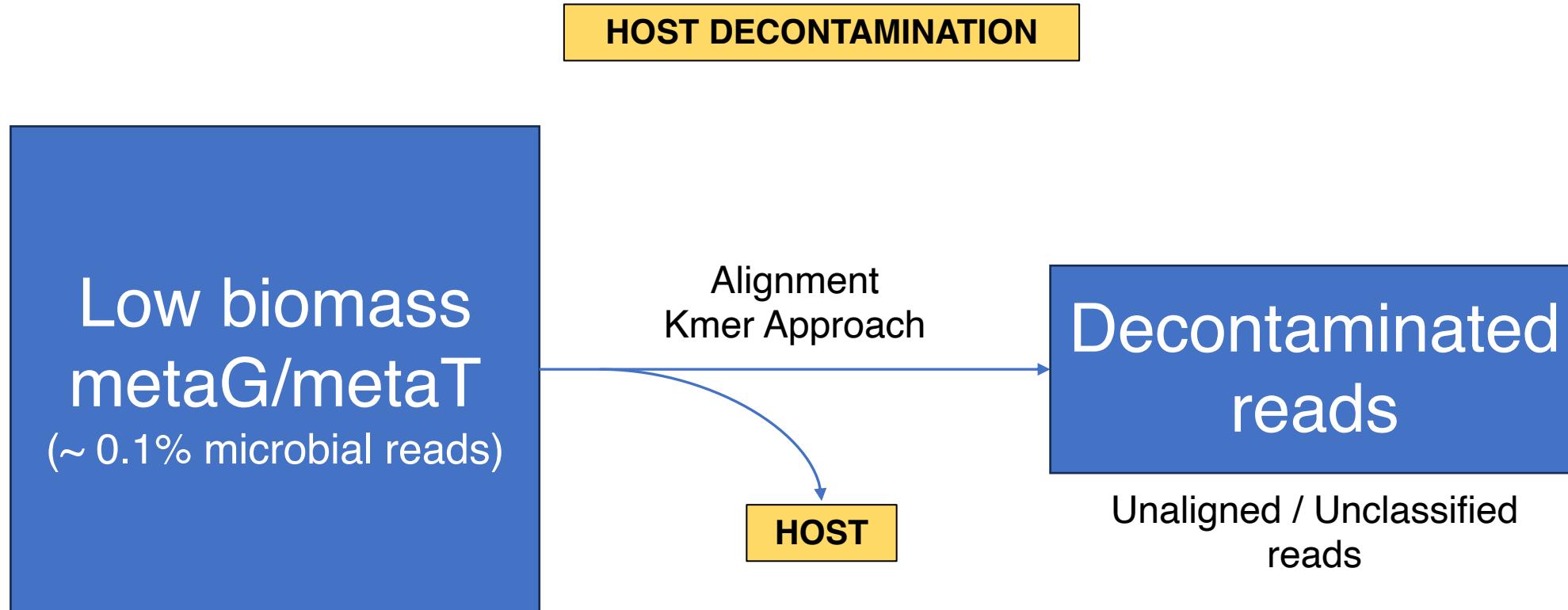
# Some of the critical issues in low biomass tissues

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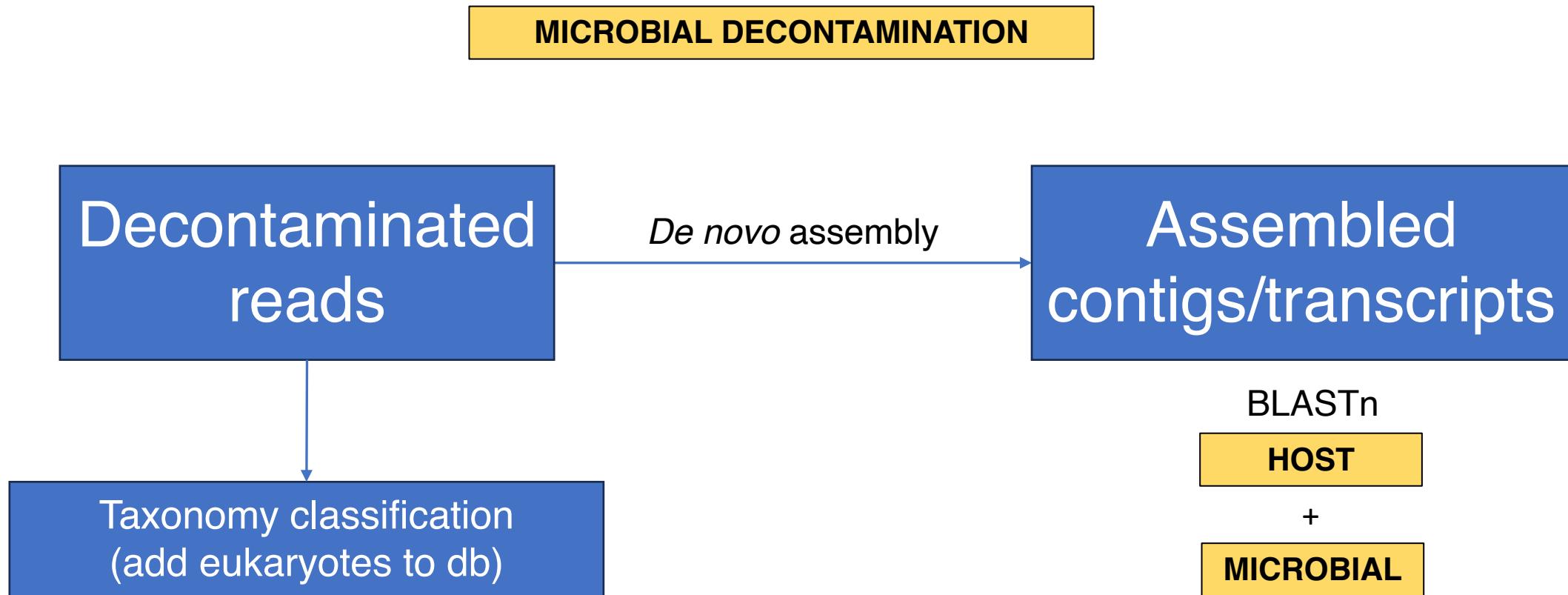
Host  
Contamination

Microbial  
Contamination

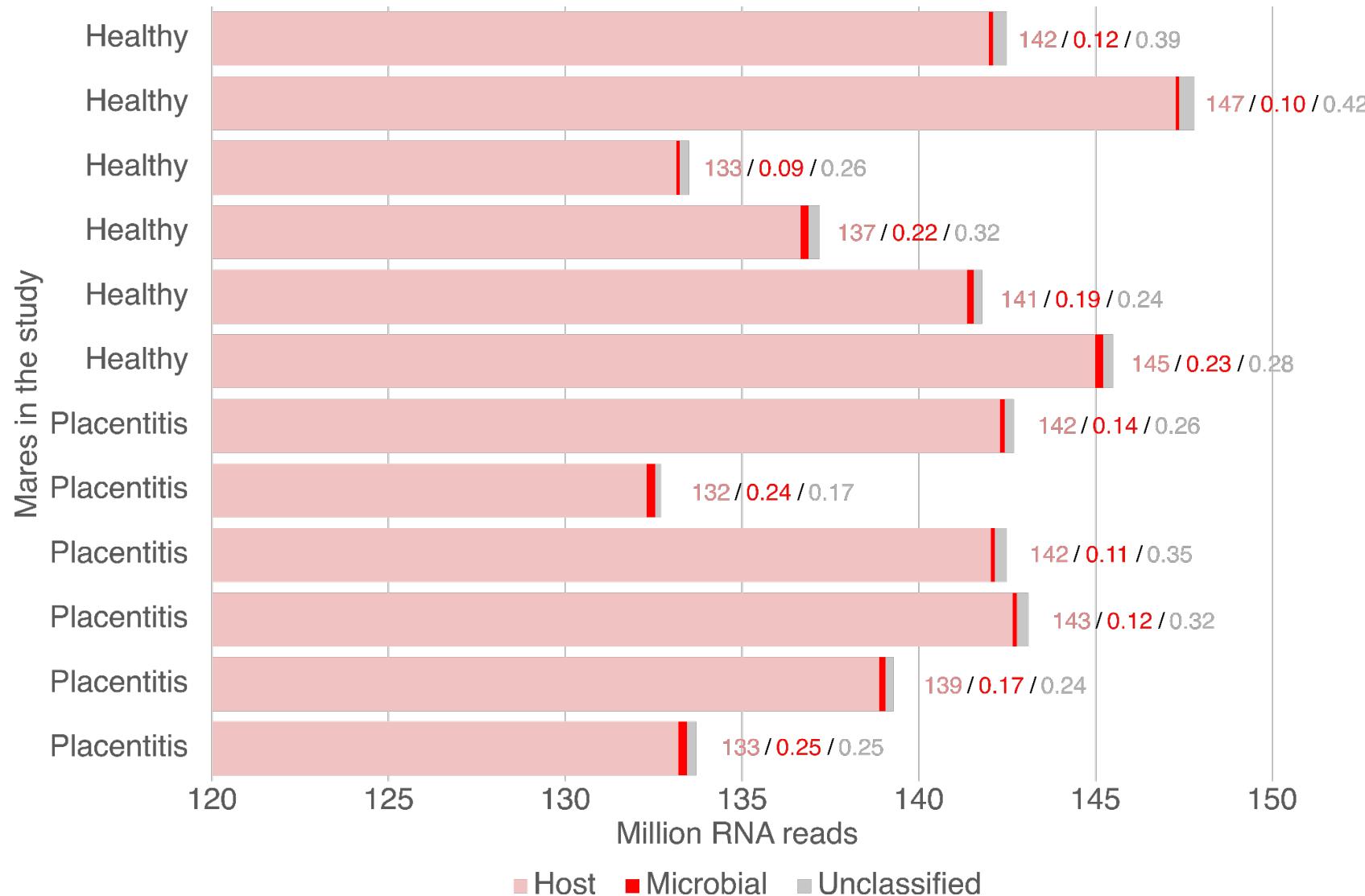
# Host contamination in low biomass tissues



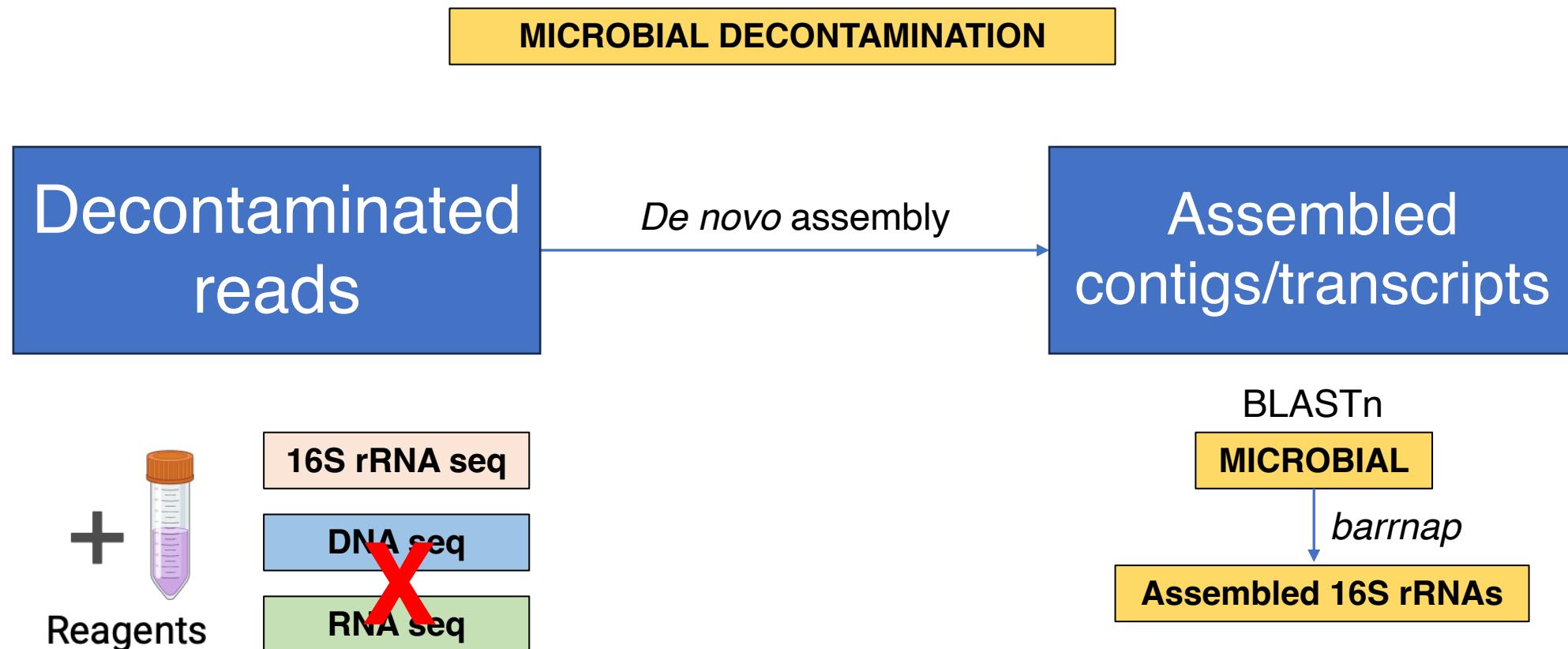
# Microbial contamination in low biomass tissues



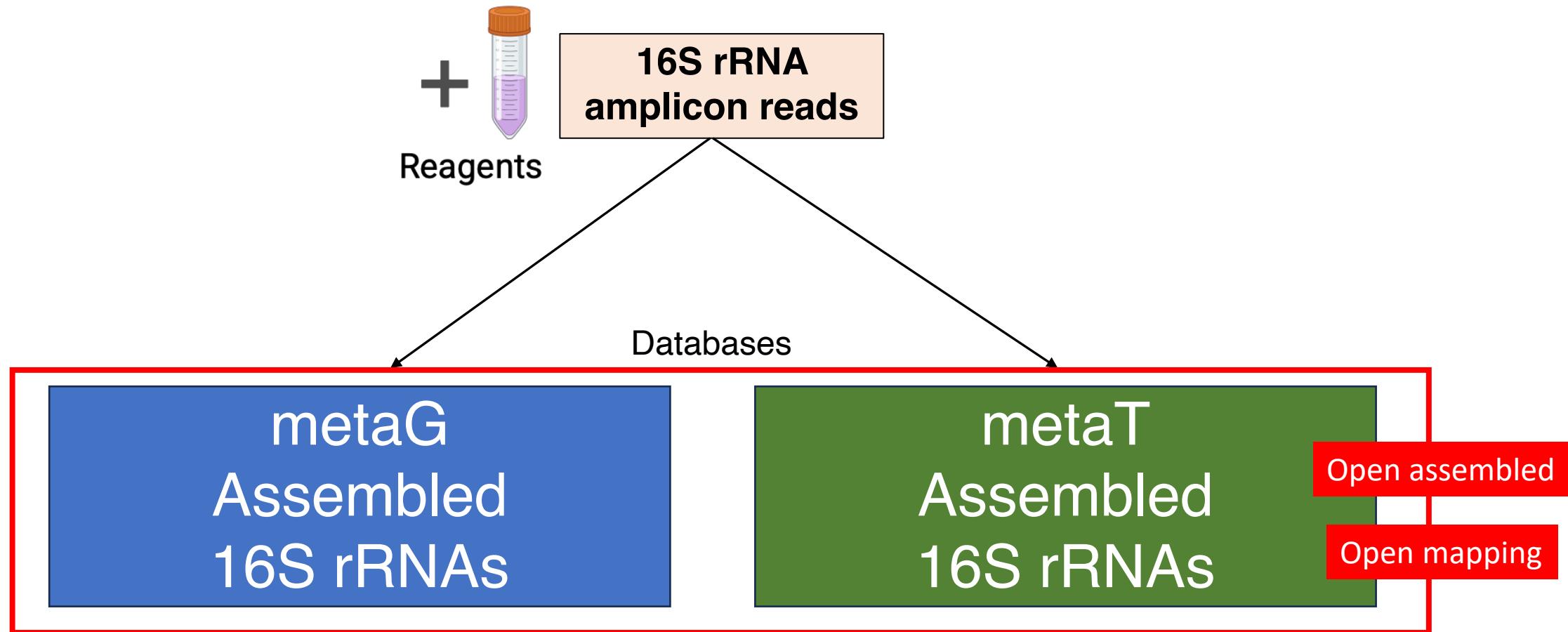
# Host contamination in low biomass tissues



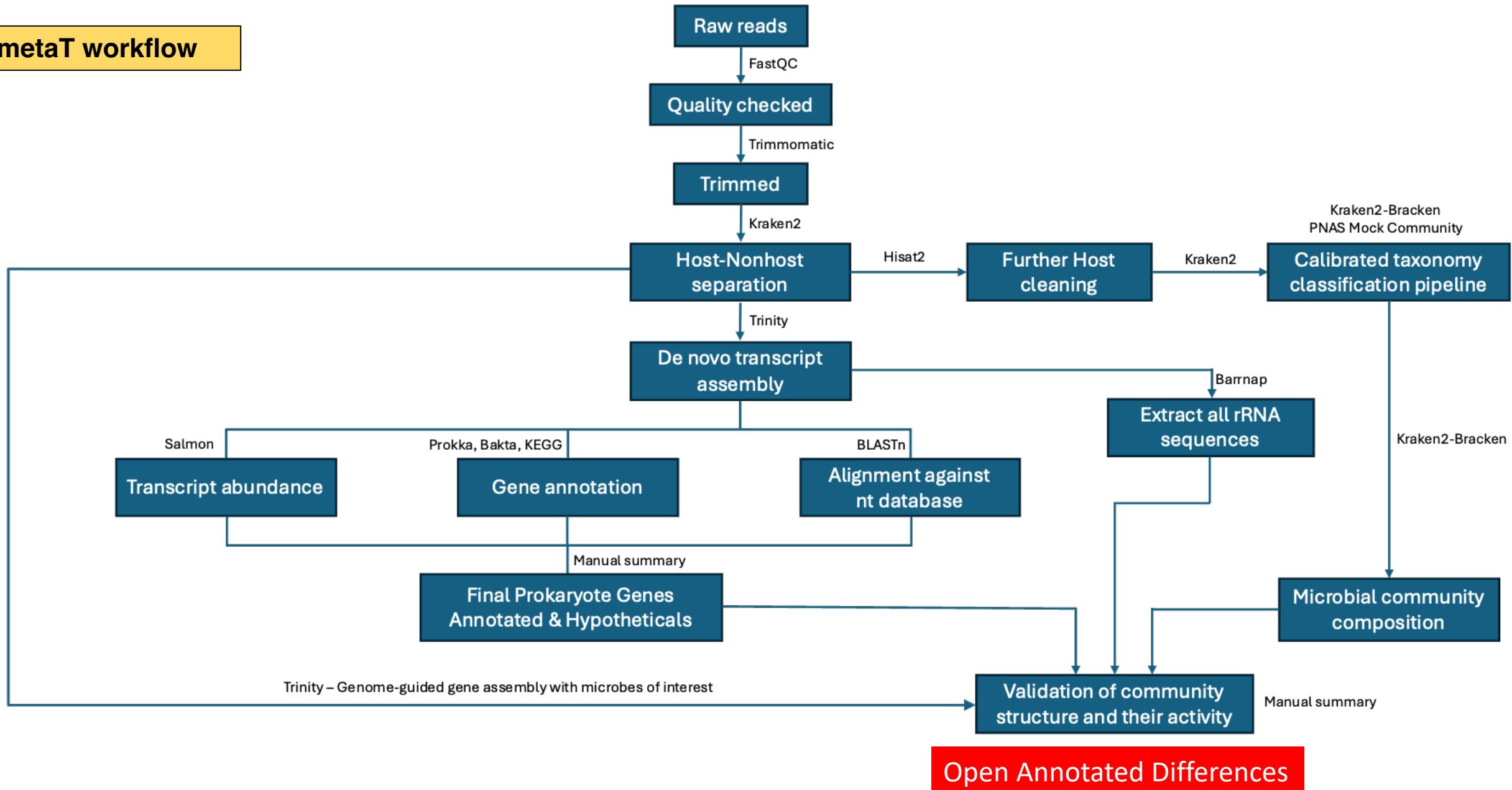
# Microbial contamination in low biomass tissues



# Microbial contamination in low biomass tissues

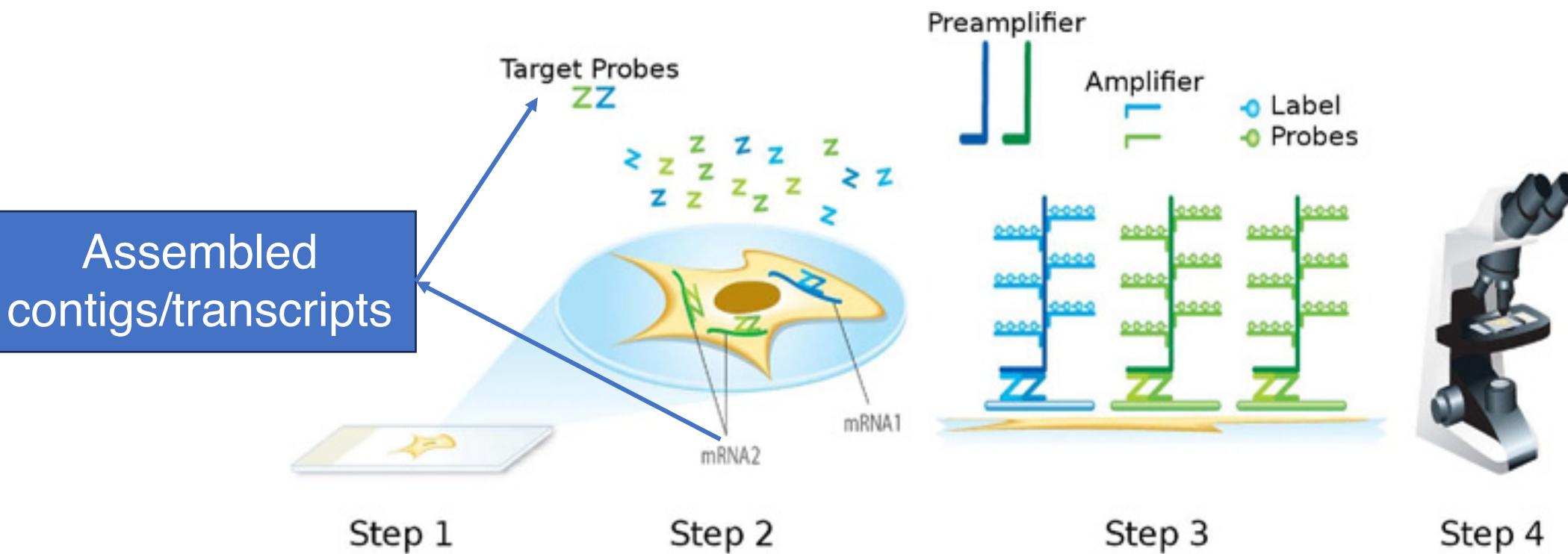


# metaT workflow



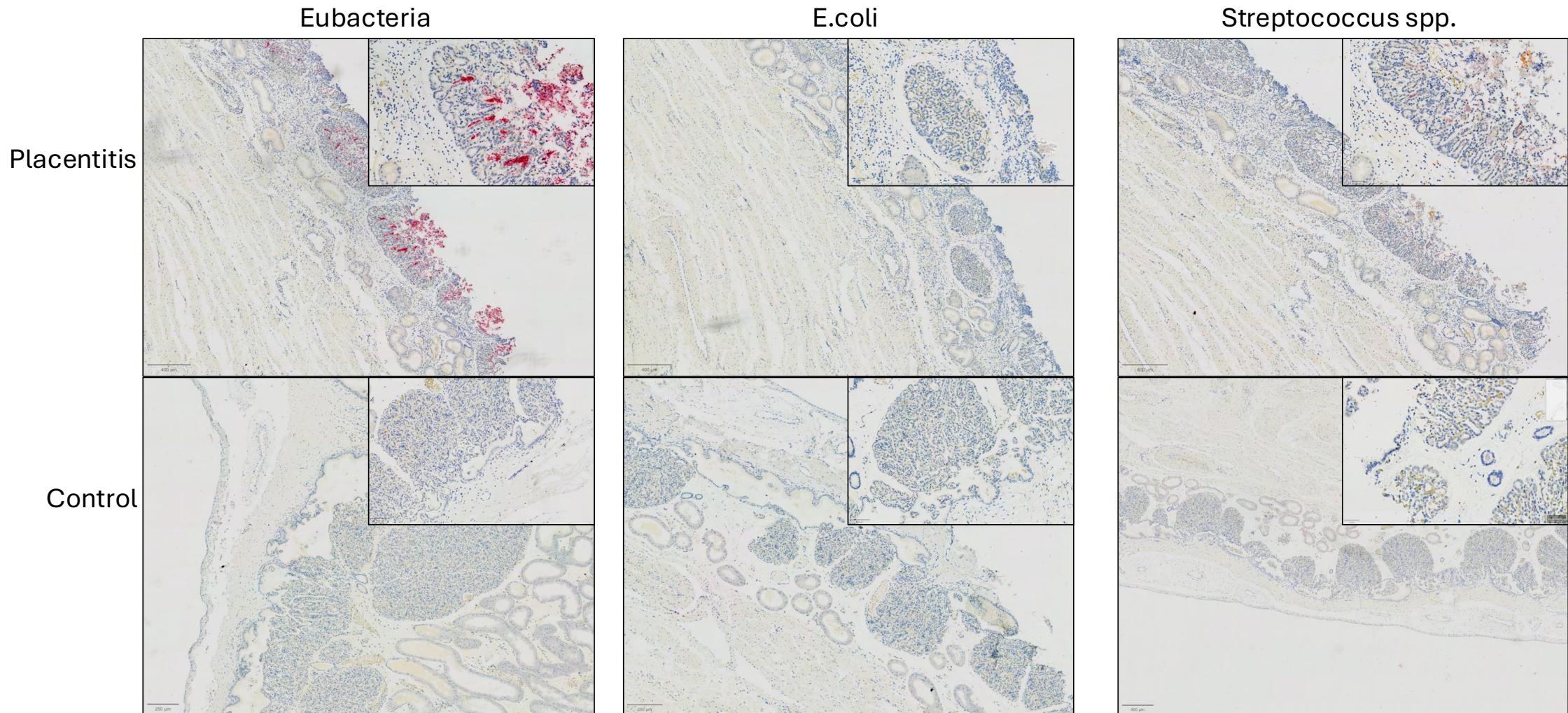
## Visual validation

## In Situ Hybridization (ISH): RNAscope



## Visual validation

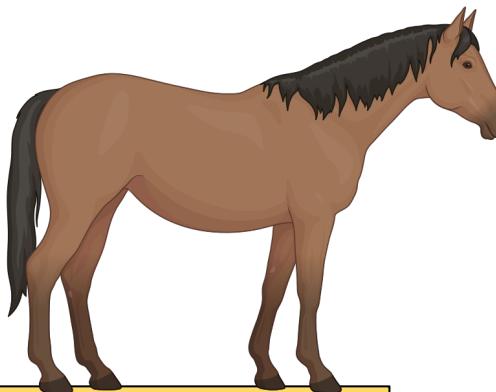
## In Situ Hybridization (ISH): RNAscope



## SOME COMPLEX HOST-MICROBIOME INTERACTION STUDIES:

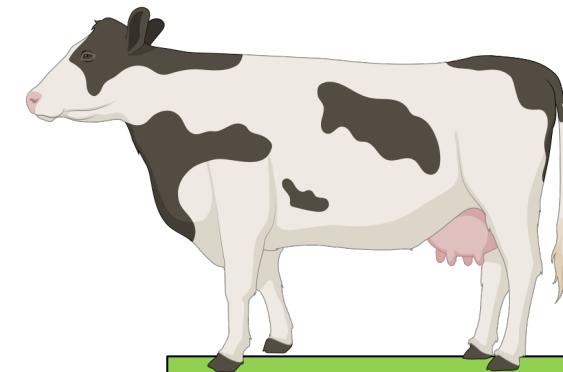


Pouya Dini  
UC Davis



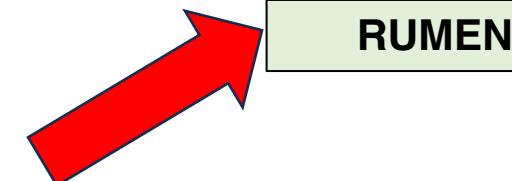
**Host-Pathogen** Interactions  
in **LOW BIOMASS** tissue

**PLACENTA**



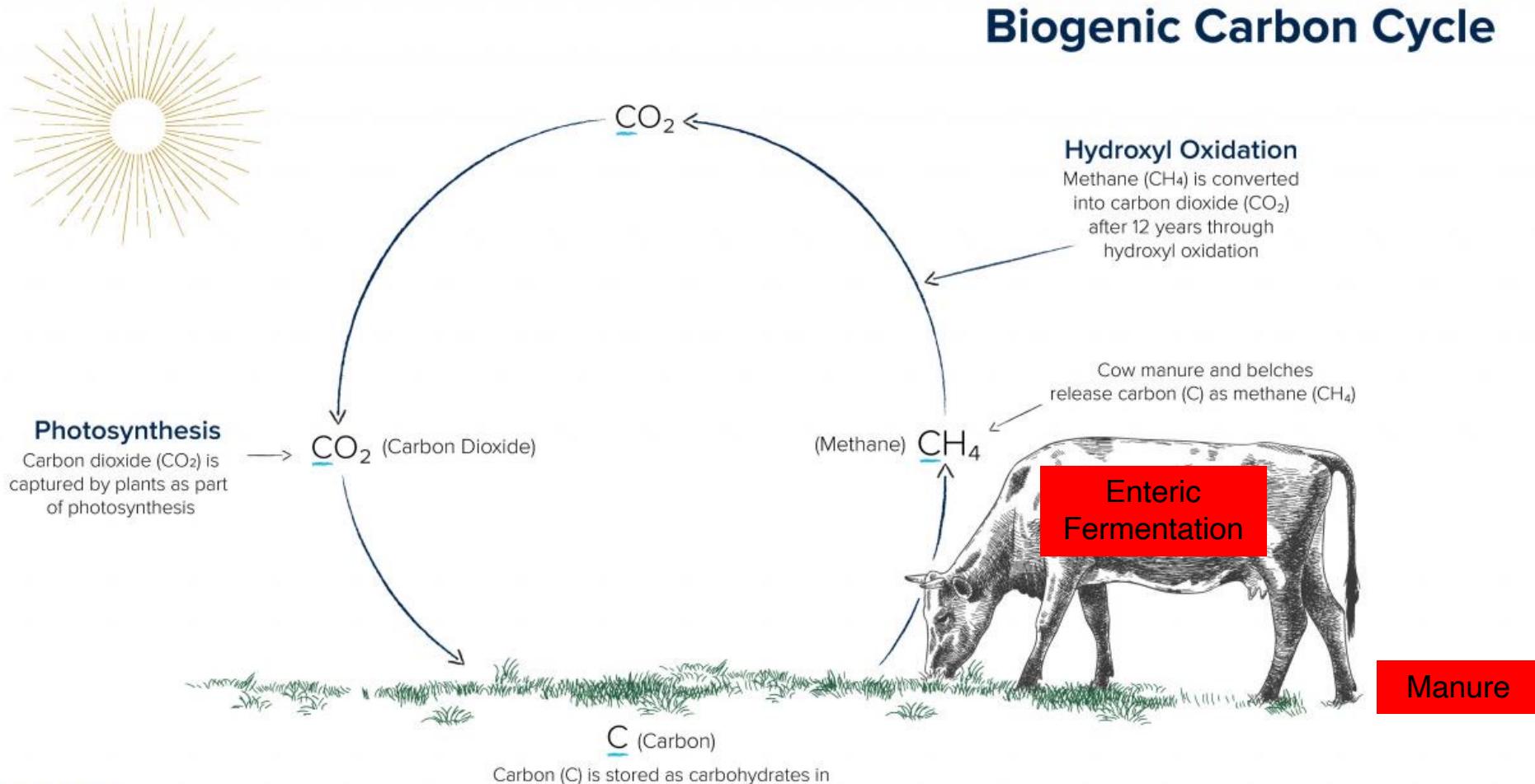
Fabio Lima  
UC Davis

**Host-Microbiome** Interactions  
in **HIGH BIOMASS** sample

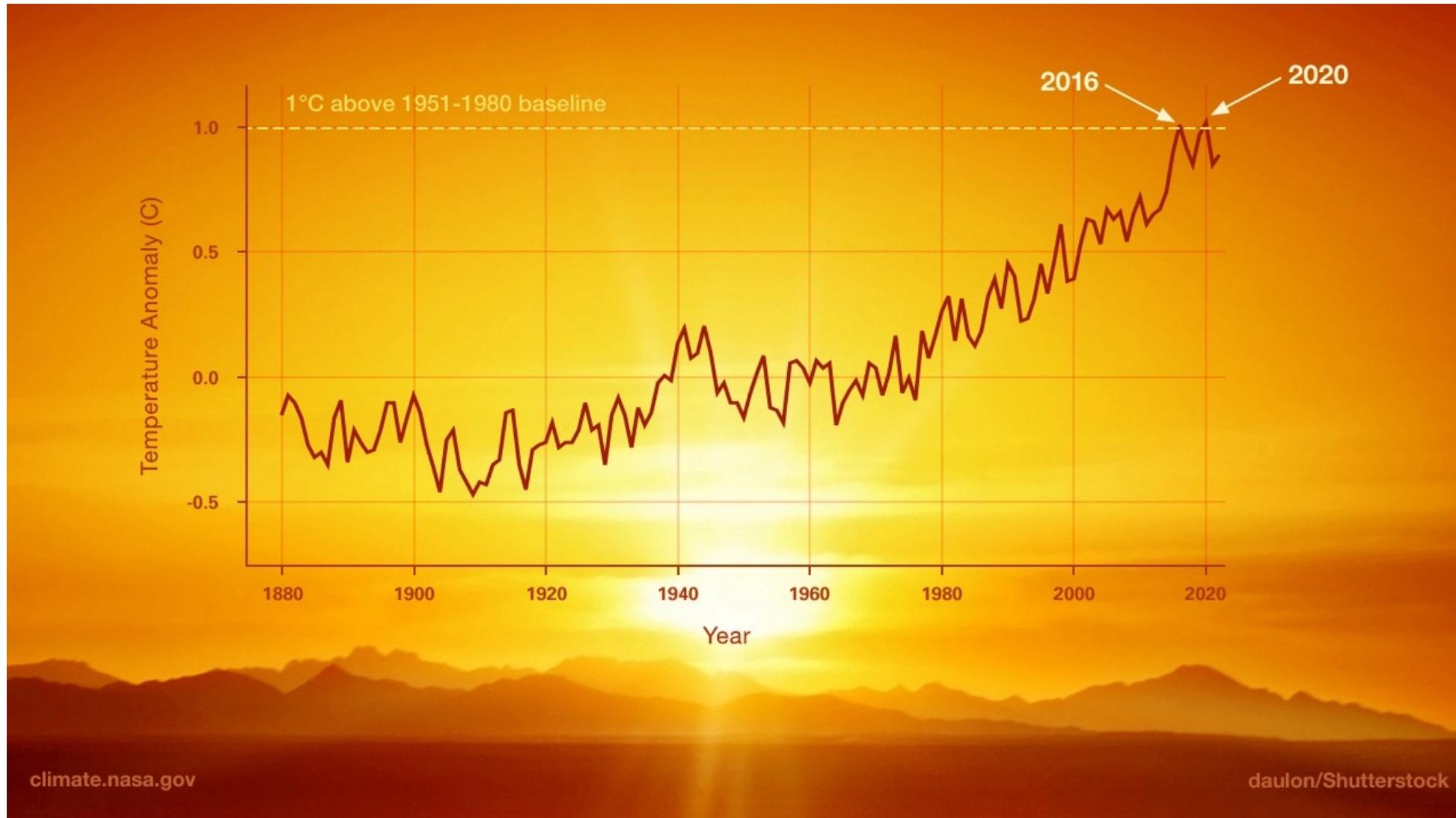


**RUMEN**

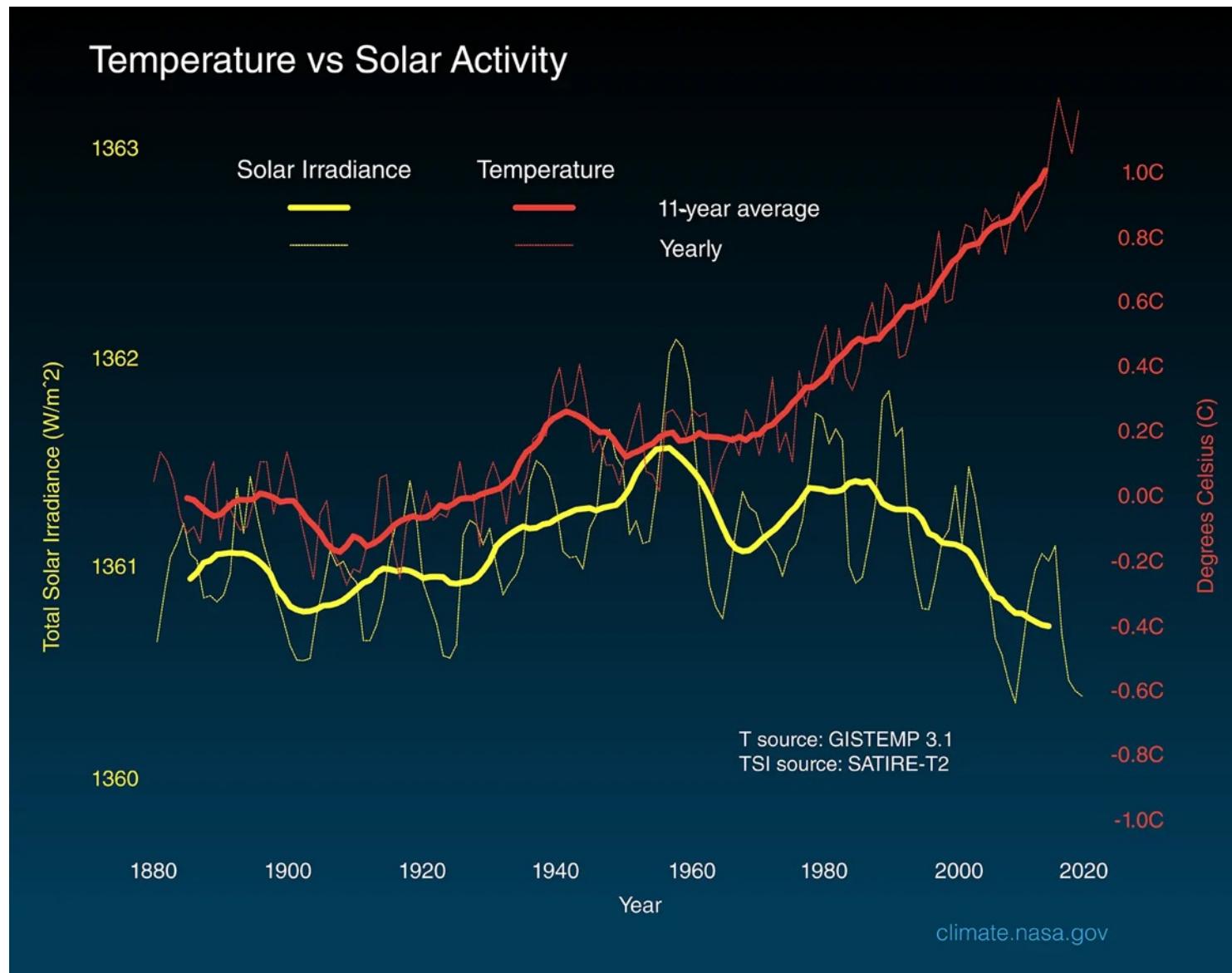
# Ruminants are key players in the carbon cycle



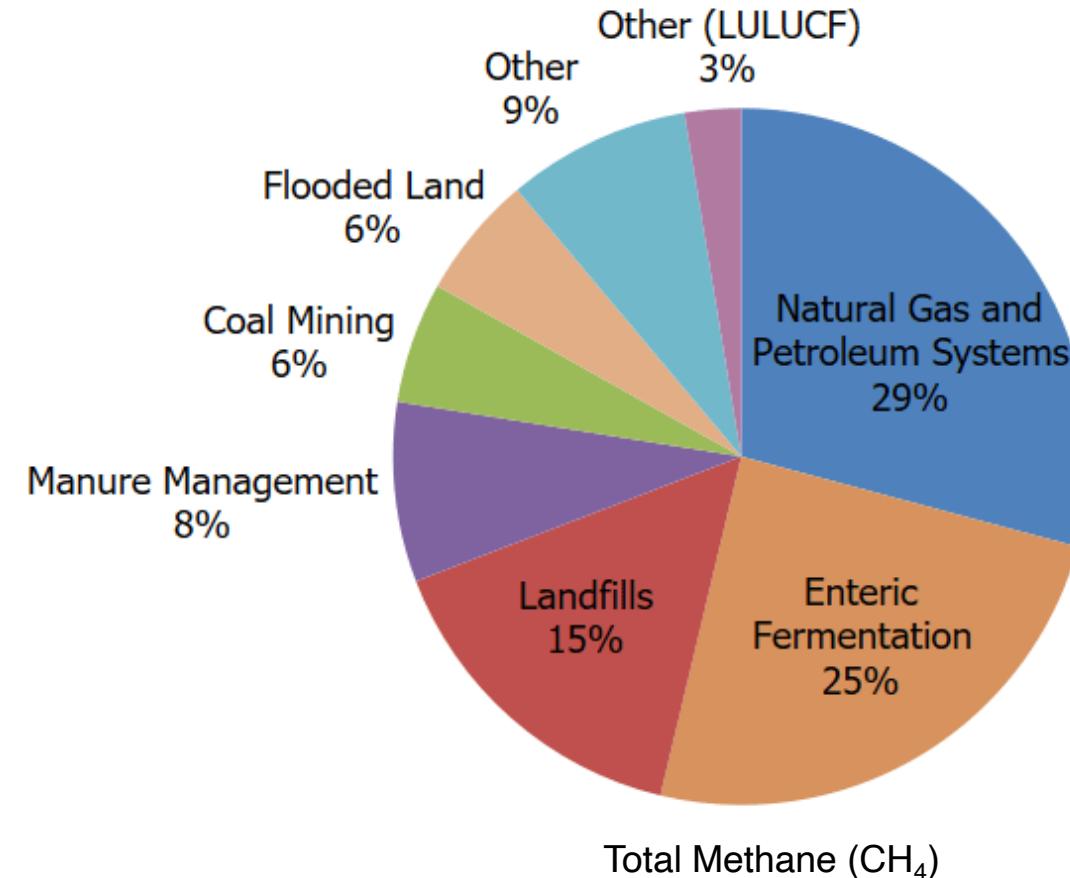
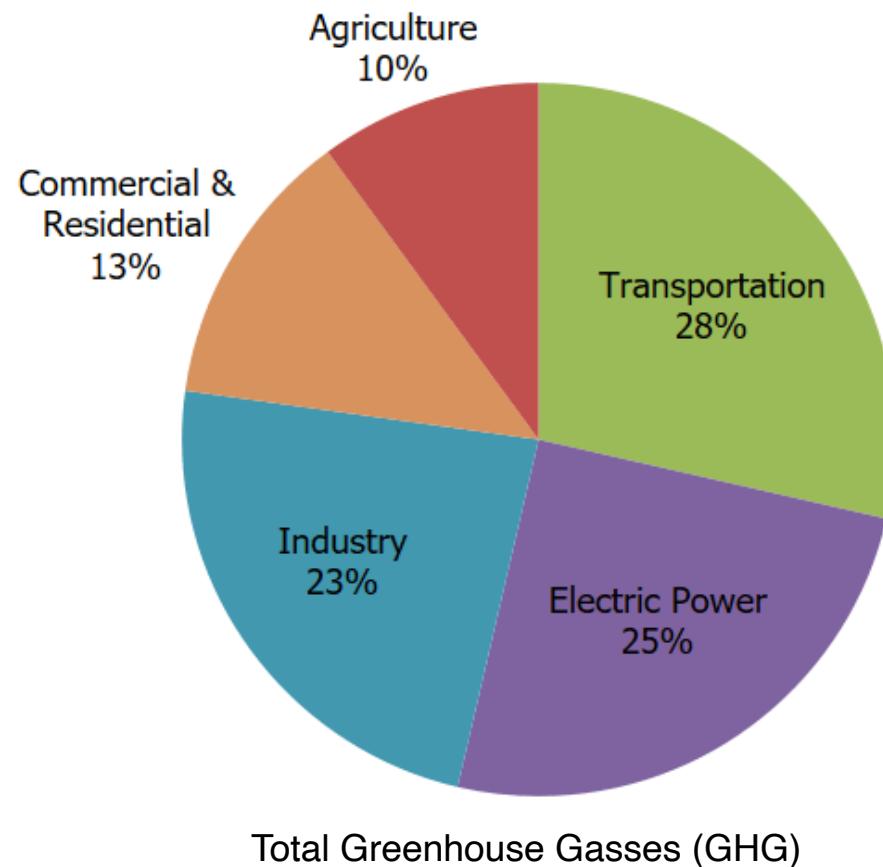
# Global temperature is consistently increasing



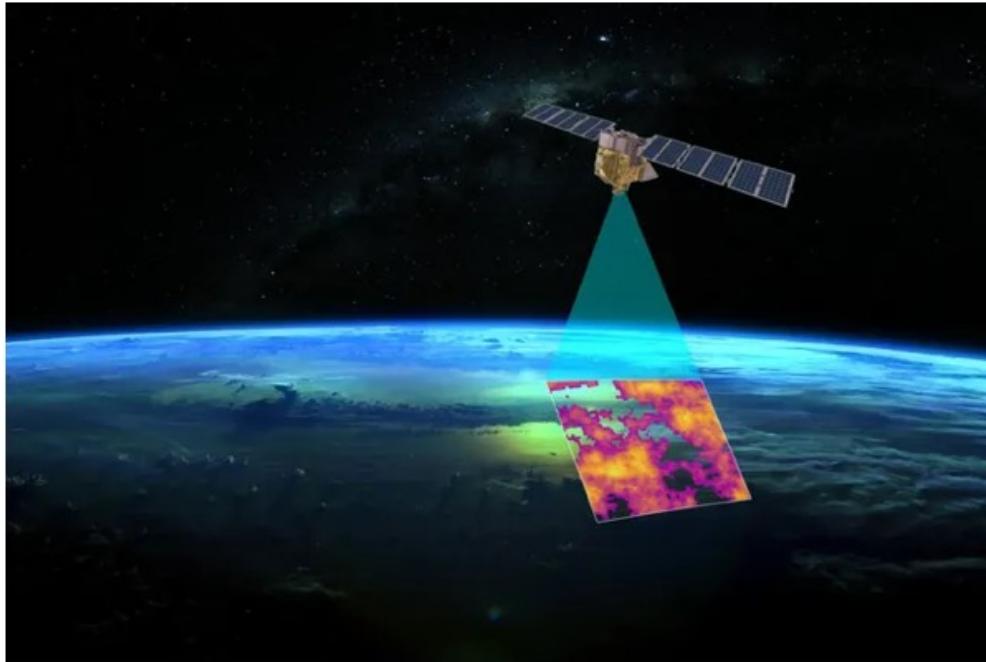
# Solar irradiance contribution to global warming



# Contribution of livestock to CH<sub>4</sub>



# Google joins a mission to map global methane emissions from space



A rendering of MethaneSat Image courtesy of Google

/ Google is joining a big initiative to track methane emissions from space.

By [Justine Calma](#), a senior science reporter covering climate change, clean energy, and environmental justice with more than a decade of experience. She is also the host of *Hell or High Water: When Disaster Hits Home*, a podcast from Vox Media and Audible Originals.

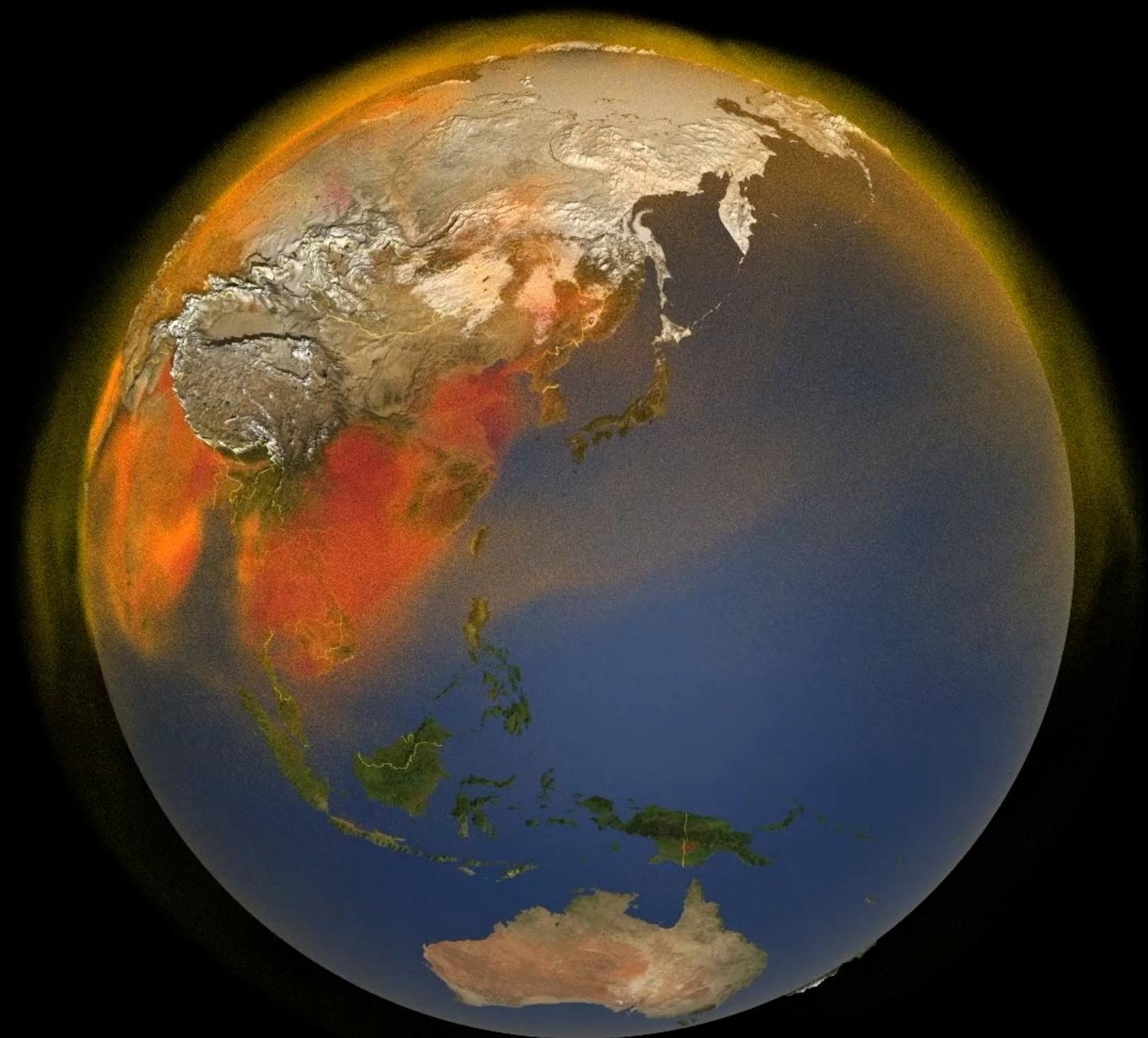
Feb 14, 2024 at 5:00 AM PST



[1 Comment \(1 New\)](#)

Google [announced](#) a partnership with the nonprofit Environmental Defense Fund (EDF) today to map methane pollution and oil and gas infrastructure from space.

Google and EDF hope to be able to pinpoint where much of those methane emissions are leaking from — which could perhaps help put a plug in those leaks. Next month, EDF plans to launch its MethaneSAT, a



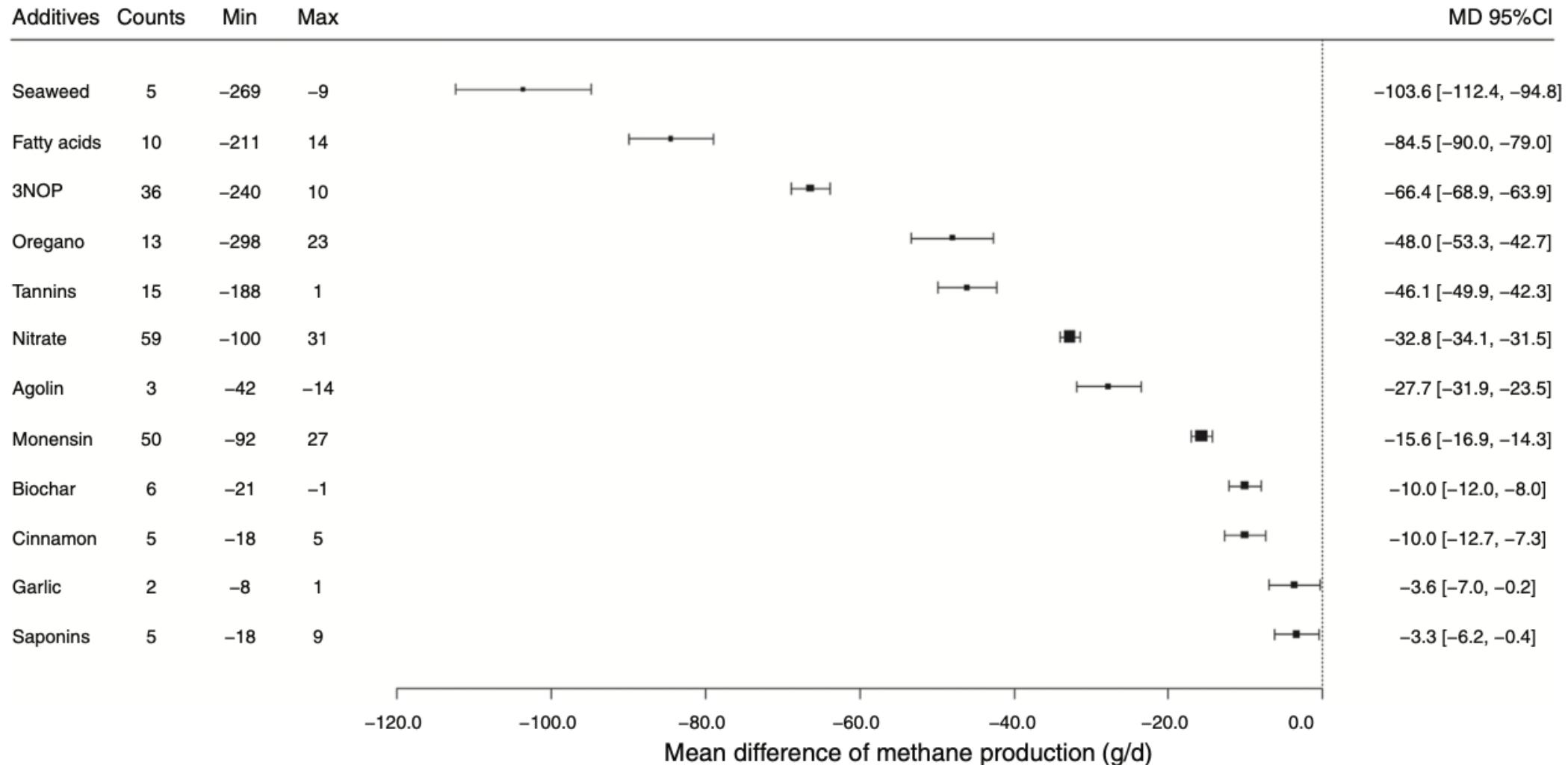
# Seaweed as a solution to enteric methane emissions



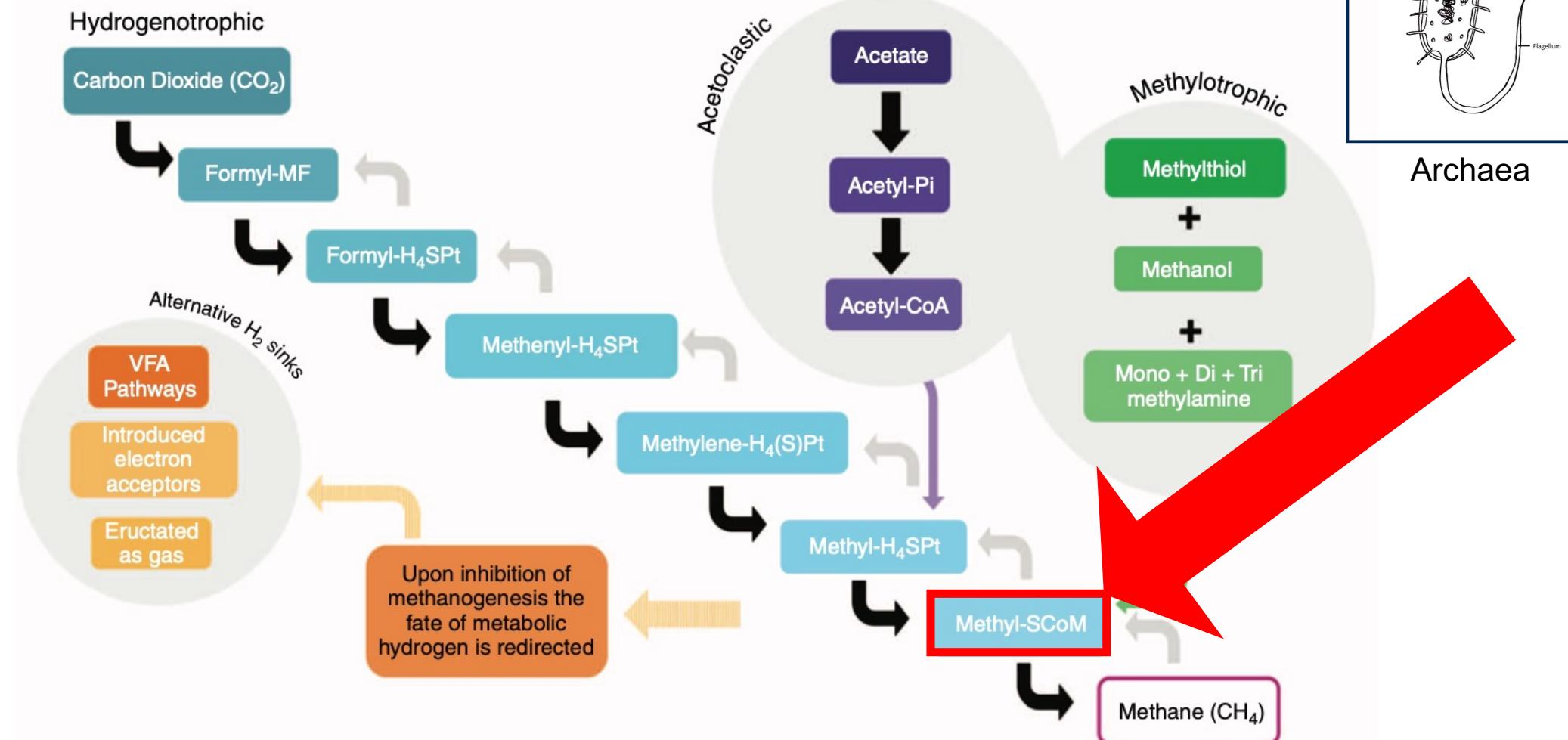
TED Talk: What seaweed and cow burps have to do with climate change. 2021.

[https://www.ted.com/talks/ermias\\_kebreab\\_what\\_seaweed\\_and\\_cow\\_burps\\_have\\_to\\_do\\_with\\_climate\\_change?language=en](https://www.ted.com/talks/ermias_kebreab_what_seaweed_and_cow_burps_have_to_do_with_climate_change?language=en)

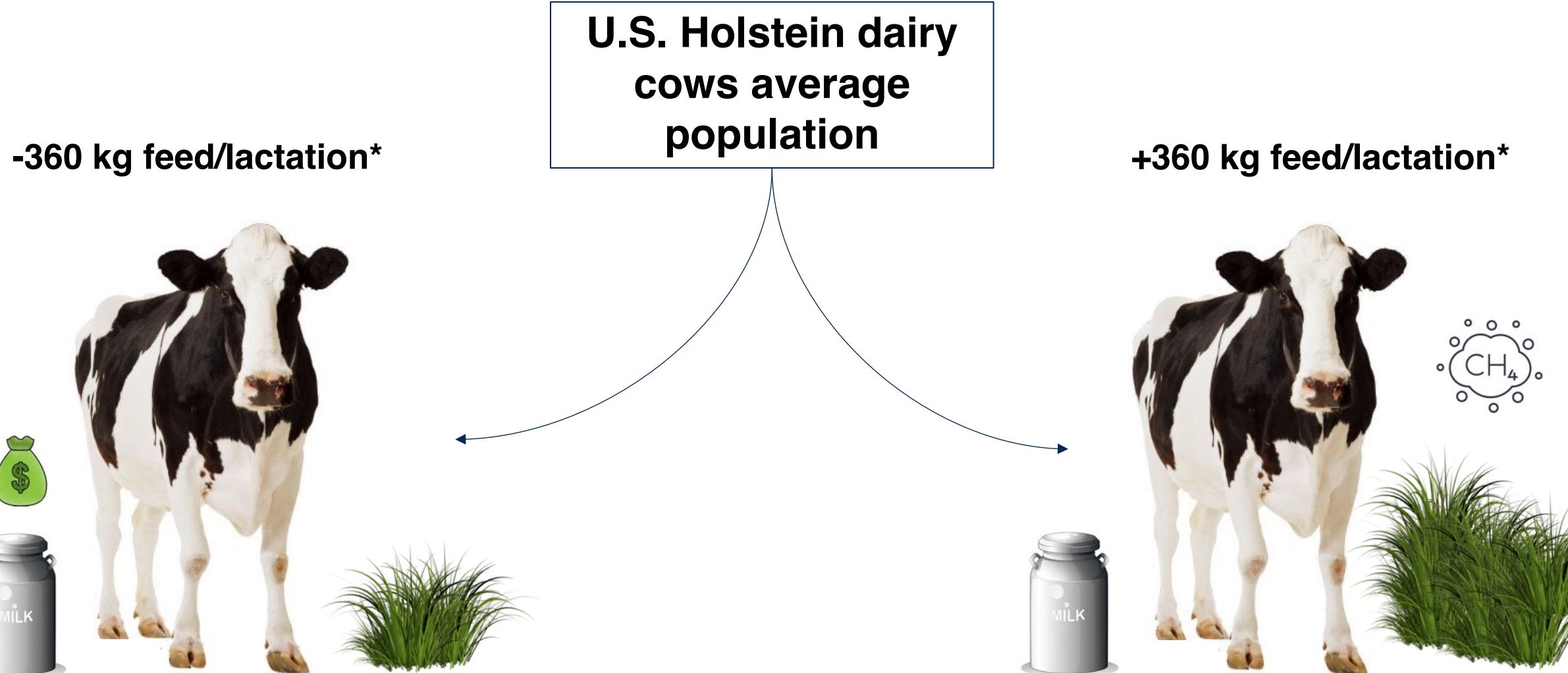
# Seaweed as a solution to enteric methane emissions



# Role of seaweed in stopping methanogenesis



# Feed efficiency in ruminants



# Selecting cows for feed efficiency as alternative to reduce methane emissions



**J. Dairy Sci.** 107:4704–4713  
<https://doi.org/10.3168/jds.2023-23751>

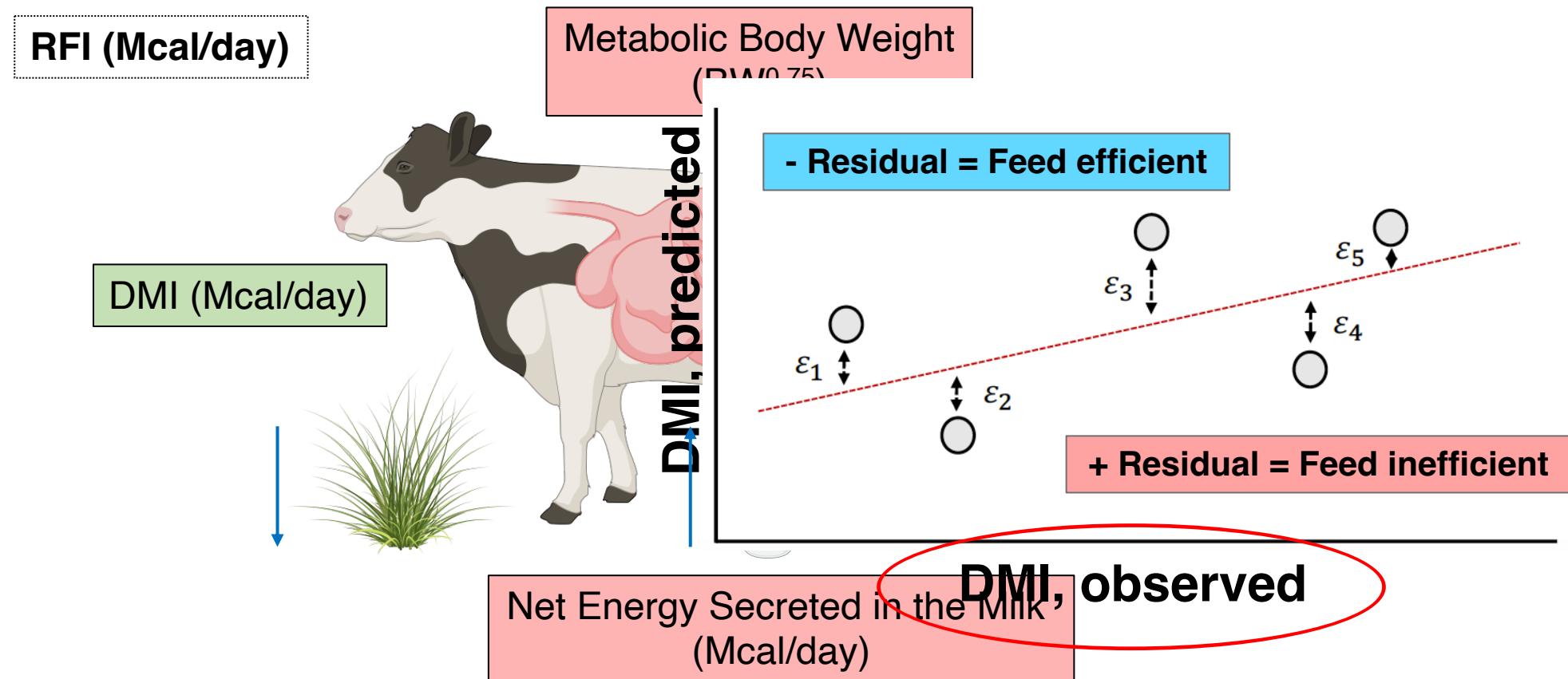
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## Estimates of genetic parameters for rumination time, feed efficiency, and methane production traits in first-lactation Holstein cows

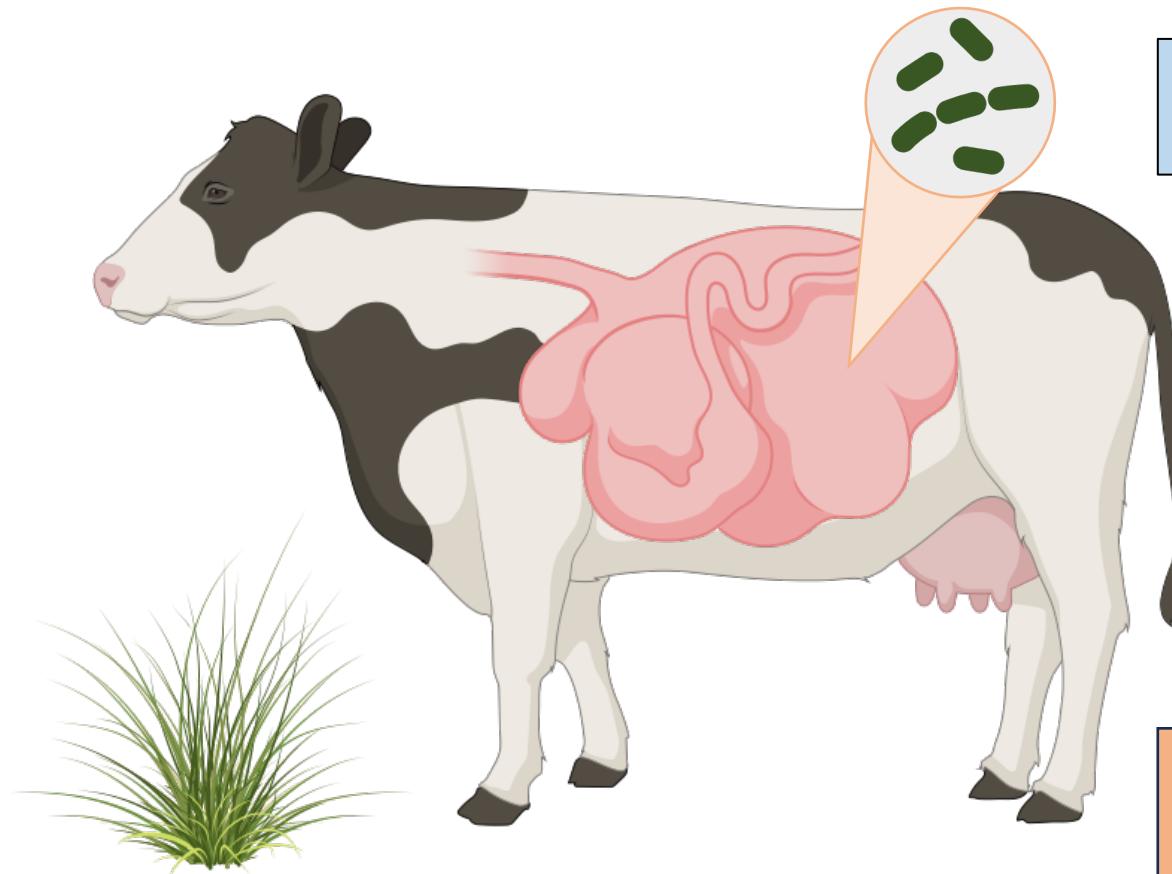
L. S. P. **Table 3.** Genetic correlation (above diagonal), heritability<sup>1</sup> (diagonal), and phenotypic correlation (below diagonal) for production, efficiency, L. M. A. methane production, and rumination time traits in first-lactation Holstein cows

Trait <sup>2</sup>	ECM	MBW	DMI	FE	MY	FY	PY	MeP	RT
ECM (kg/d)	0.28 (0.07)	-0.04 (0.21)	0.57 (0.16)	0.20 (0.26)	0.74 (0.07)	0.87 (0.04)	0.89 (0.03)	0.74 (0.13)	0.49 (0.11)
MBW (kg <sup>0.75</sup> )	-0.03 (0.04)	0.43 (0.11)	0.37 (0.21)	0.34 (0.28)	-0.10 (0.19)	-0.05 (0.20)	0.07 (0.20)	0.68 (0.10)	-0.24 (0.13)
DMI (kg/d)	0.34 (0.03)	0.21 (0.04)	0.23 (0.07)	0.93 (0.04)	0.51 (0.06)	0.40 (0.08)	0.33 (0.14)	0.83 (0.11)	0.21 (0.13)
FE (kg/d)	0.05 (0.03)	0.07 (0.04)	0.94 (0.004)	0.15 (0.07)	0.16 (0.06)	0.55 (0.02)	0.29 (0.06)	0.63 (0.17)	-0.01 (0.17)
MY (kg/d)	0.83 (0.01)	0.91 (0.01)	0.06 (0.04)	0.40 (0.08)	0.33 (0.14)	0.80 (0.05)	0.55 (0.12)	0.52 (0.12)	
FY (kg/d)	0.91 (0.01)	0.01 (0.04)	0.35 (0.03)	0.02 (0.03)	0.55 (0.02)	0.29 (0.06)	0.61 (0.10)	0.89 (0.12)	0.36 (0.11)
PY (kg/d)	0.90 (0.01)	0.01 (0.04)	0.35 (0.03)	0.08 (0.03)	0.88 (0.01)	0.67 (0.02)	0.32 (0.07)	0.55 (0.14)	0.54 (0.11)
MeP (g/d)	0.37 (0.06)	0.45 (0.07)	0.46 (0.05)	0.26 (0.08)	0.18 (0.06)	0.41 (0.05)	0.23 (0.06)	0.36 (0.12)	-0.53 (0.24)
RT (min/d)	0.25 (0.07)	-0.17 (0.08)	0.20 (0.07)	0.08 (0.09)	0.30 (0.07)	0.16 (0.07)	0.29 (0.07)	-0.09 (0.06)	0.45 (0.14)

# How we measured feed efficiency



## The reason why the rumen microbiome may be dictating feed efficiency:

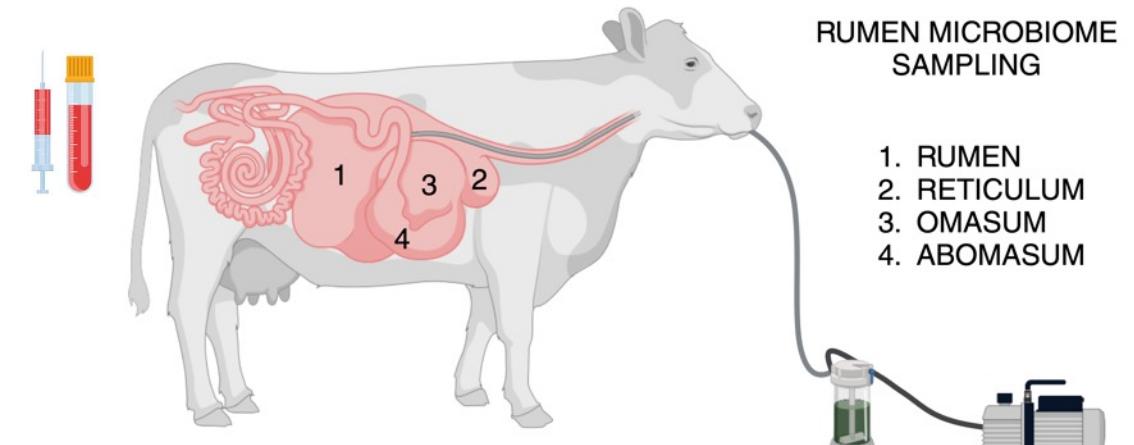
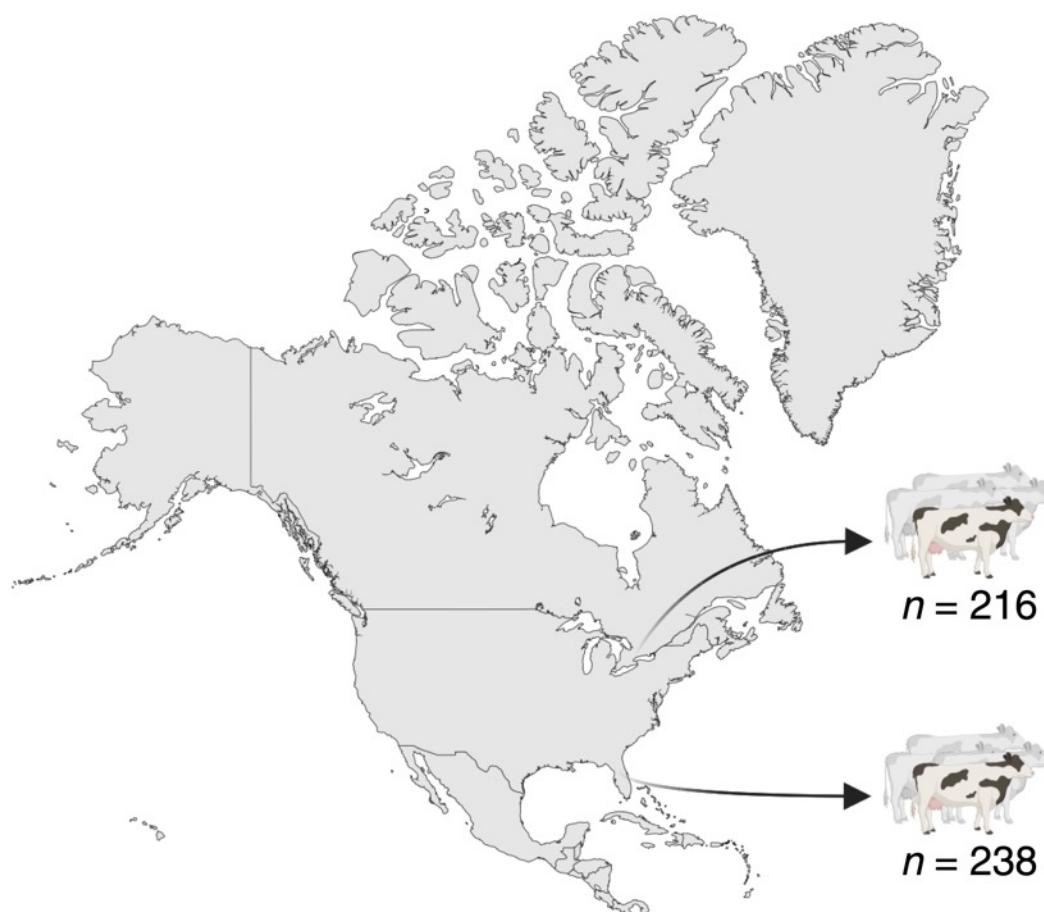


### SOME KEY REASONS THE MICROBIOME IS CRITICAL TO THE RUMINANT:

1. Deep extraction of glucose from fiber
2. Volatile Fatty Acids (VFA)
3. 100's potentially bioactive molecules
4. Microbial Protein (MCP)
5. Rumen undegraded protein (RUP)
6. Vitamin synthesis
7. Methane ( $\text{CH}_4$ ) synthesis
8. And many others...

SEVERAL DRAWBACKS IN EACH OF THESE MECHANISMS

# Feed efficiency study

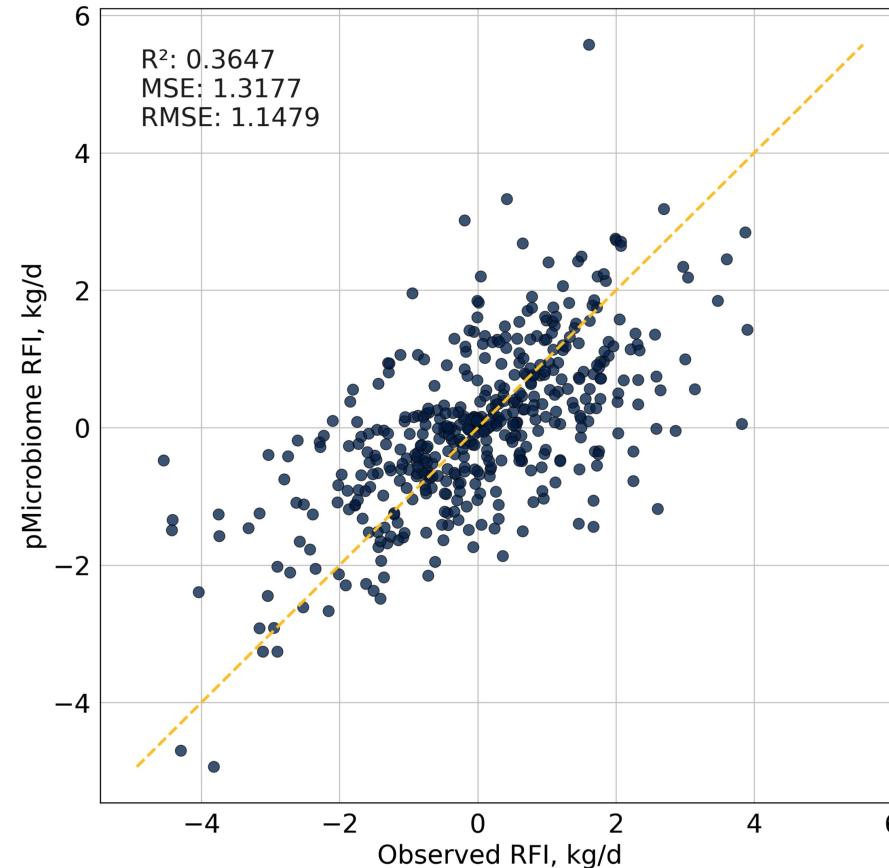


## HYPOTHESES

1. The rumen microbiome improves feed efficiency by increasing gluconeogenic precursors to the cows
2. The mechanism by which the microbiome improves feed efficiency is through an interaction between the rumen microbiome and the host

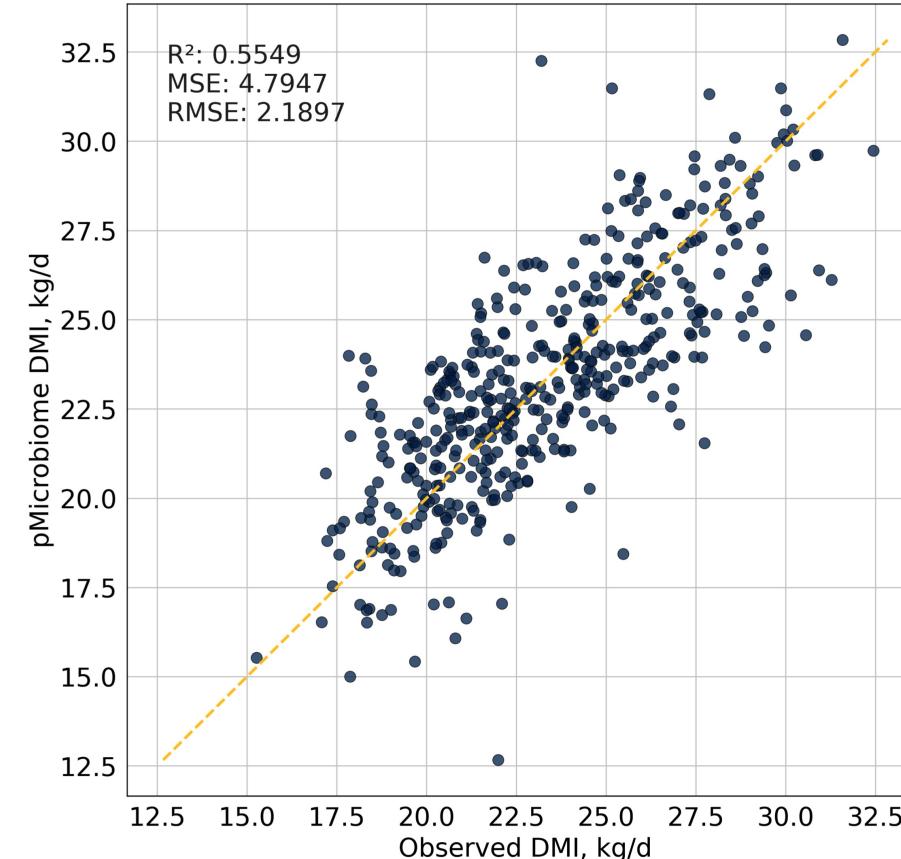
# Importance of rumen fermentation to ruminants

## FEED EFFICIENCY



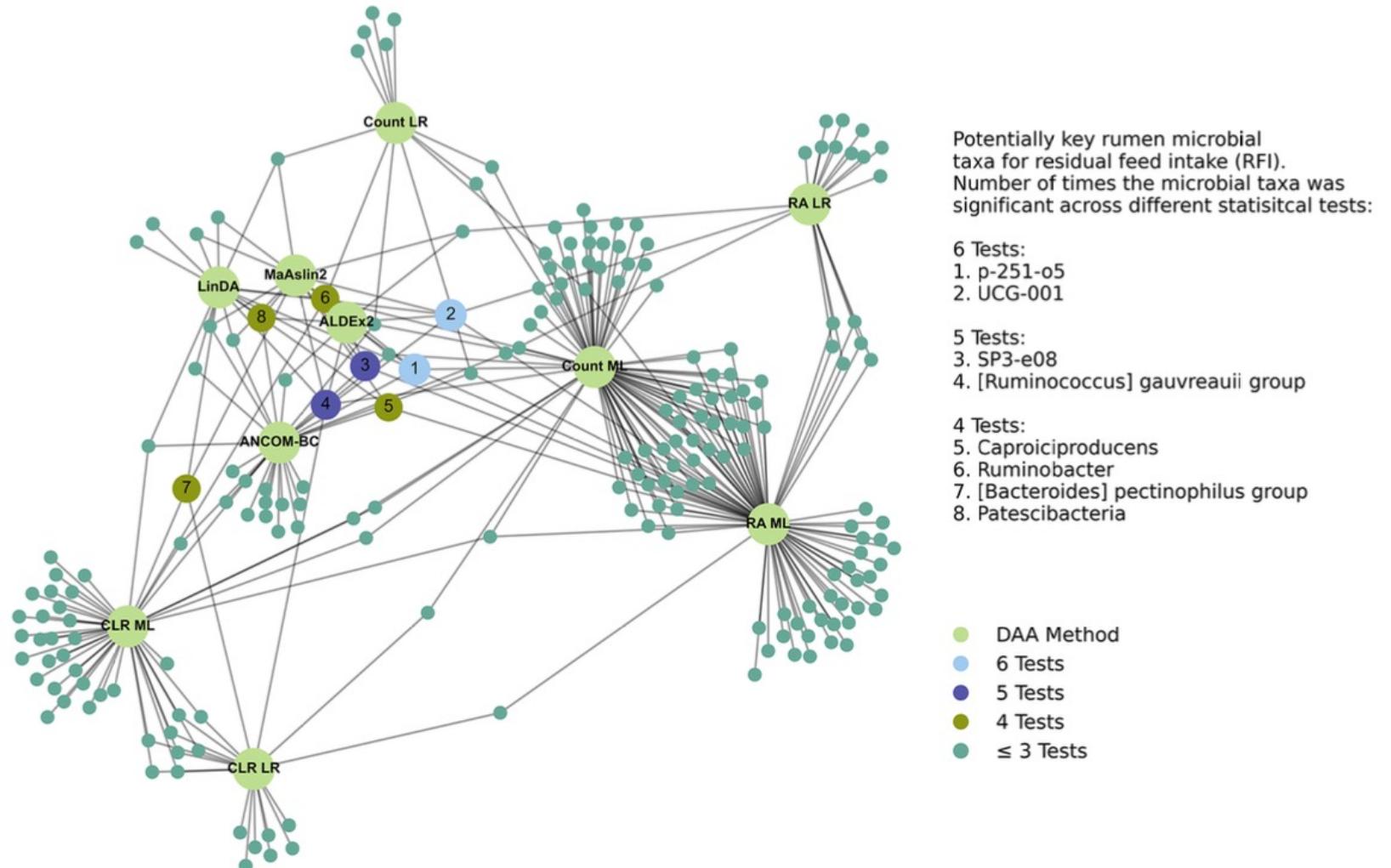
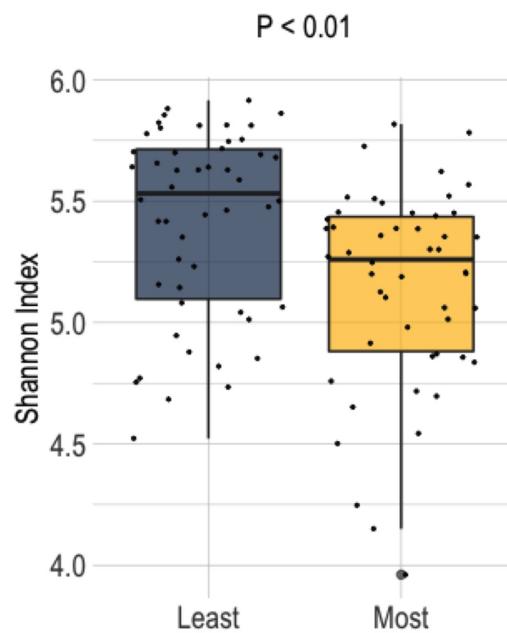
$$\text{RFI, kg/d} = \text{Microbiome} + \epsilon$$

## DMI

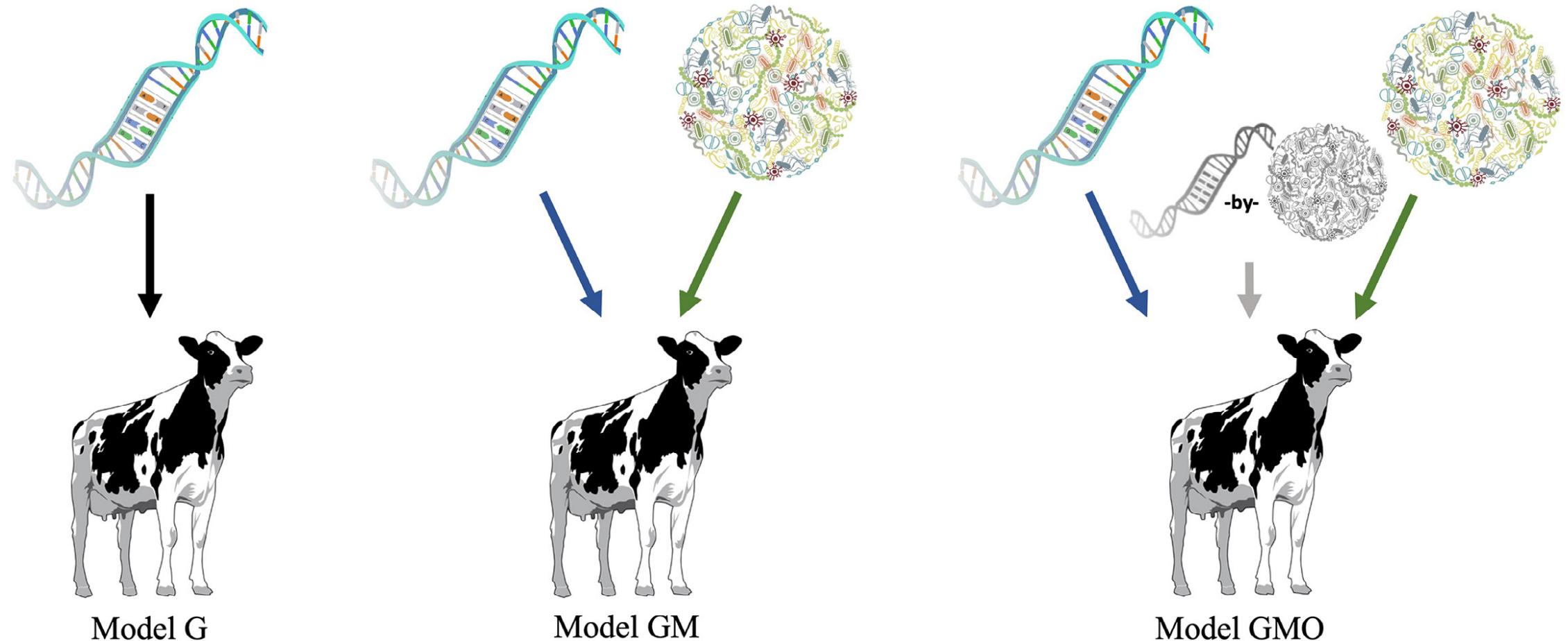


$$\text{DMI, kg/d} = \text{Microbiome} + \epsilon$$

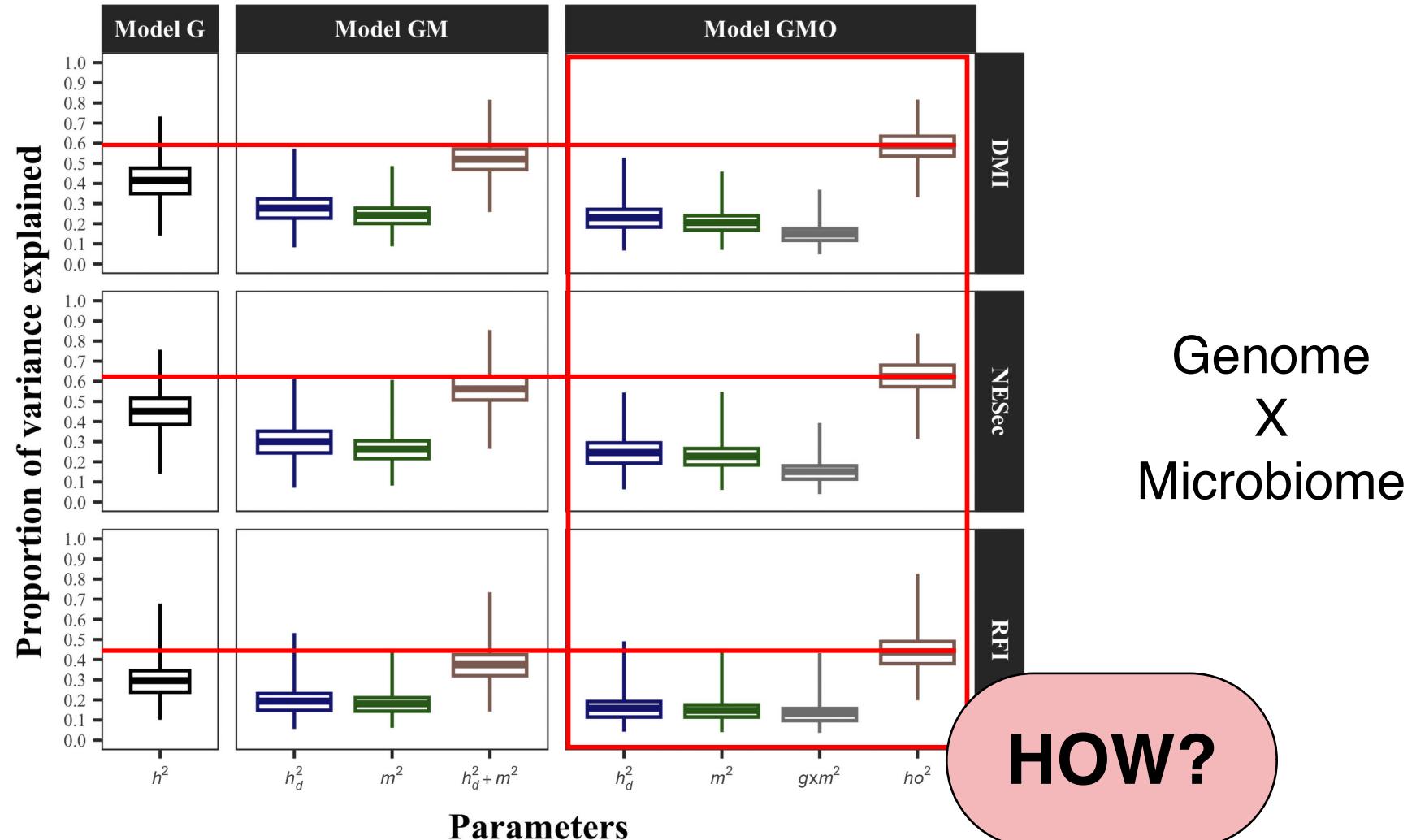
# Importance of rumen fermentation to ruminants



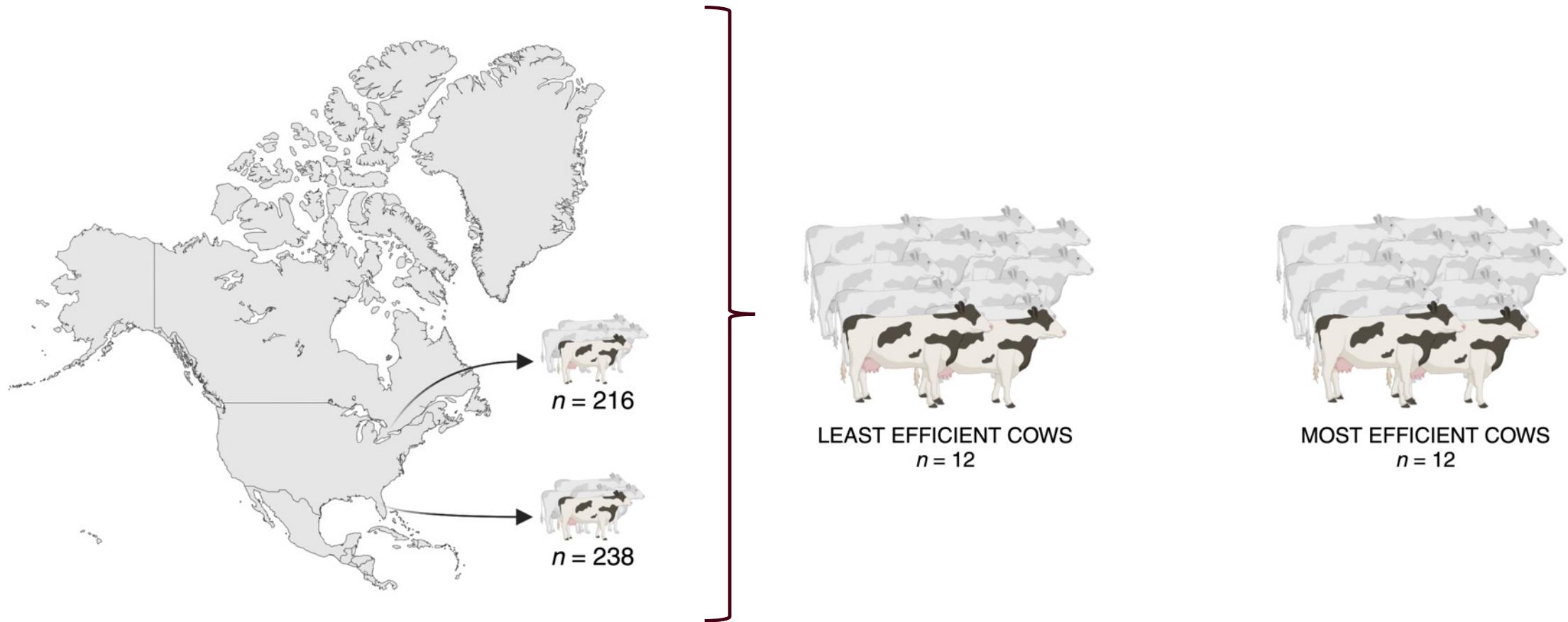
# Importance of rumen fermentation to ruminants



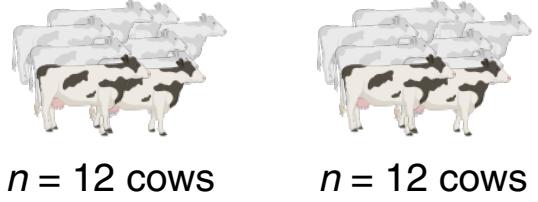
# Importance of rumen fermentation to ruminants



# In-depth exploration of the feed efficiency mechanism



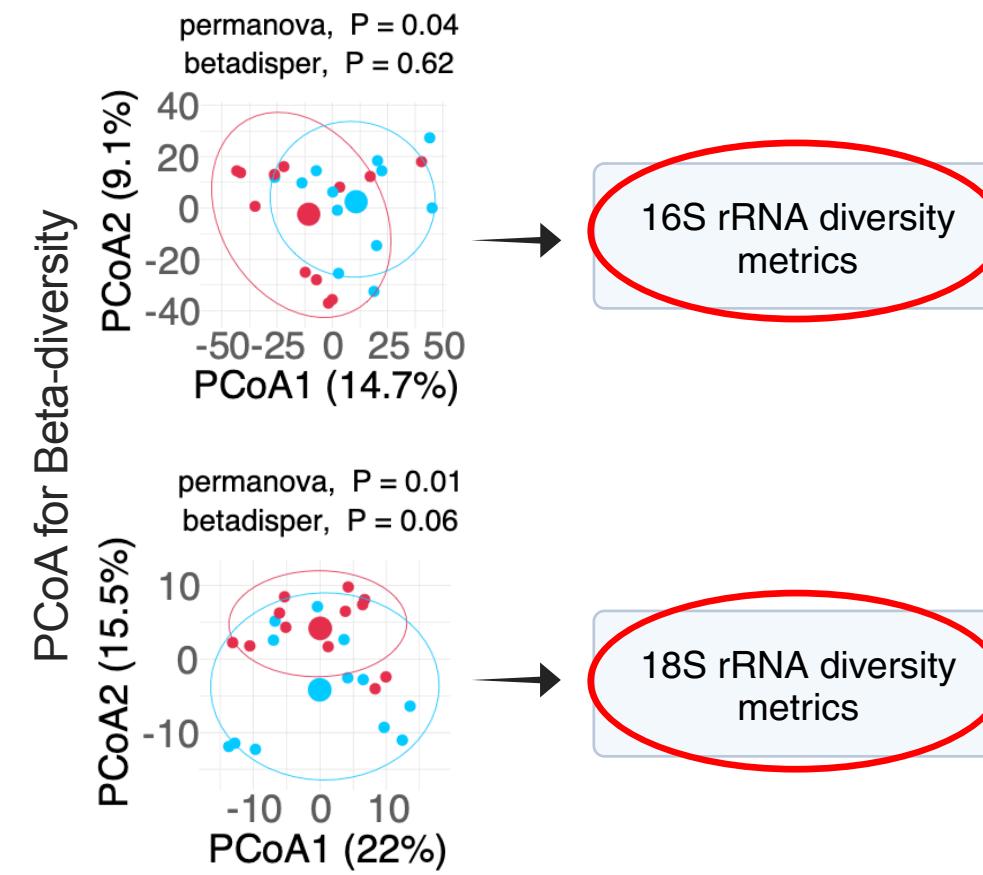
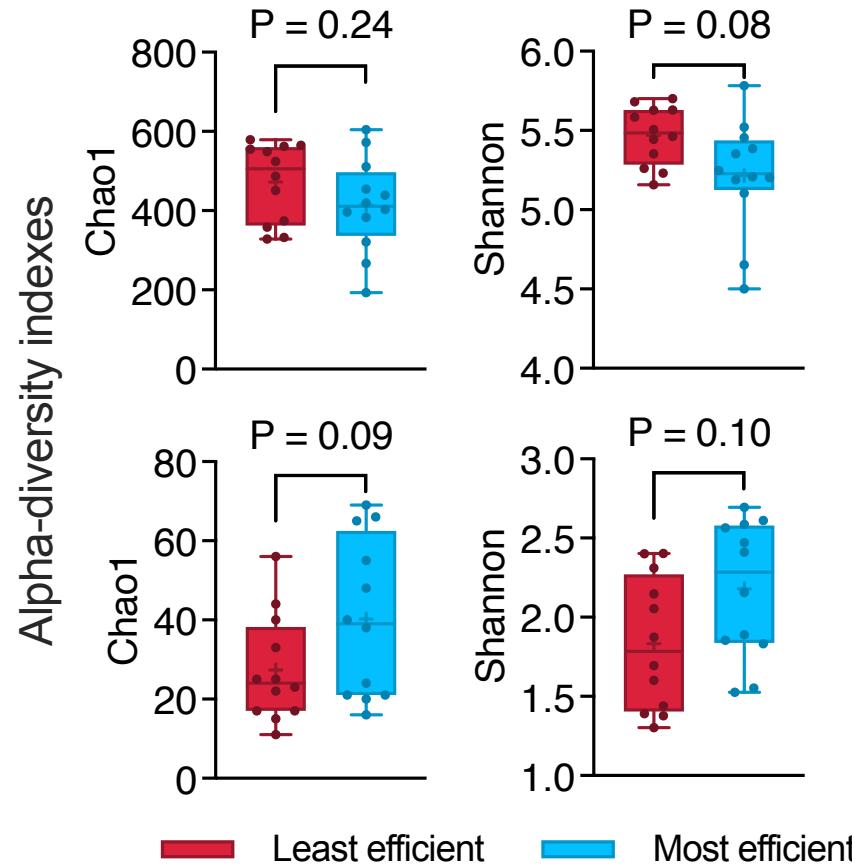
# Selection of a strictly homogeneous group of cows



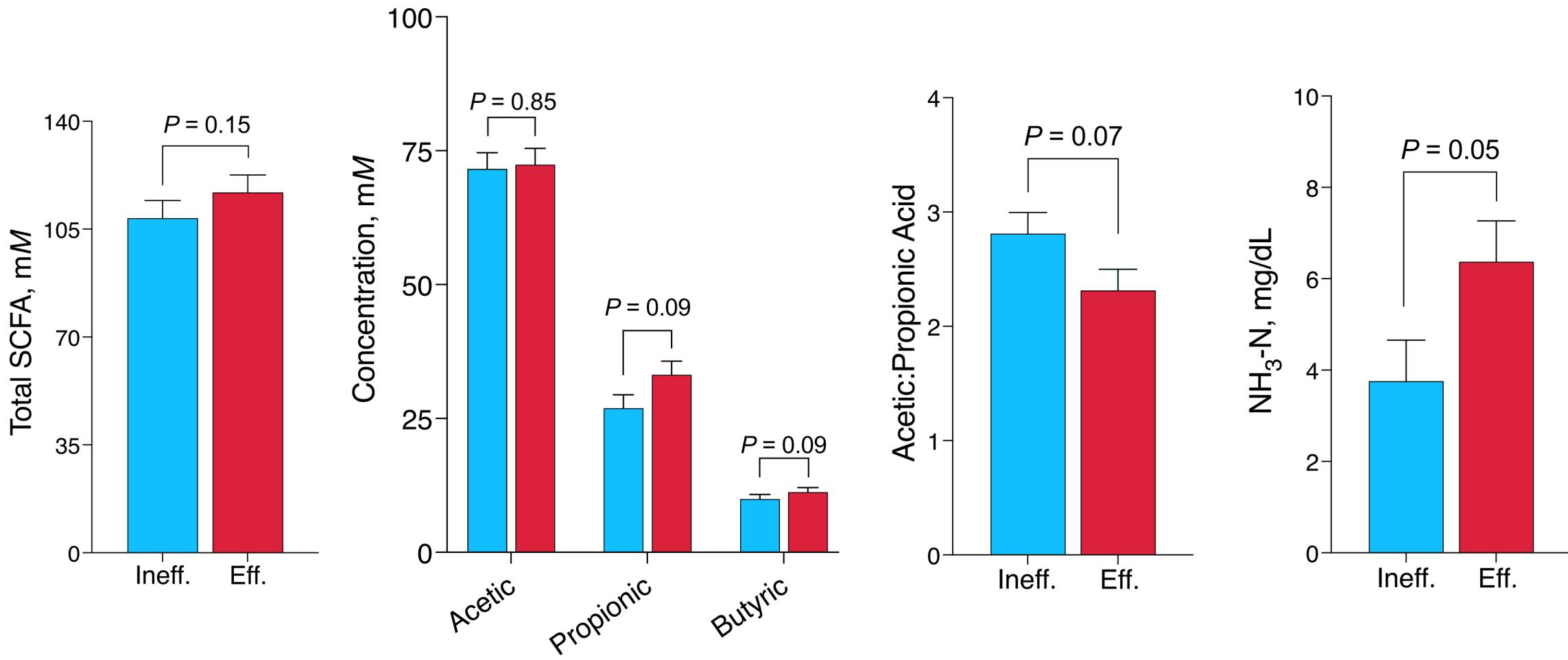
COHORT USED FOR THE STUDY

	EFFICIENCY STATUS		SEM	P-Values
	LEAST	MOST		
FEED INTAKE, kg/d	26.1	20.7	1.05	<0.001
N° LACTATION, cycles	1.58	1.58	0.17	0.99
BODY WEIGHT (BW), kg	668	677	34	0.79
METABOLIC BW CHANGES, kg/d	0.39	0.26	0.18	0.48
ENERGY CHANGES, Mcal/d	2.56	1.76	1.19	0.52
MILK PRODUCTION, kg/d	40.5	39.8	2.10	0.81
MILK PROTEIN, %	3.06	2.93	0.11	0.28
MILK FAT, %	3.81	3.62	0.24	0.46
MILK LACTOSE, %	4.84	4.83	0.06	0.86

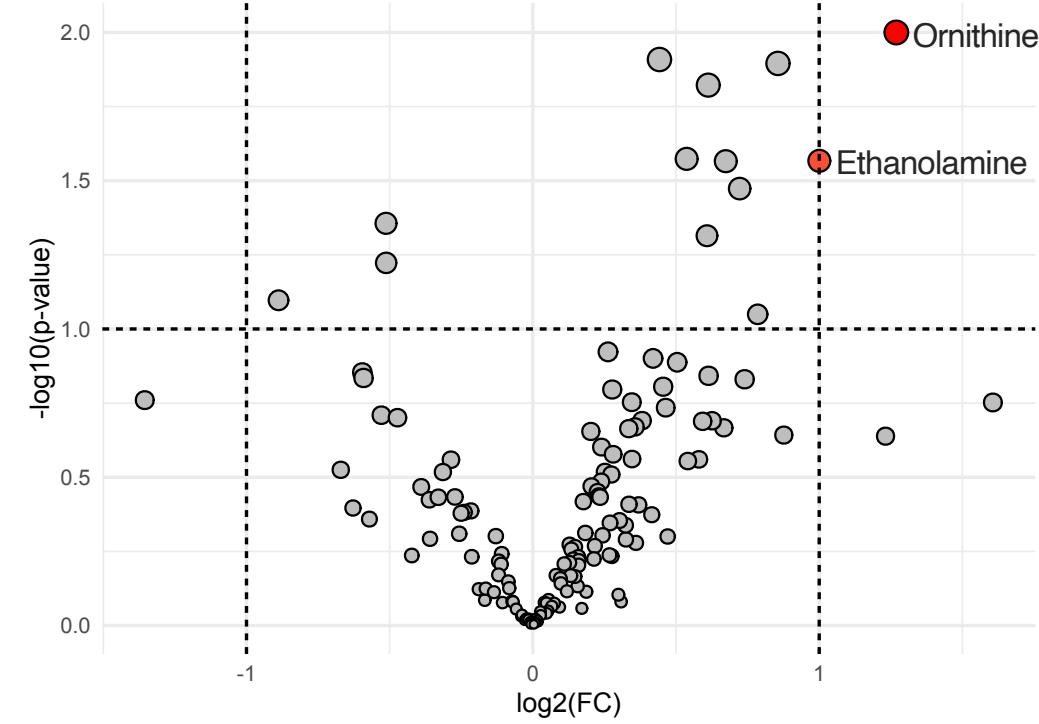
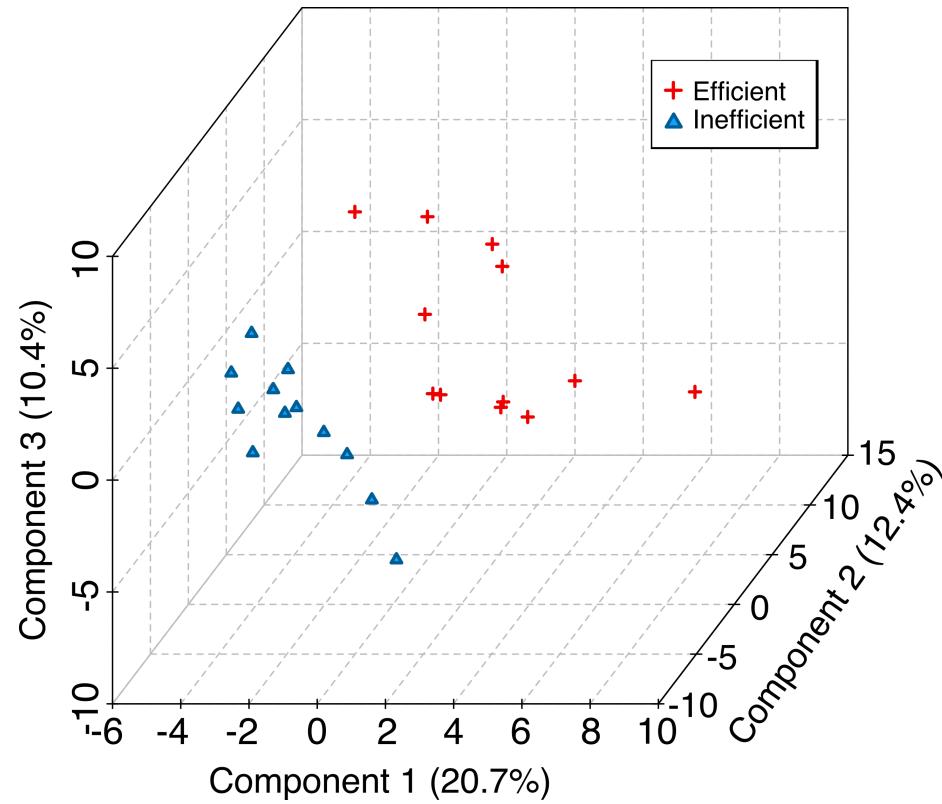
# First screening of the rumen microbiome



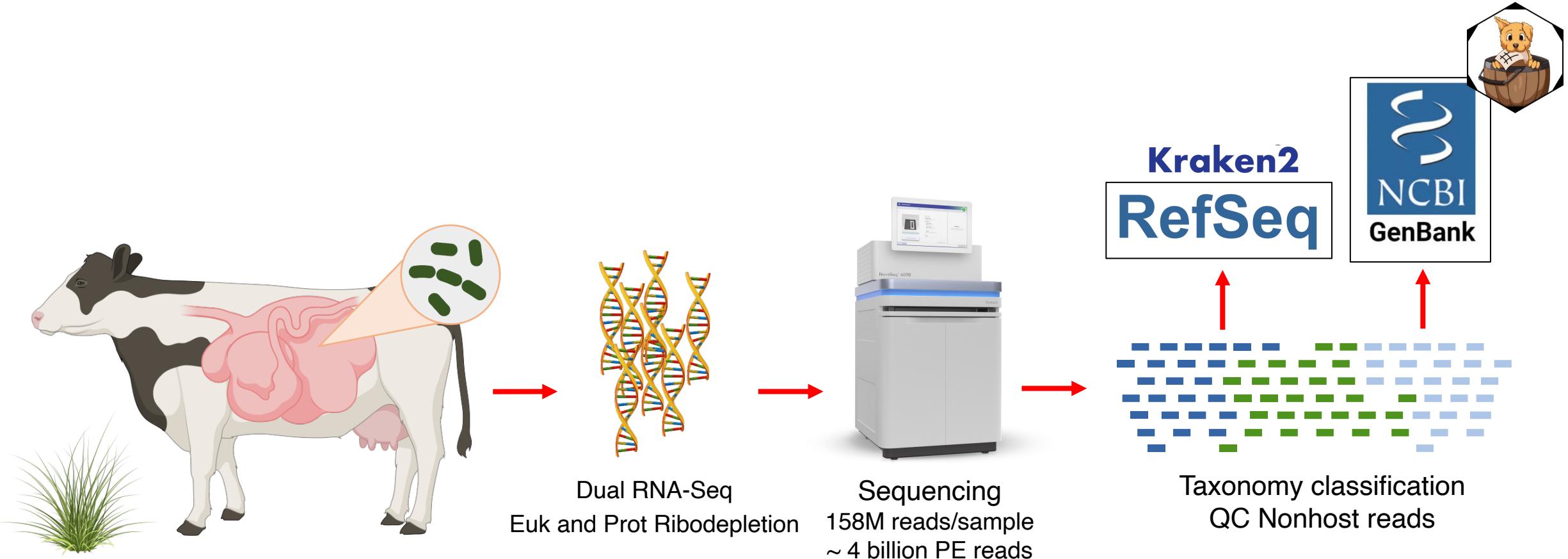
# Targeted Short-chain FA



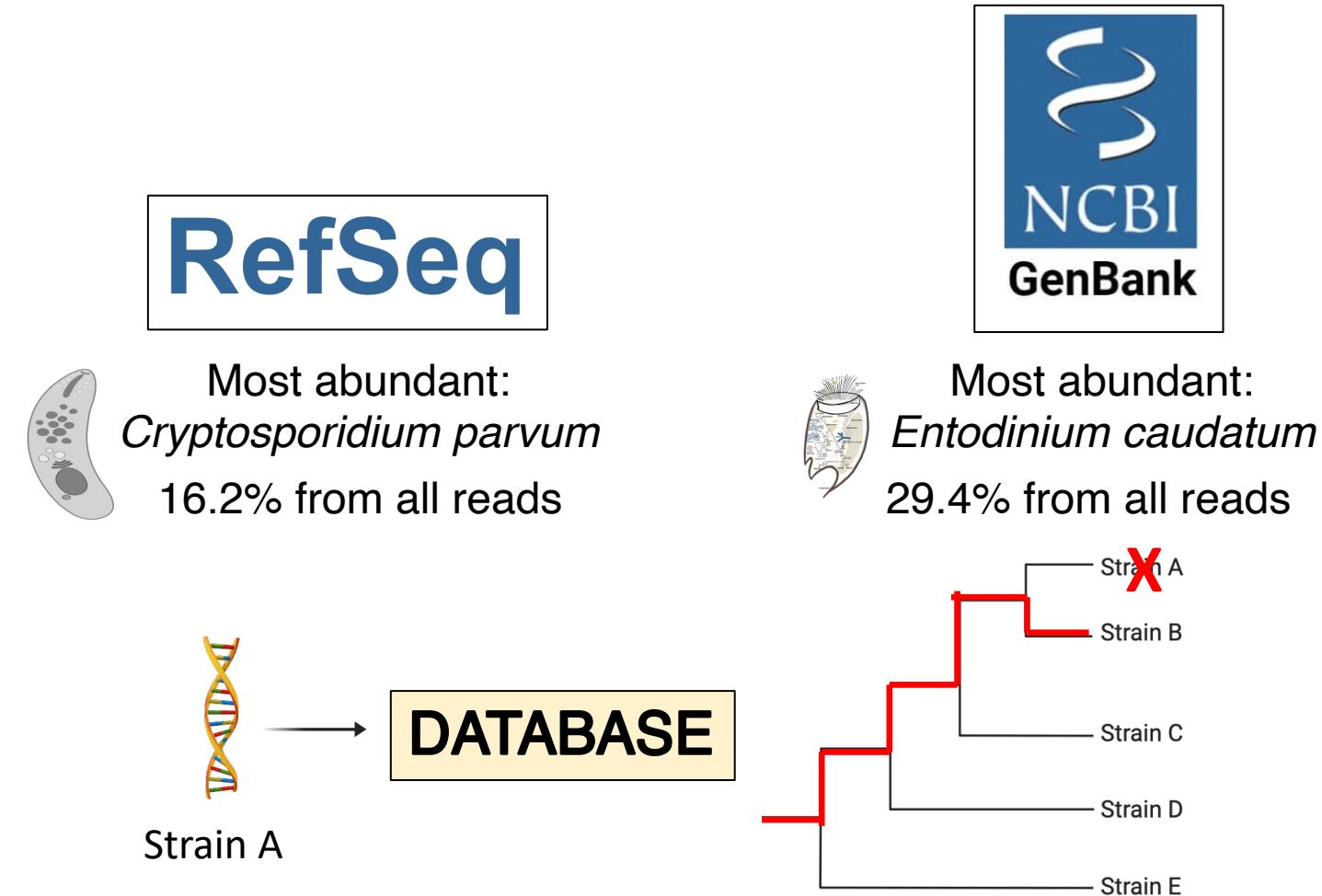
# Untargeted-primary metabolites



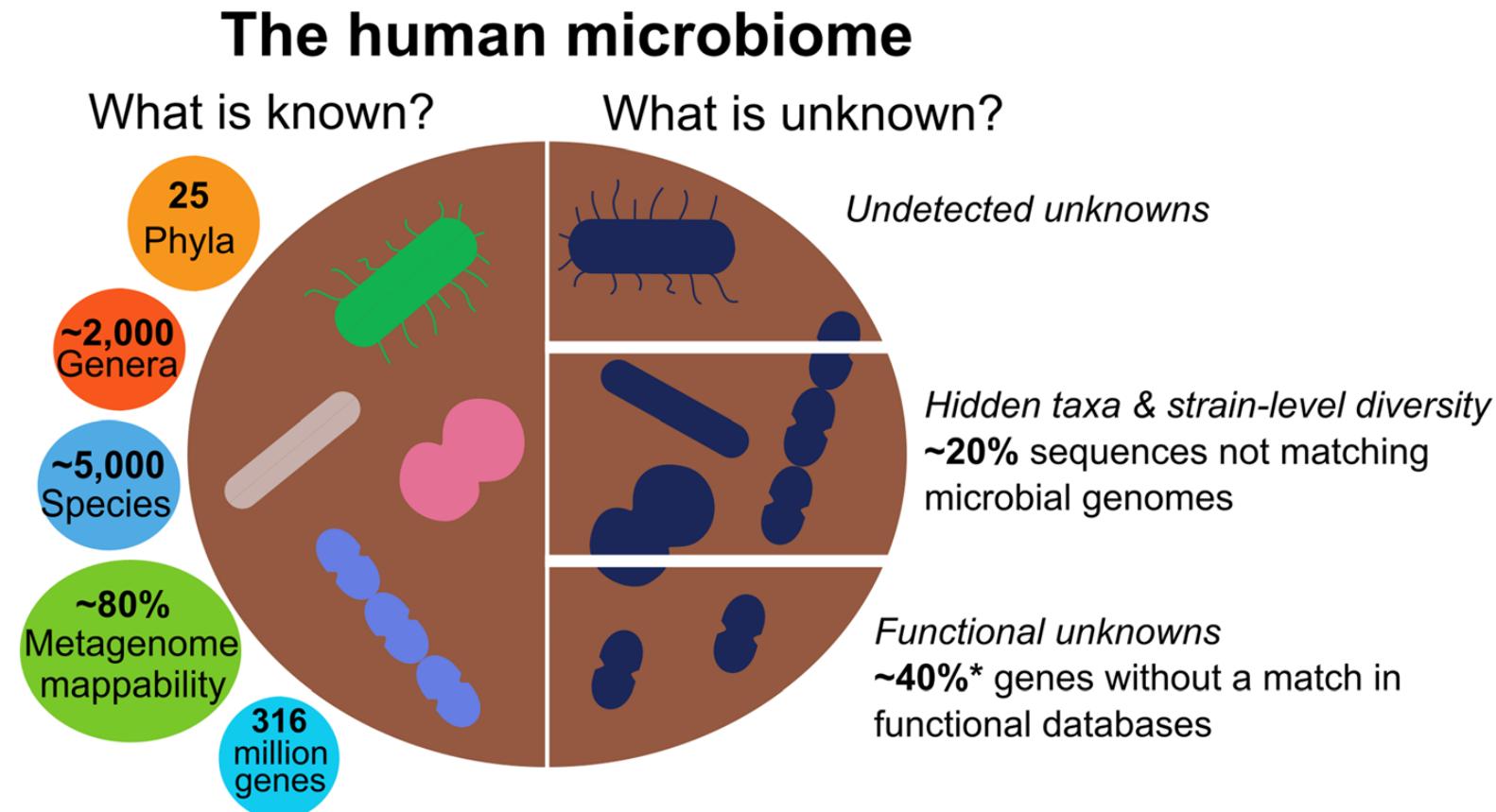
# Characterization of the active rumen microbiome



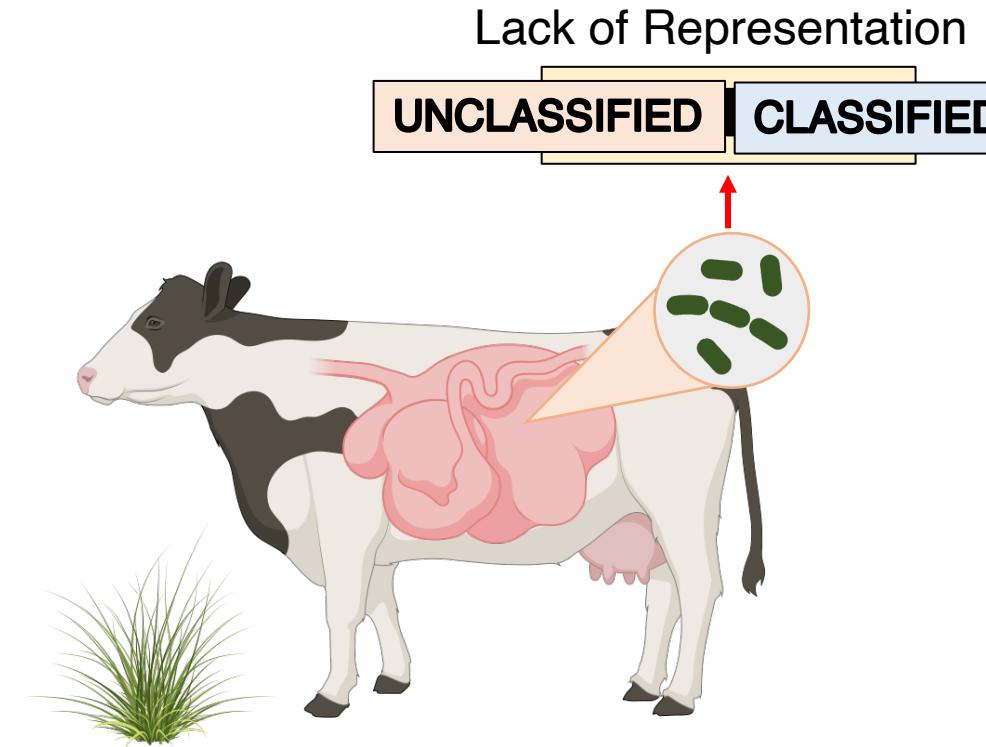
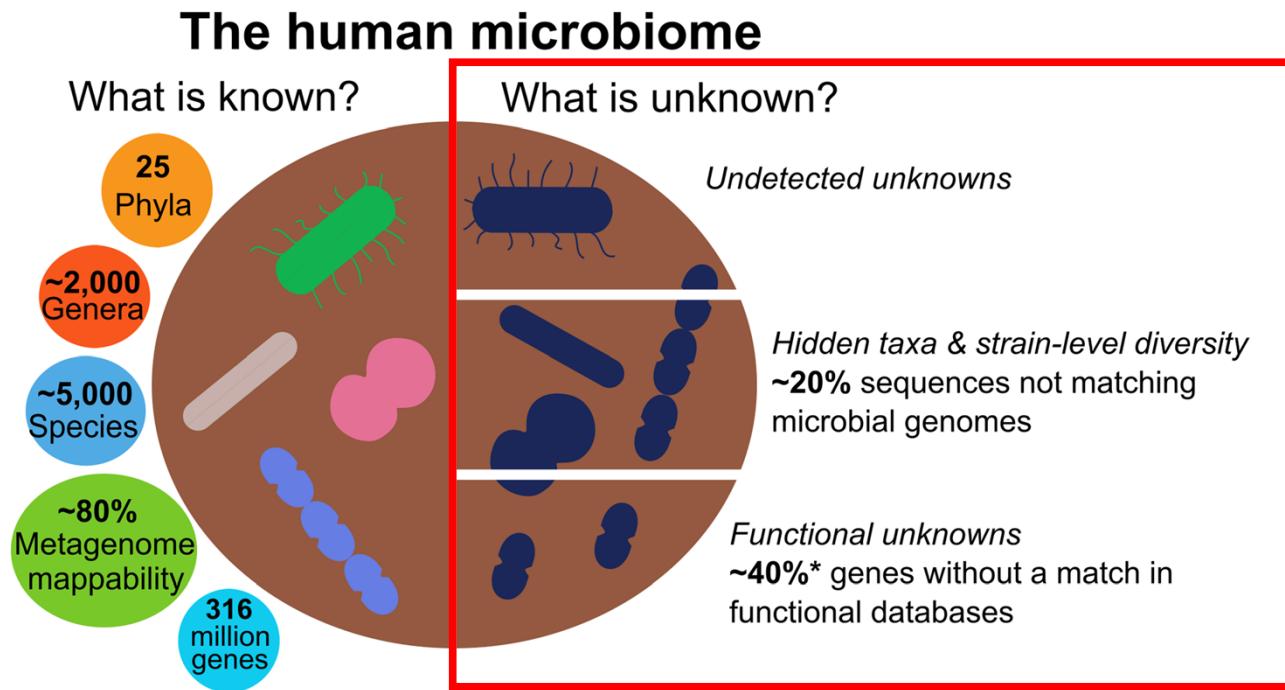
# Impact of genomic database on analysis



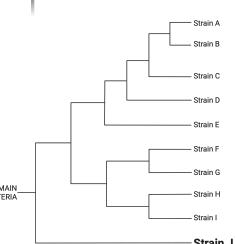
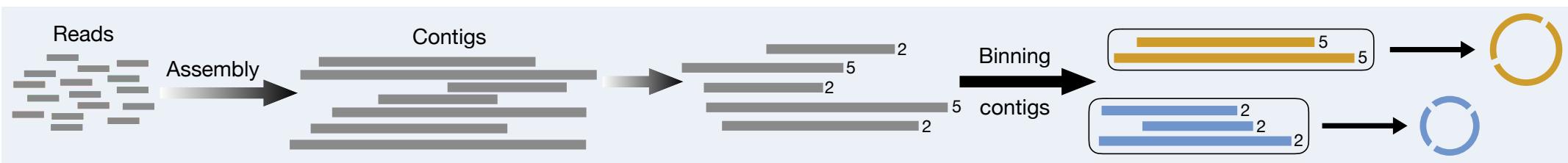
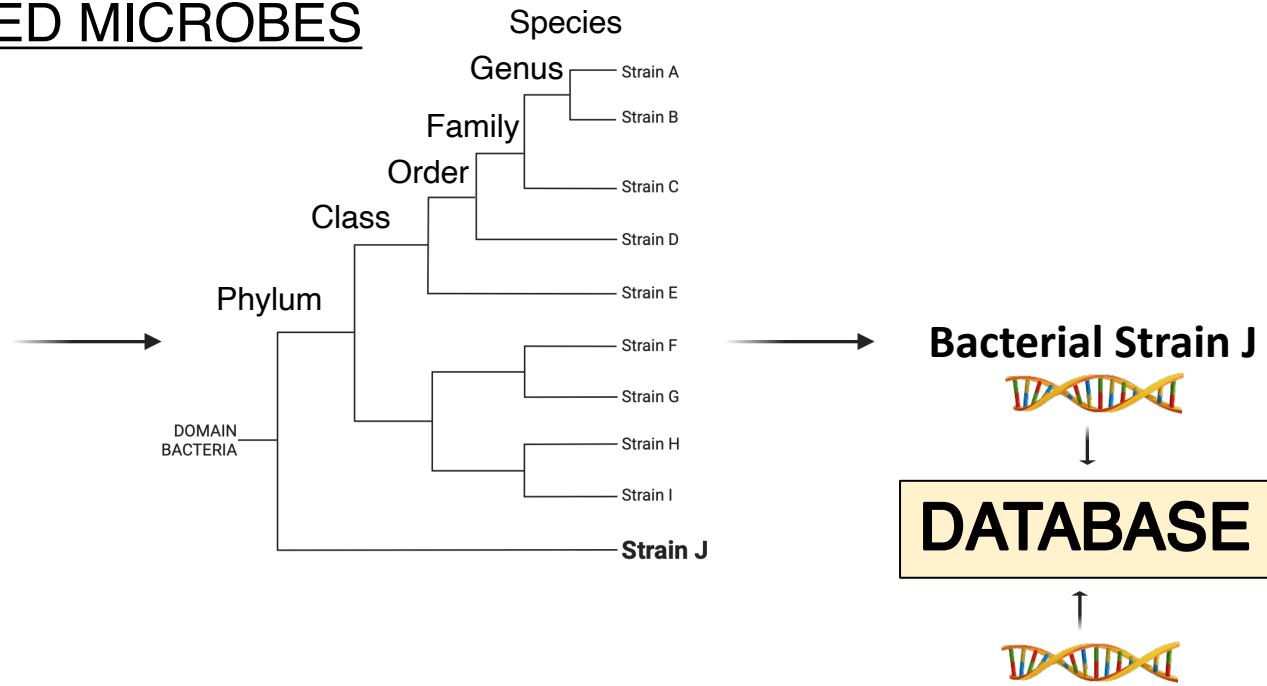
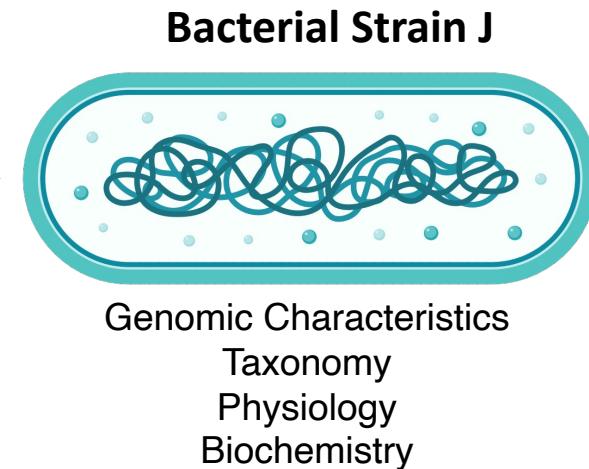
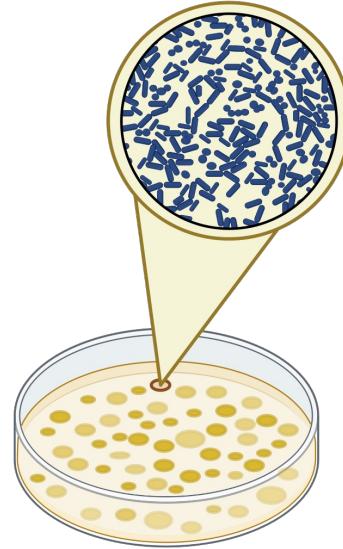
# A considerably well-characterized community



# Challenges of an uncharacterized community



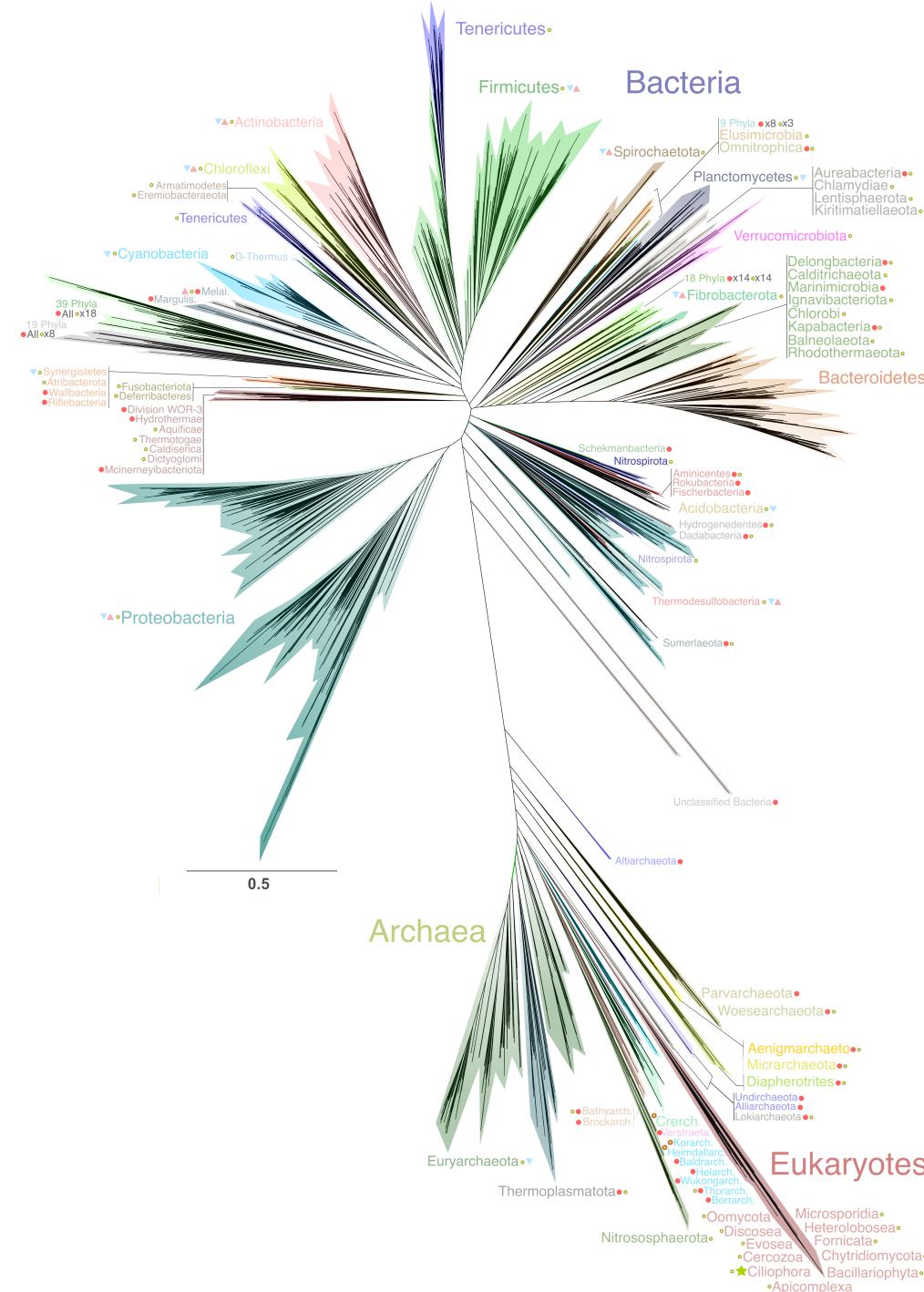
# Comprehensive db is critical to uncharacterized communities



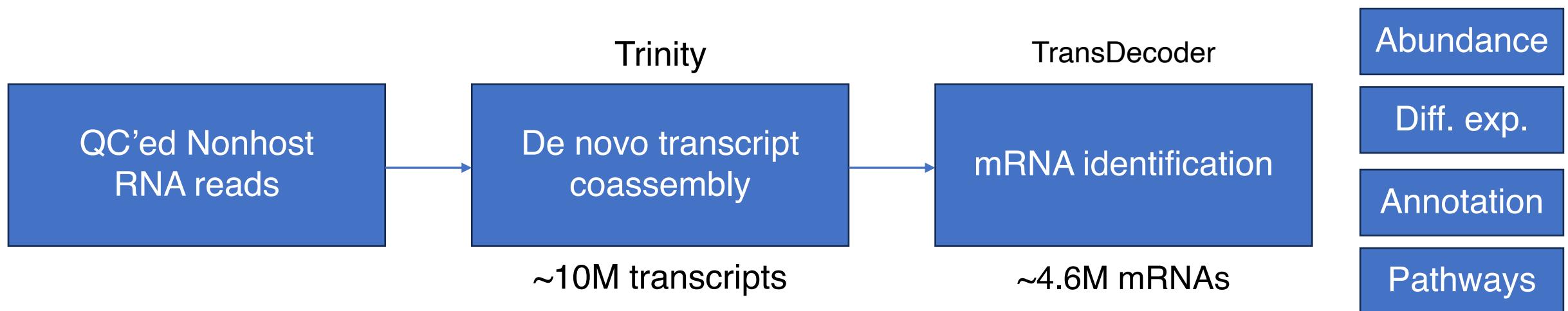
# The active rumen microbiome

Sourmash w/ GenBank

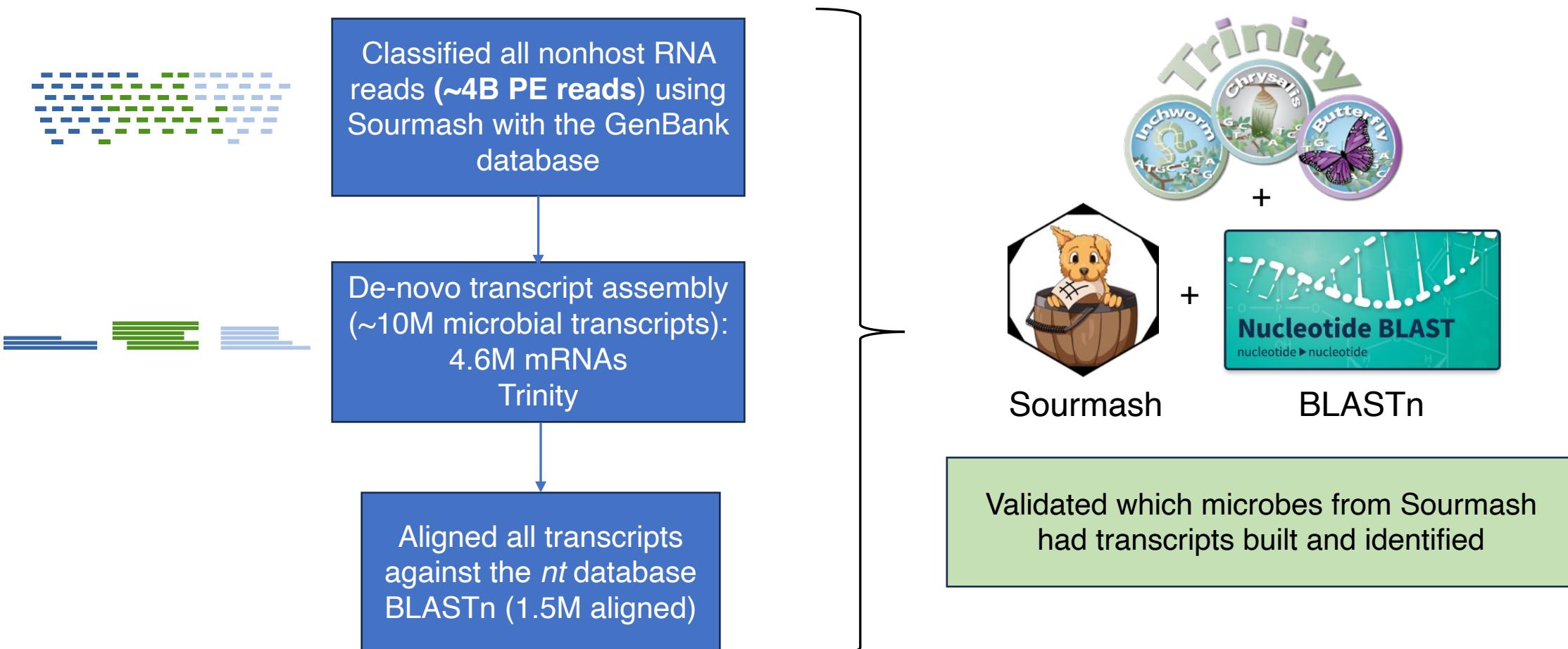
**GTo**  **TREE**



# *De novo* transcript assembly



# Characterization of the rumen microbiome activity



# The active rumen microbiome

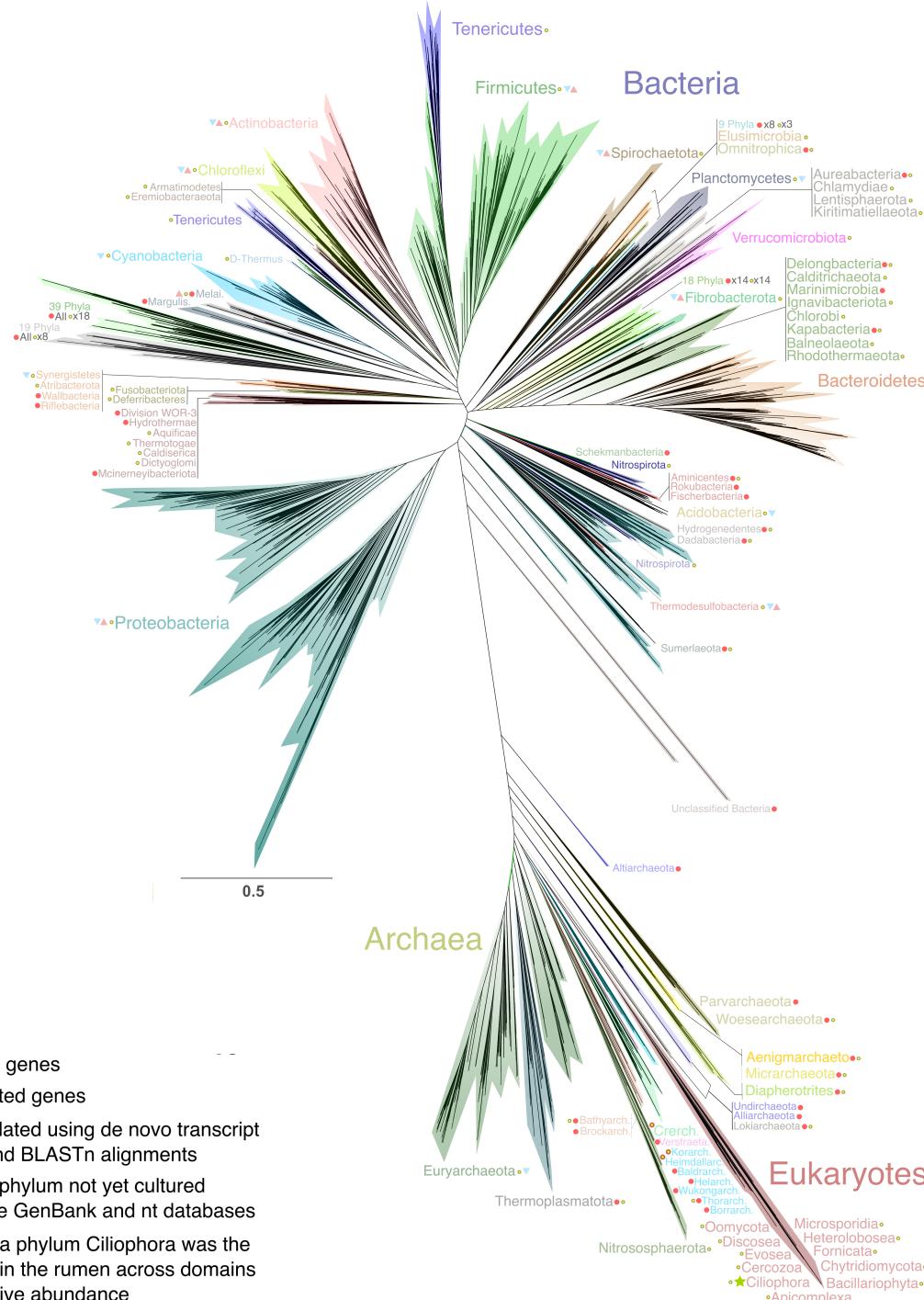
Dual-RNA seq

GT 

RNAseq with Salmon  
and Deseq2

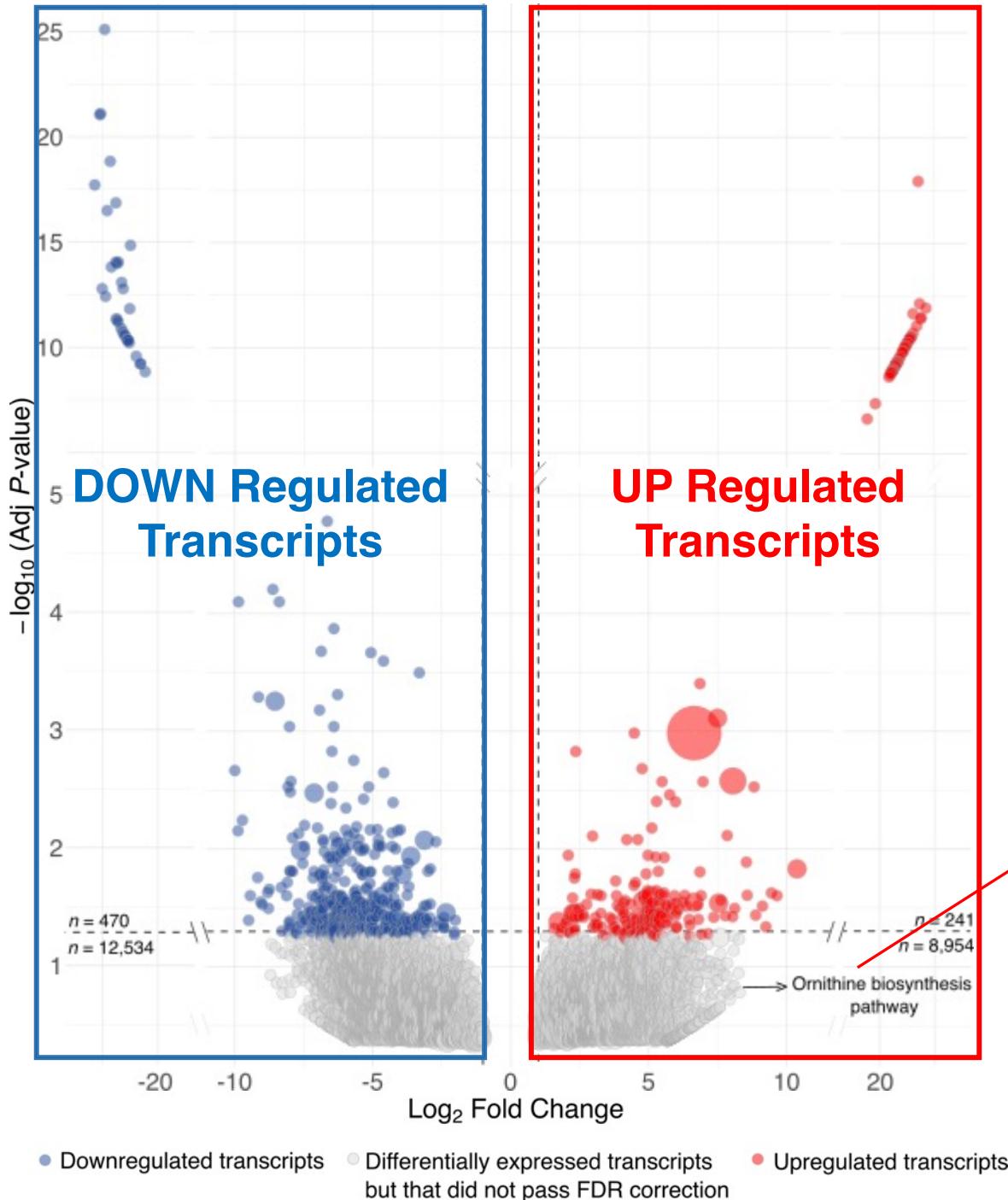


- ▲ Upregulated genes
- ▼ Downregulated genes
- Furhter validated using de novo transcript assembly and BLASTn alignments
- Candidatus phylum not yet cultured based on the GenBank and nt databases
- ★ The protozoa phylum Ciliophora was the most active in the rumen across domains at 30% relative abundance



## Feed efficient vs. Inefficient cows

Dual-RNA seq



Pathway Analysis:



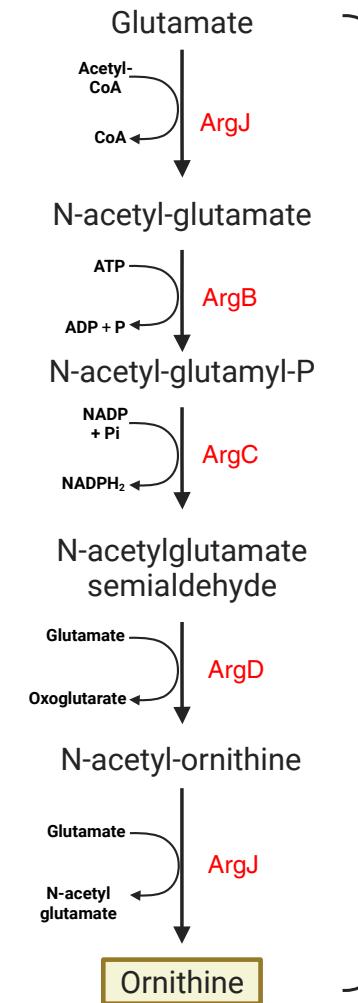
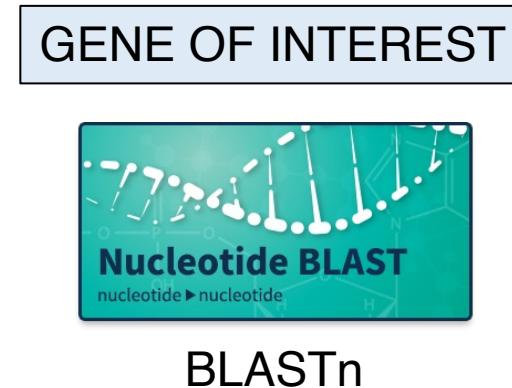
**BIOCYC**  
Genome Database Collection

Ornithine biosynthesis  
Arginine biosynthesis

Open final  
annotation file

# Upregulated ornithine biosynthesis pathway

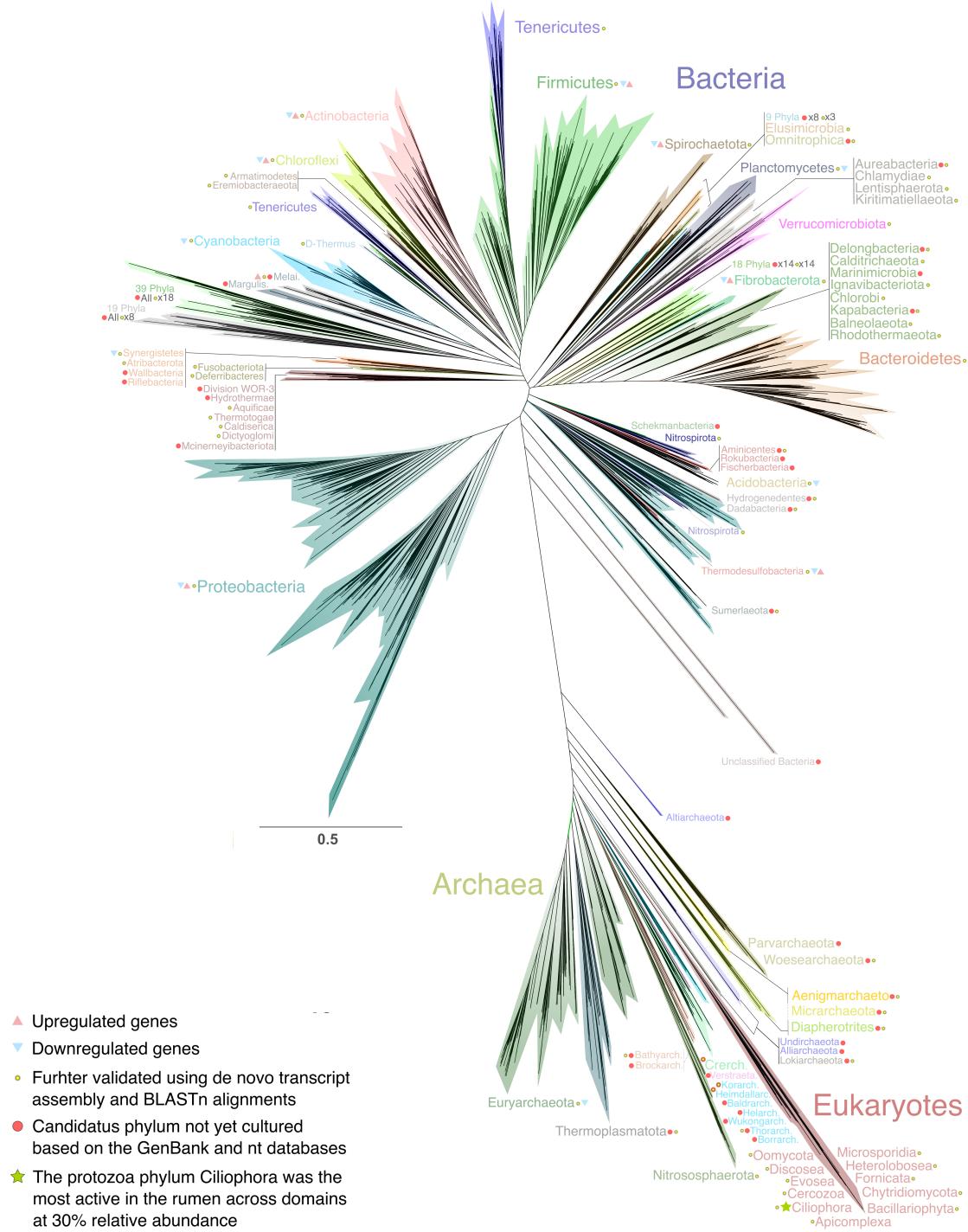
Dual-RNA seq



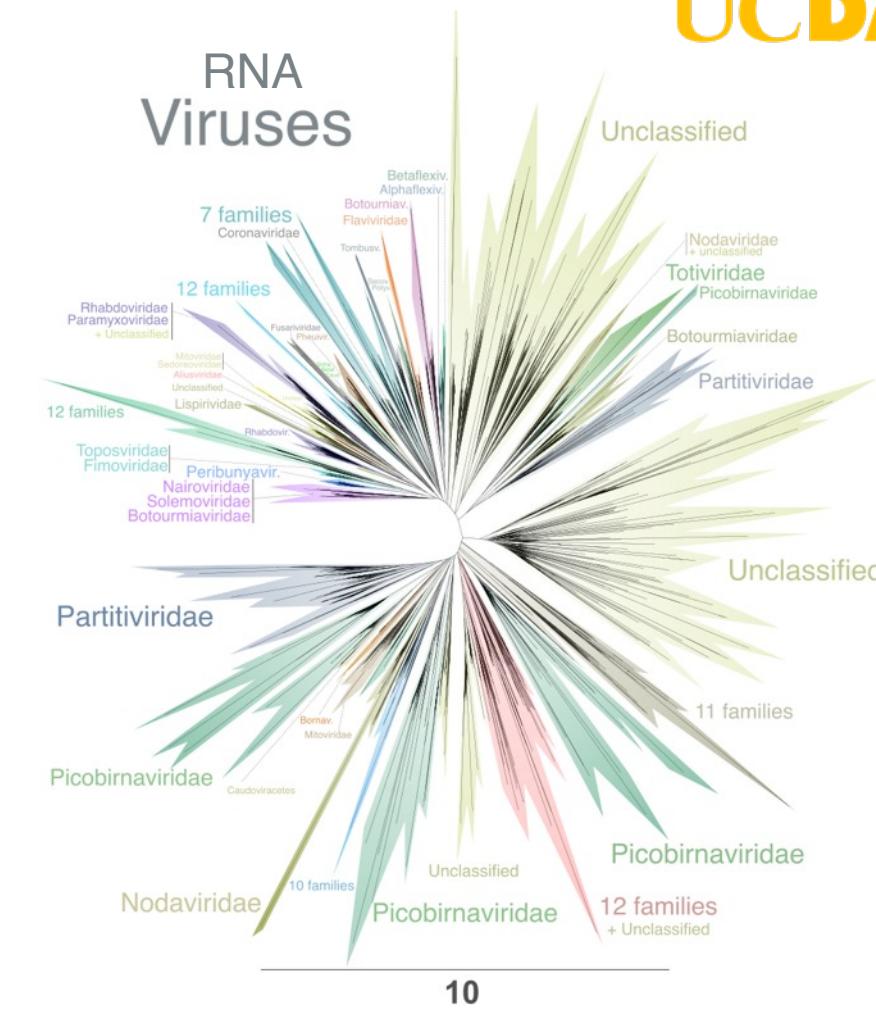
*Uncultured Ruminococcus*  
*Ruminococcus* sp. JE7B6  
*Ruminococcus bovis* strain JEA12



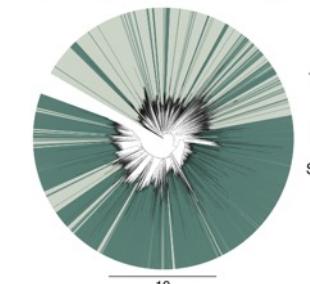
*Segatella copri*



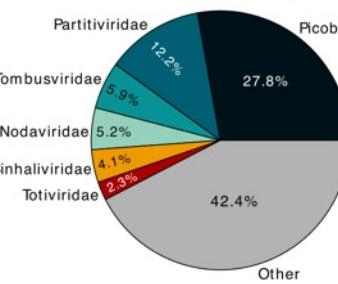
## RNA Viruses



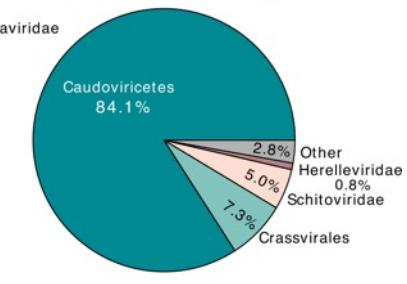
■ RefSeq RdRp ■ Novel RdRp



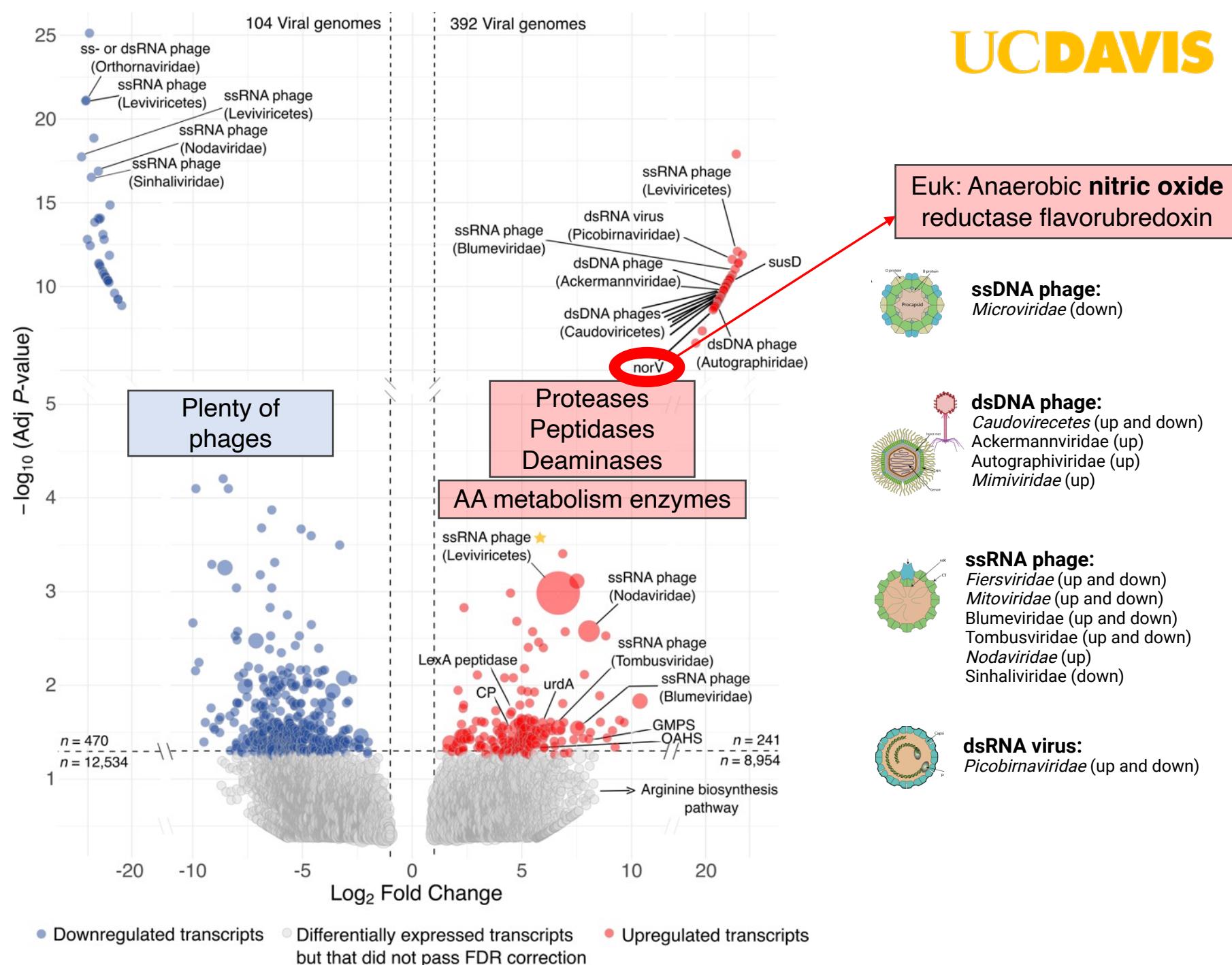
### RNA Viral Family



### Active DNA Viral Family

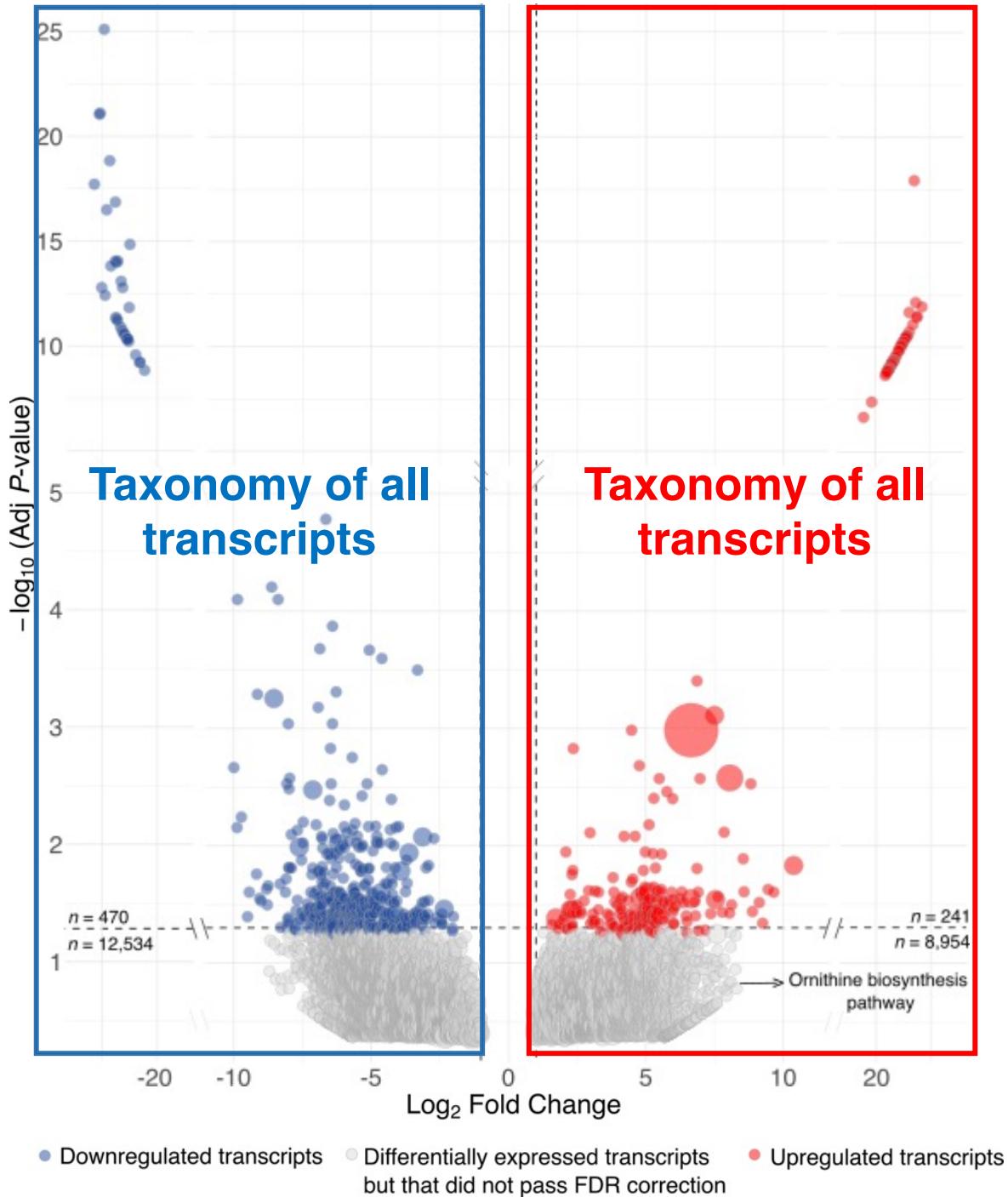


# Phages as greatest rumen predators



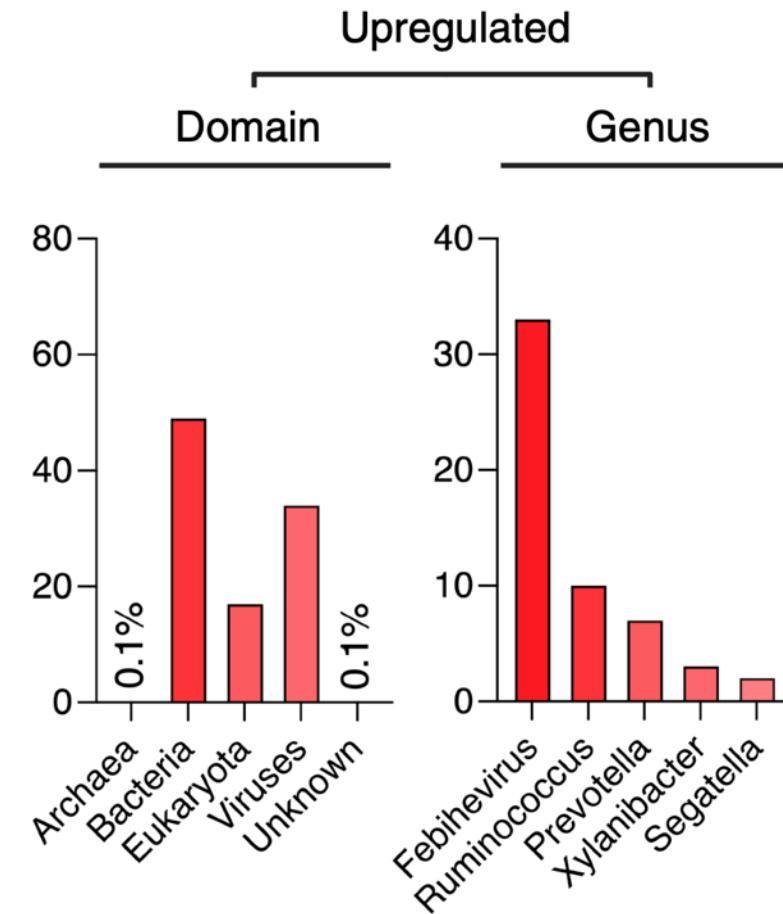
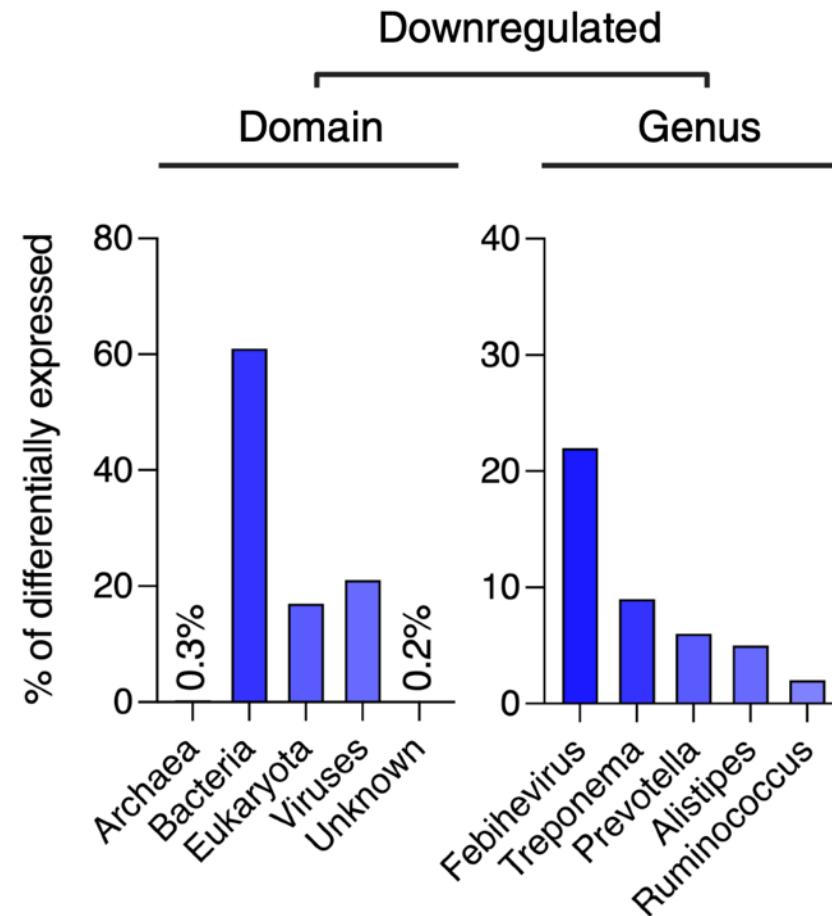
# Feed efficient vs. Inefficient cows

Dual-RNA seq



BLASTn

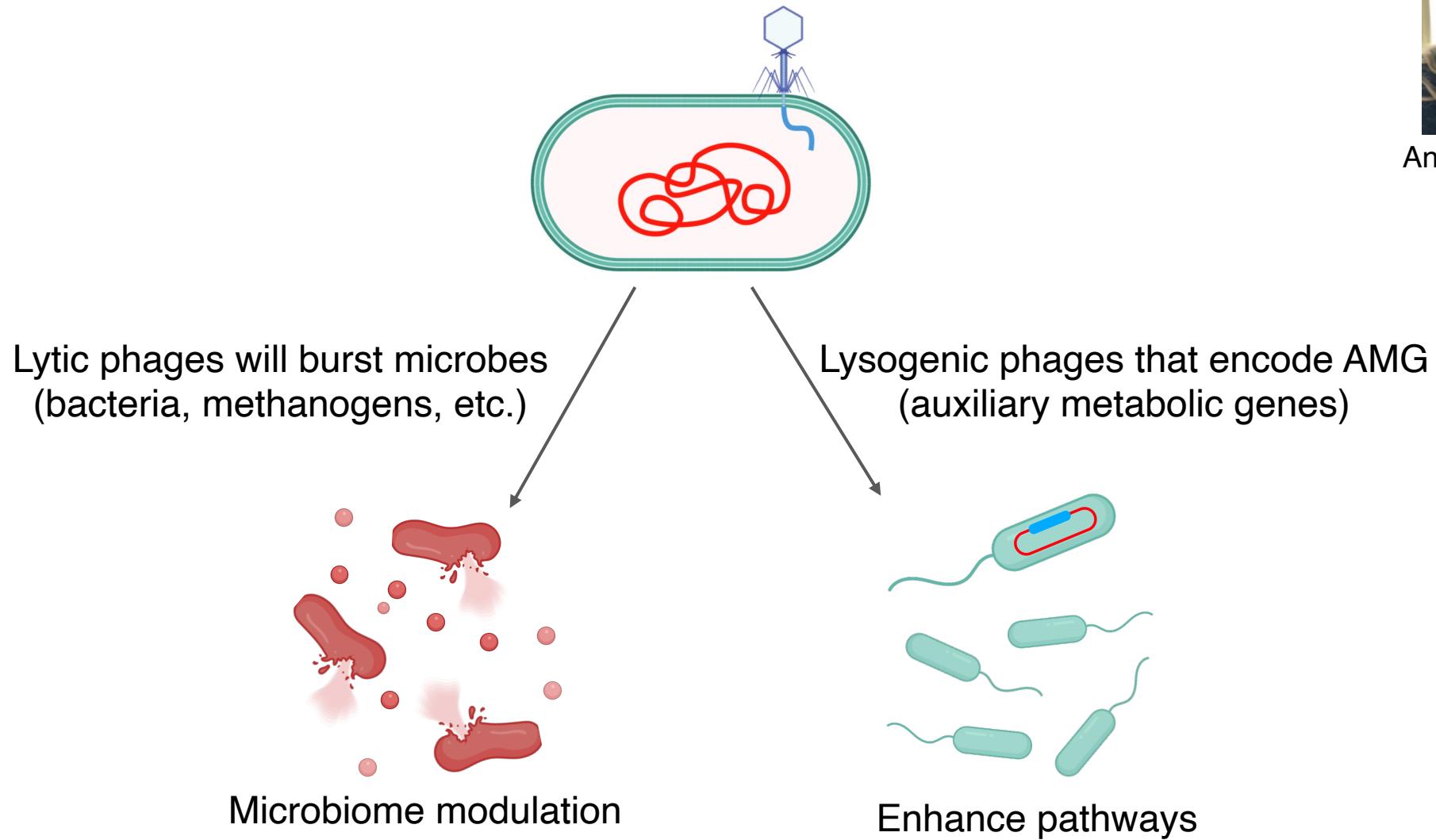
# Taxonomy of diff. expressed genes



# A viral shunt as an explanation to differences found in feed efficiency



Anneliek ter Horst  
UC Davis





## Review

# Phage therapy: From biological mechanisms to future directions

Steffanie A. Strathdee,<sup>1,\*</sup> Graham F. Hatfull,<sup>2</sup> Vivek K. Mutalik,<sup>3</sup> and Robert T. Schooley<sup>1</sup>

<sup>1</sup>Center for Innovative Phage Applications and Therapeutics, Division of Infectious Disease and Global Public Health, University of California, San Diego, La Jolla, CA 92093-0507, USA

<sup>2</sup>Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

<sup>3</sup>Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

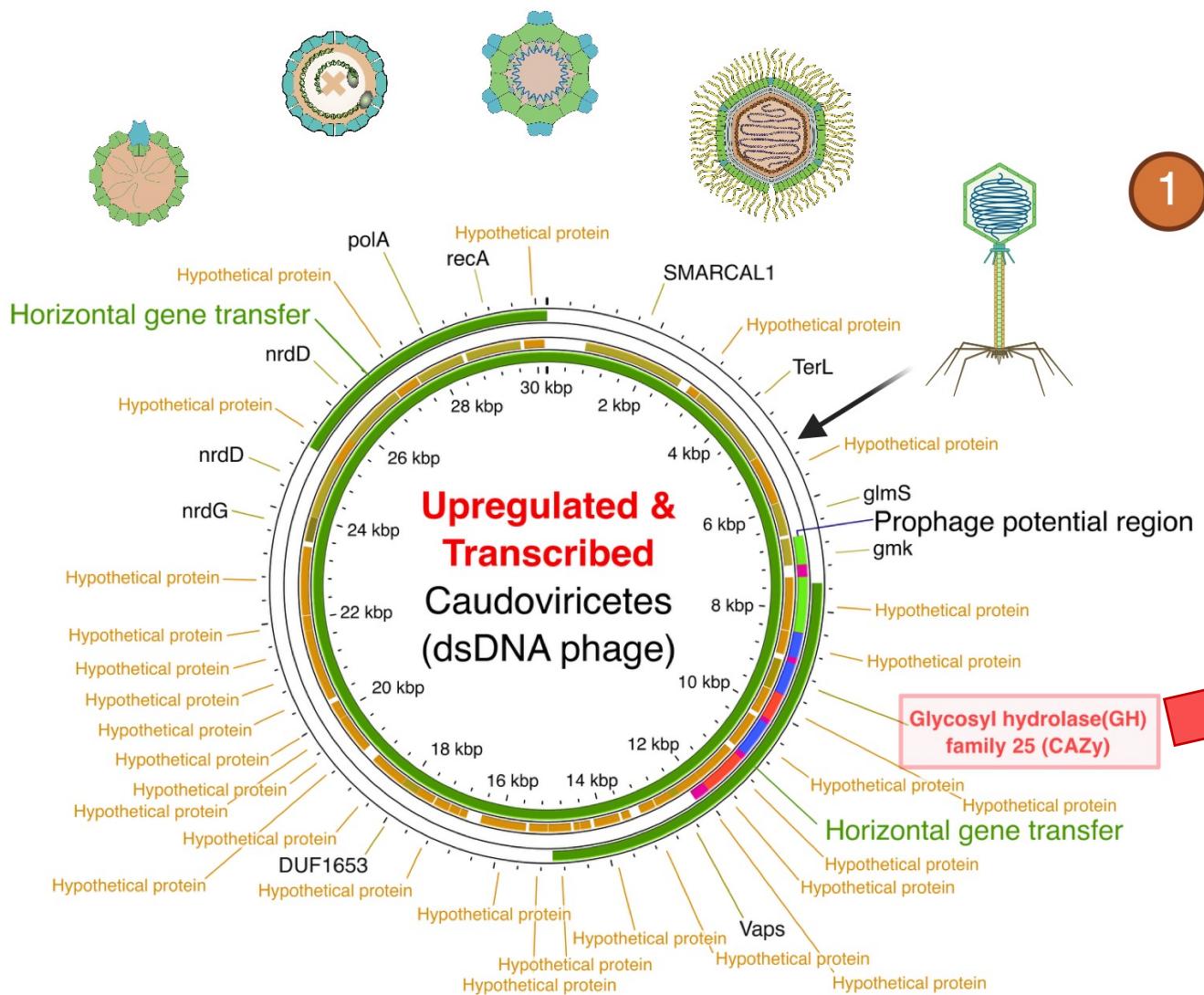
\*Correspondence: [sstrathdee@health.ucsd.edu](mailto:sstrathdee@health.ucsd.edu)

<https://doi.org/10.1016/j.cell.2022.11.017>

## SUMMARY

Increasing antimicrobial resistance rates have revitalized bacteriophage (phage) research, the natural predators of bacteria discovered over 100 years ago. In order to use phages therapeutically, they should (1) preferably be lytic, (2) kill the bacterial host efficiently, and (3) be fully characterized to exclude side effects. Developing therapeutic phages takes a coordinated effort of multiple stakeholders. Herein, we review the state of the art in phage therapy, covering biological mechanisms, clinical applications, remaining challenges, and future directions involving naturally occurring and genetically modified or synthetic phages.

# The Rumen Environment



1

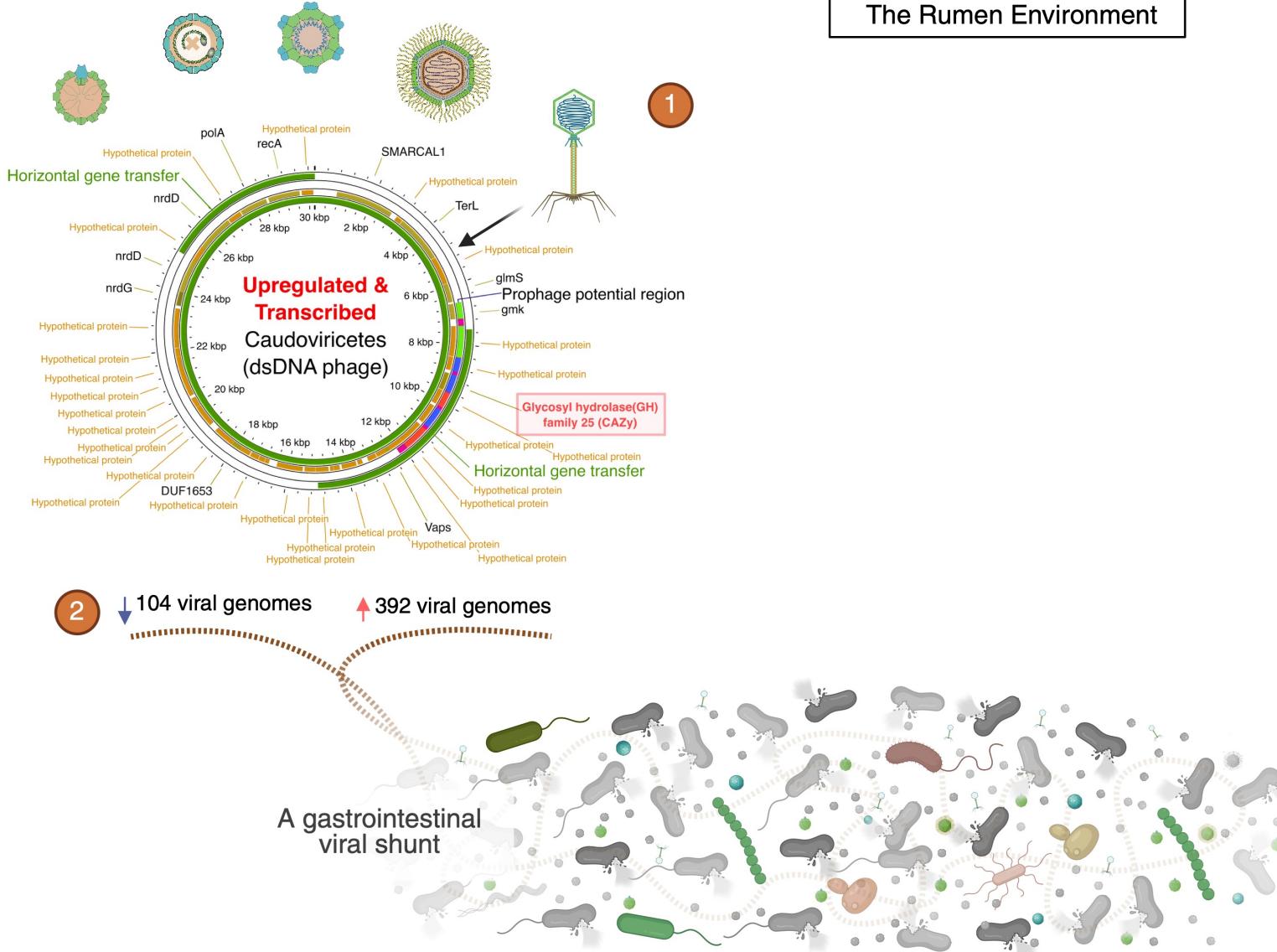
## **ssDNA phage:** *Microviridae* (down)

- dsDNA phage:
  - Caudoviretes* (up and down)
  - Ackermannviridae* (up)
  - Autographiviridae* (up)
  - Mimiviridae* (up)

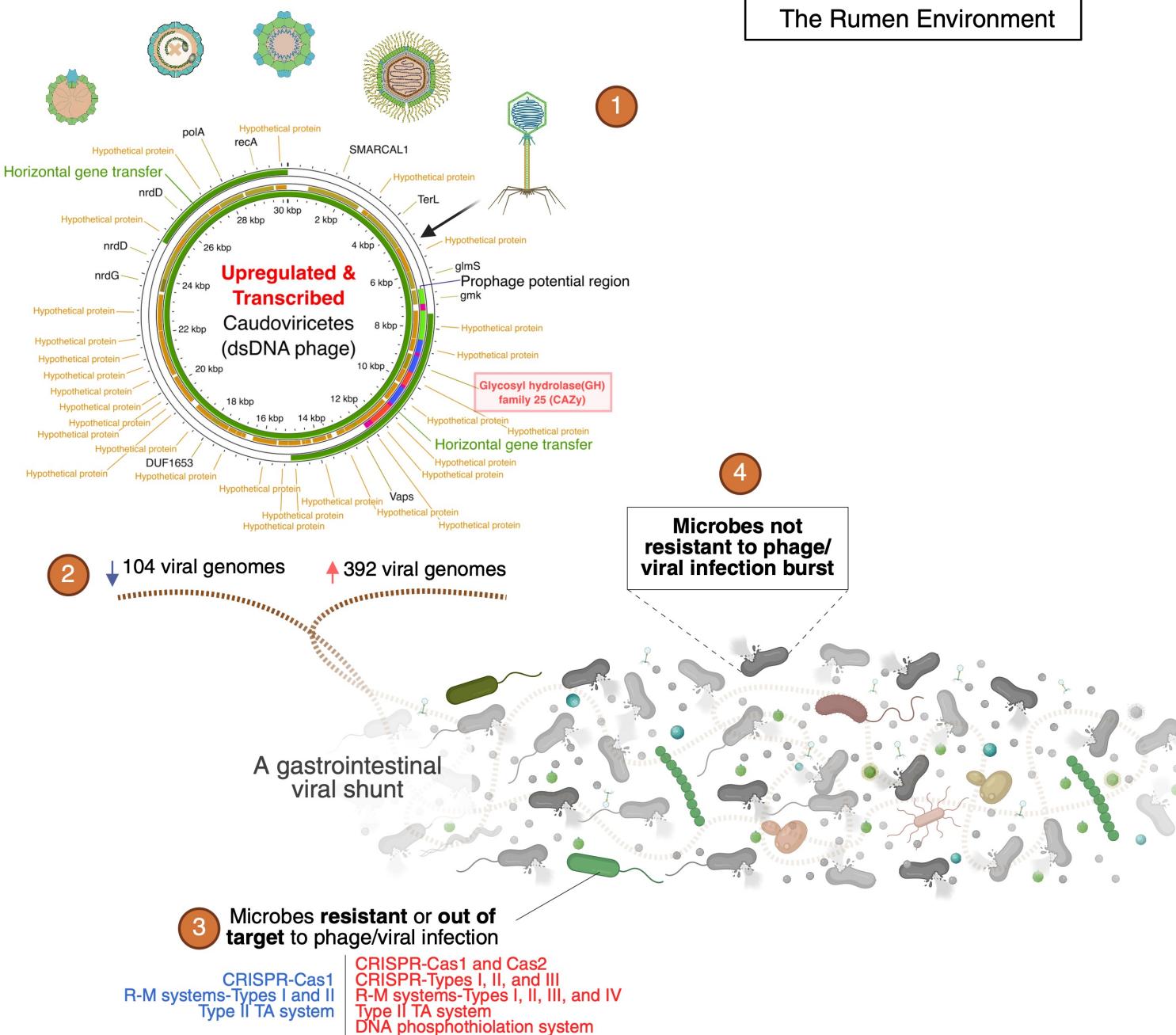
**SSRNA phage:**  
*Fiersviridae* (up and down)  
*Mitoviridae* (up and down)  
*Blumeviridae* (up and down)  
*Tombusviridae* (up and down)  
*Nodaviridae* (up and down)  
*Sinhaliviridae* (down)

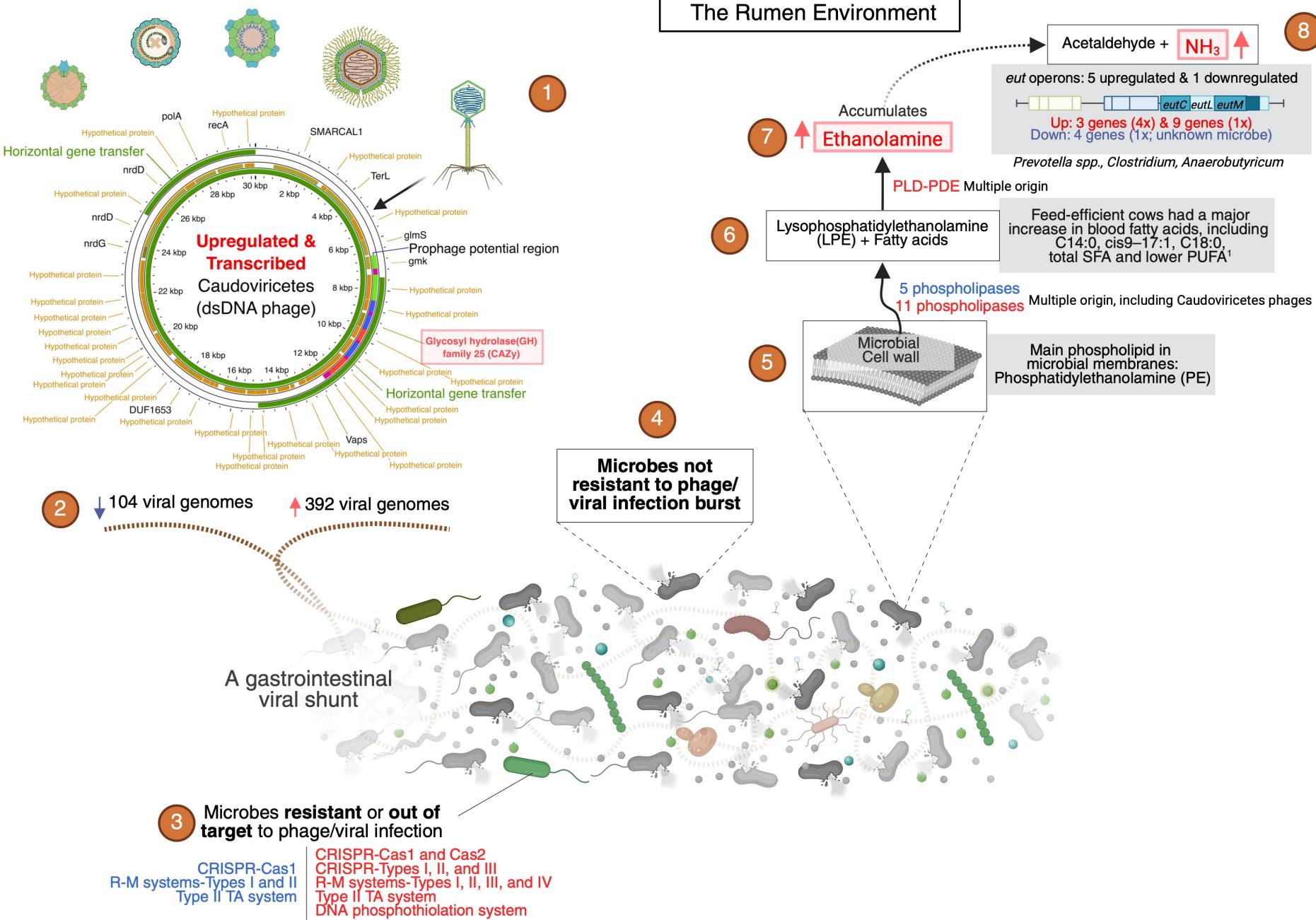
**dsRNA virus:**  
*Picobirnaviridae* (up and down)

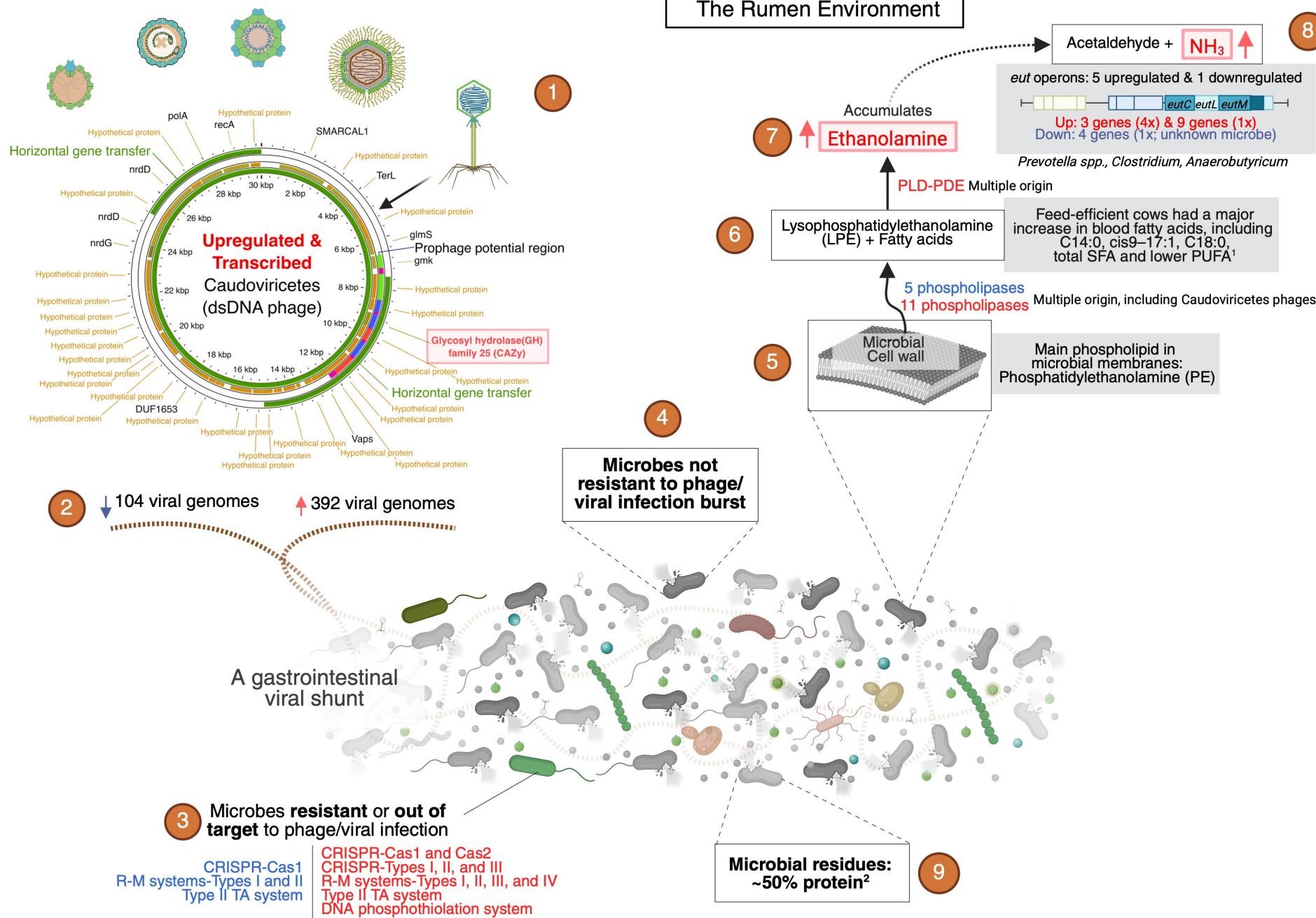
## The Rumen Environment

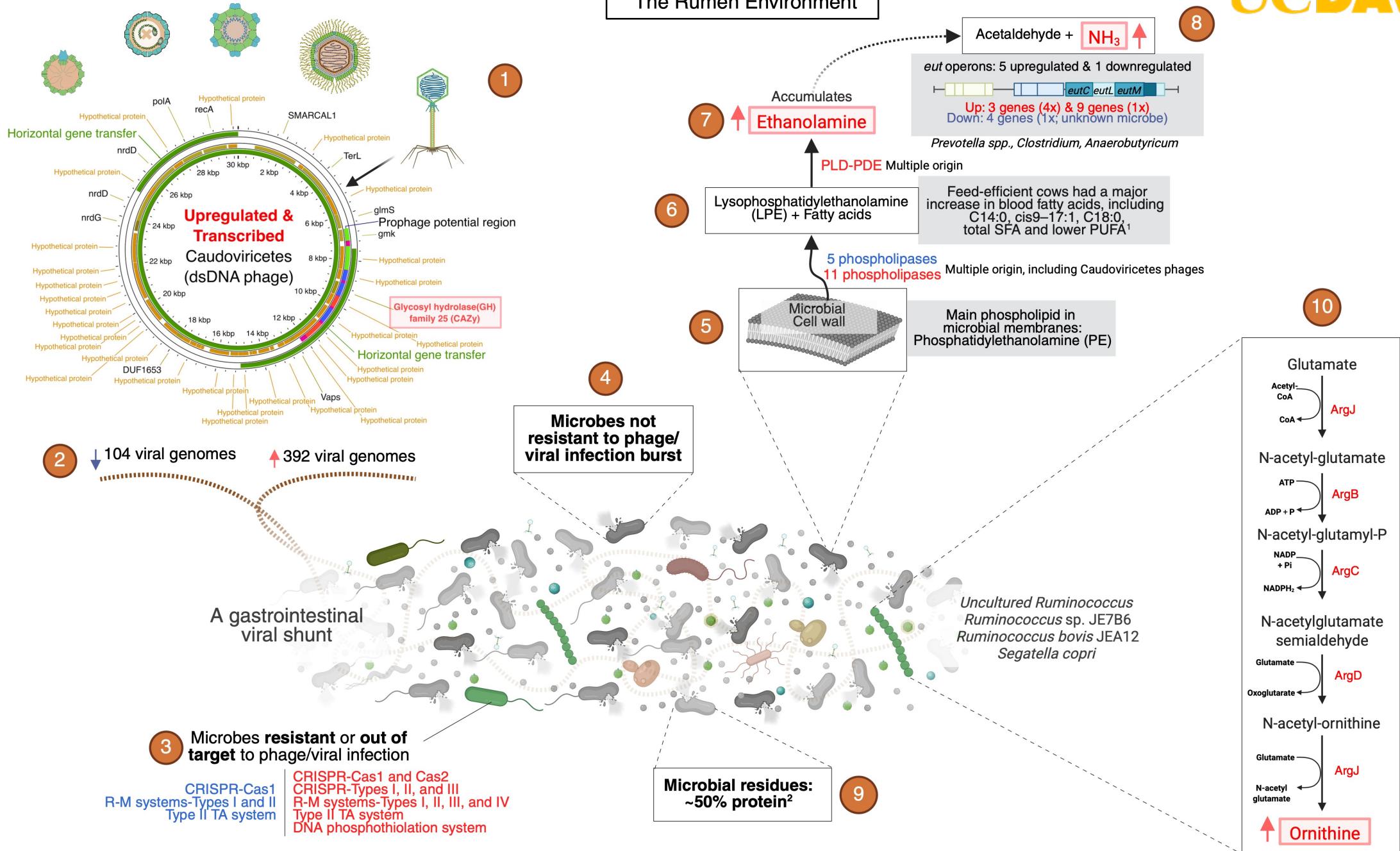


## The Rumen Environment

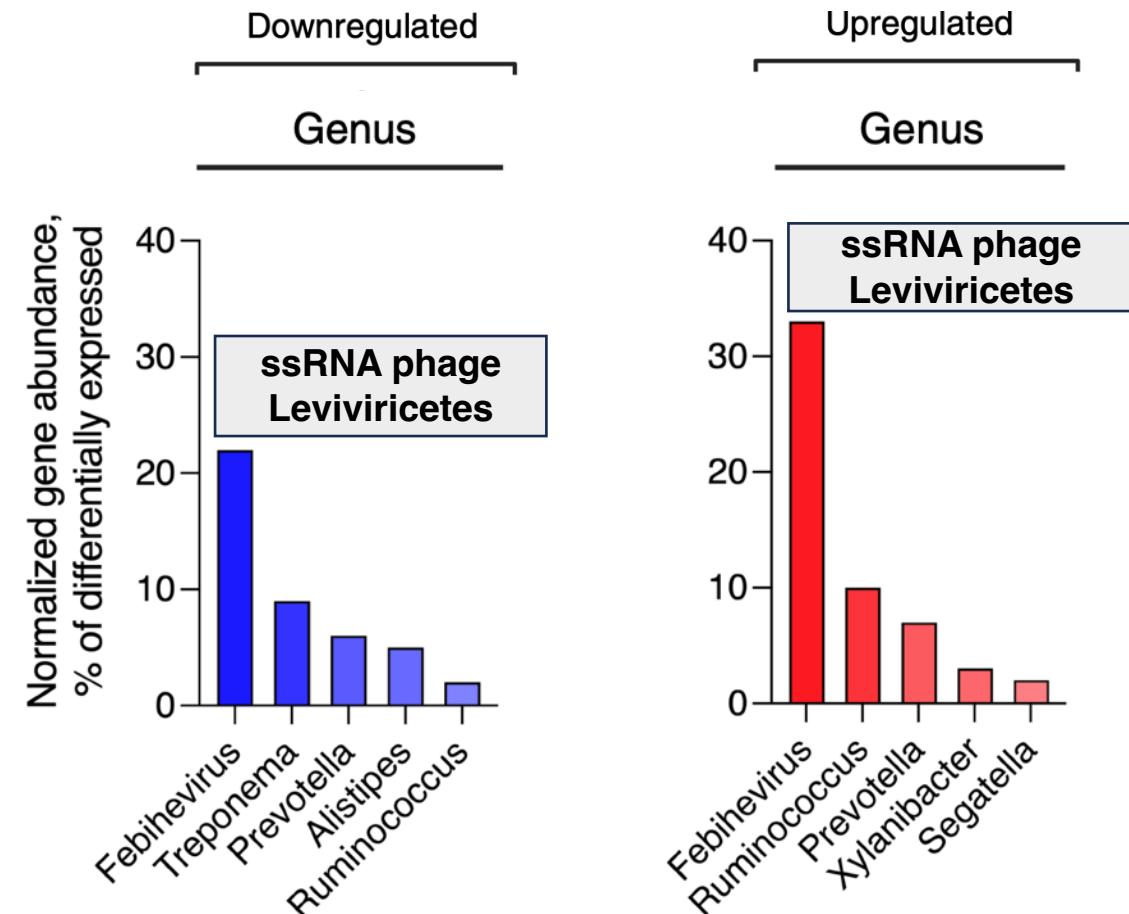
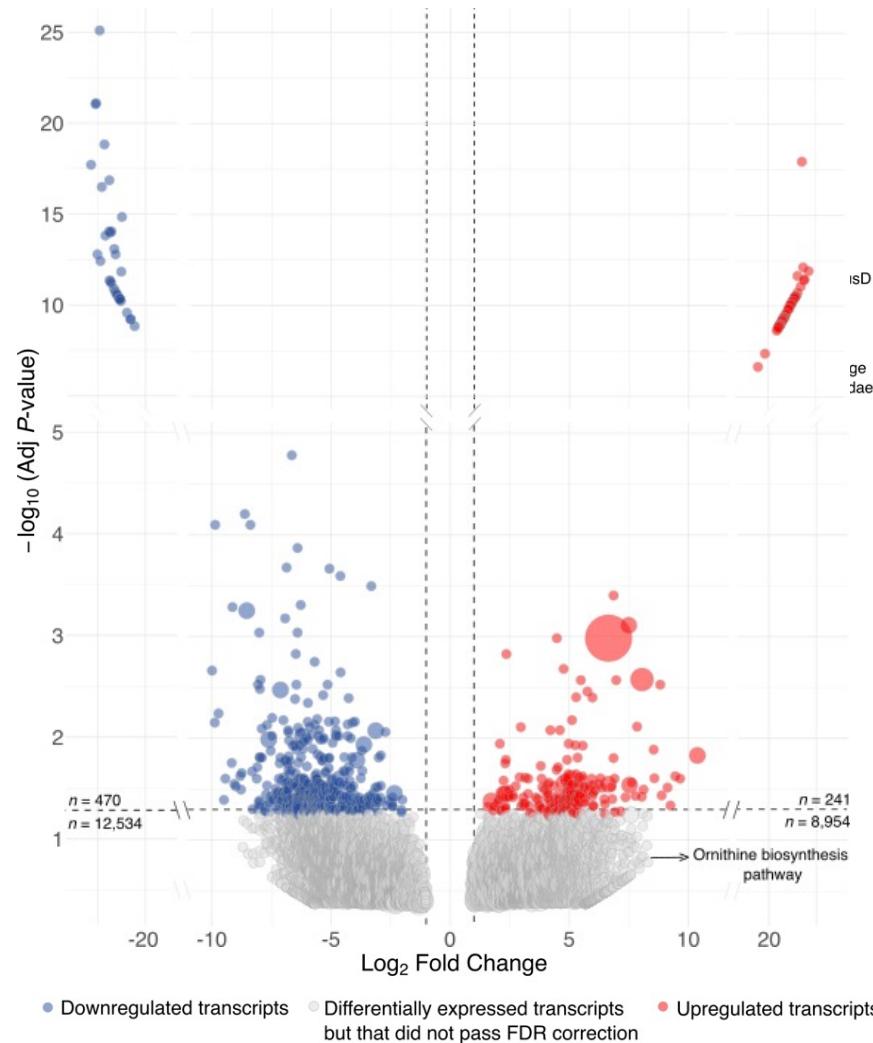




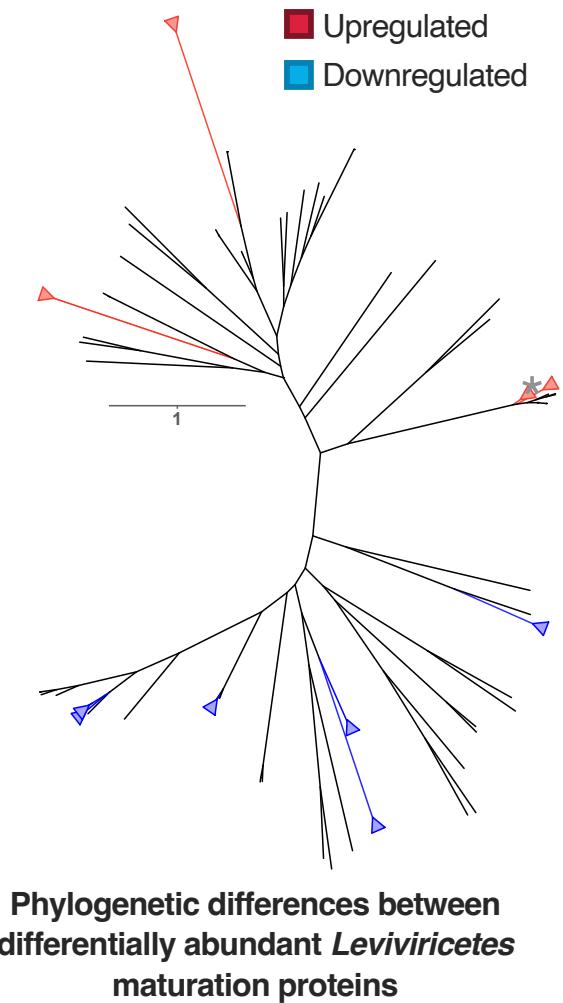
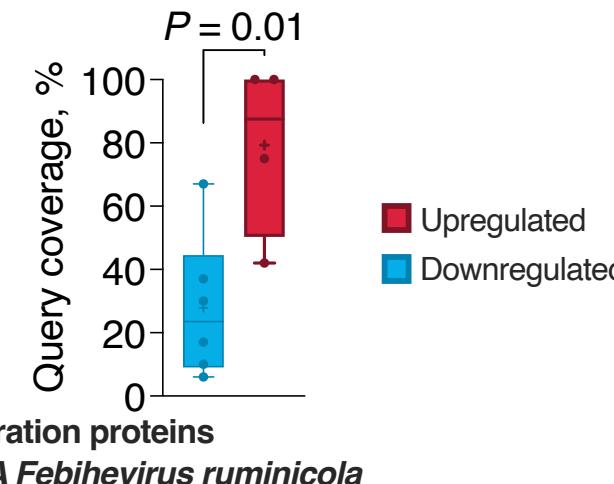
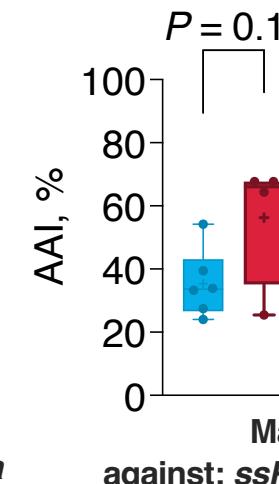
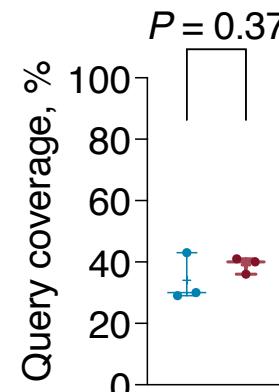
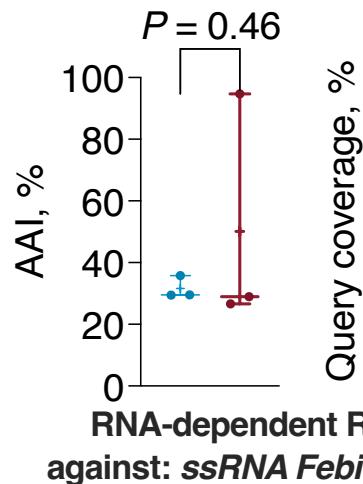
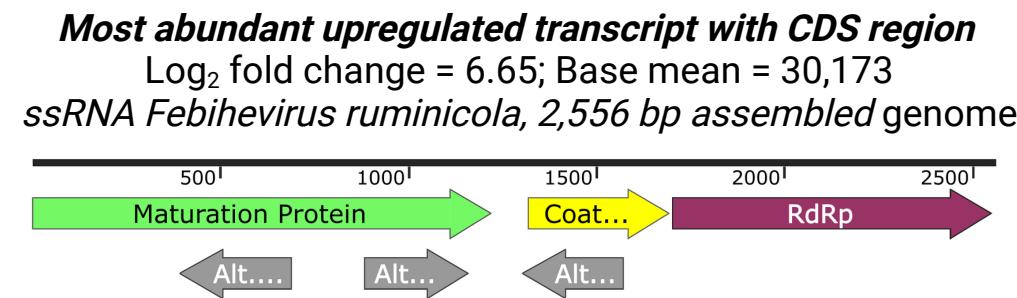
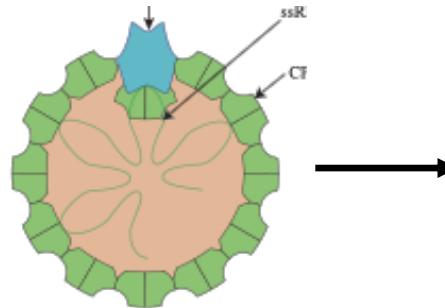




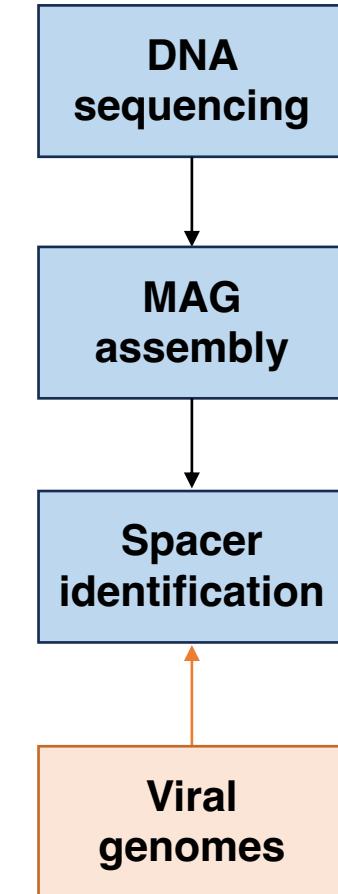
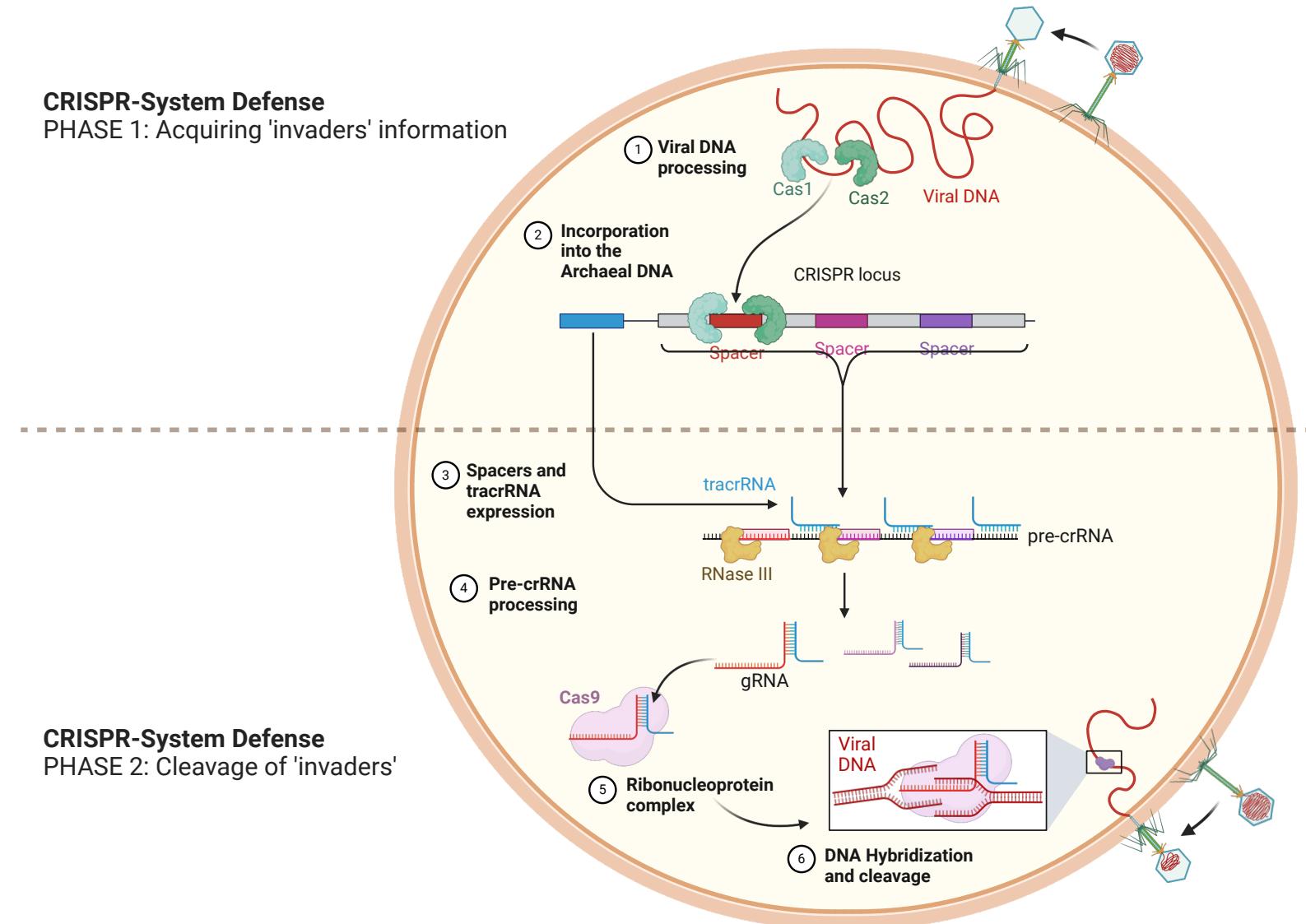
# Summary of taxonomy from diff. expression



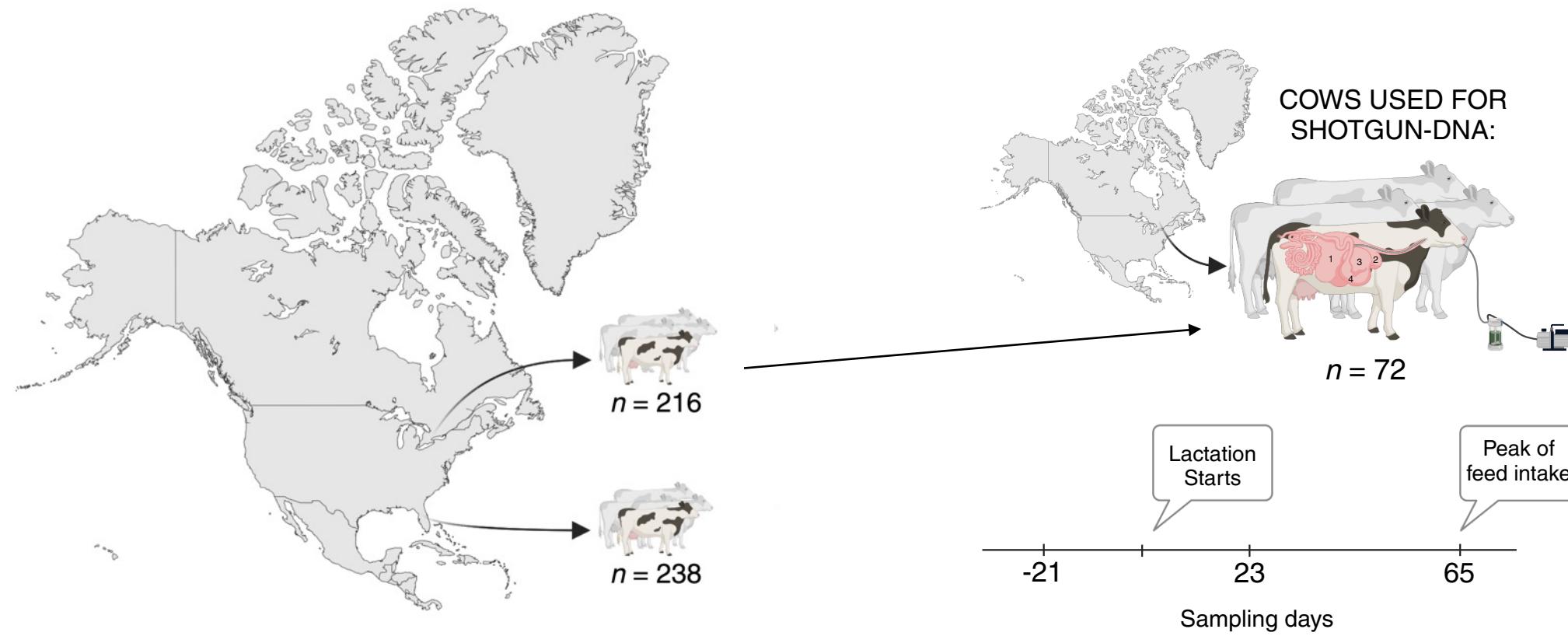
# A look into the most abundant ssRNA phage in the rumen



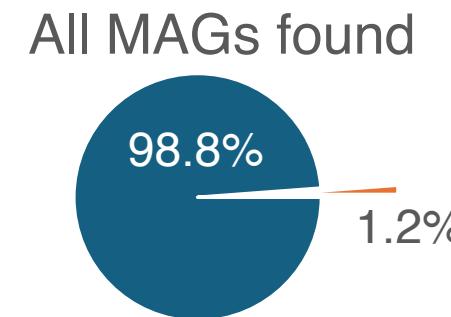
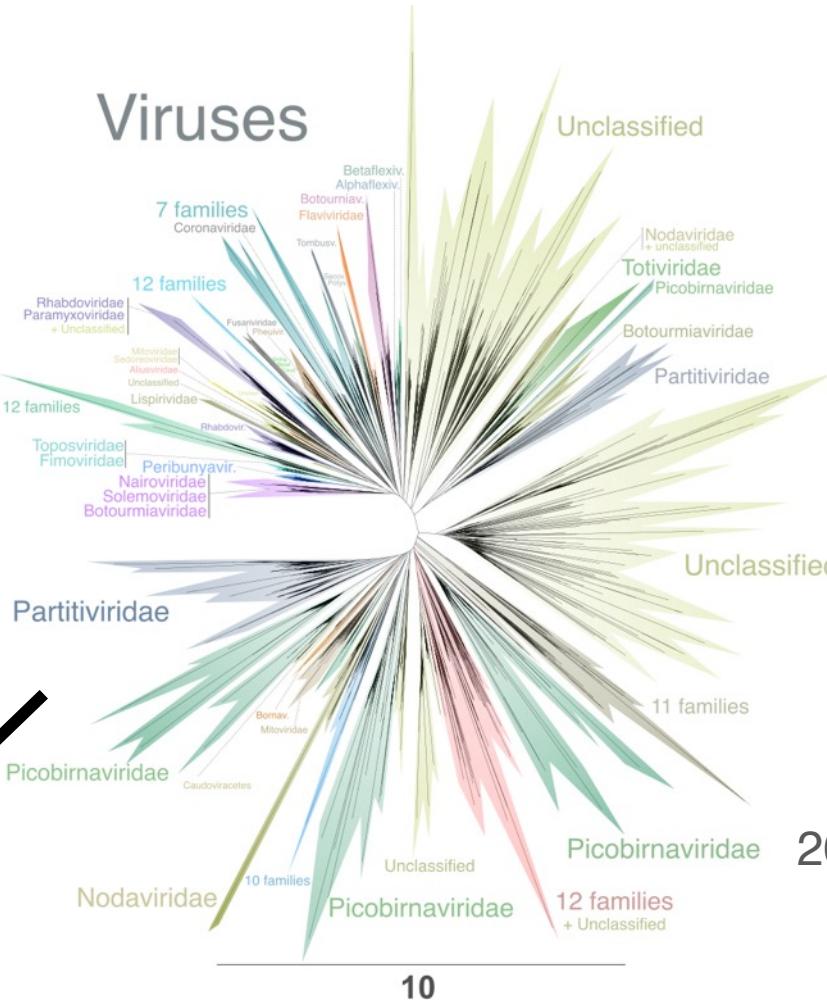
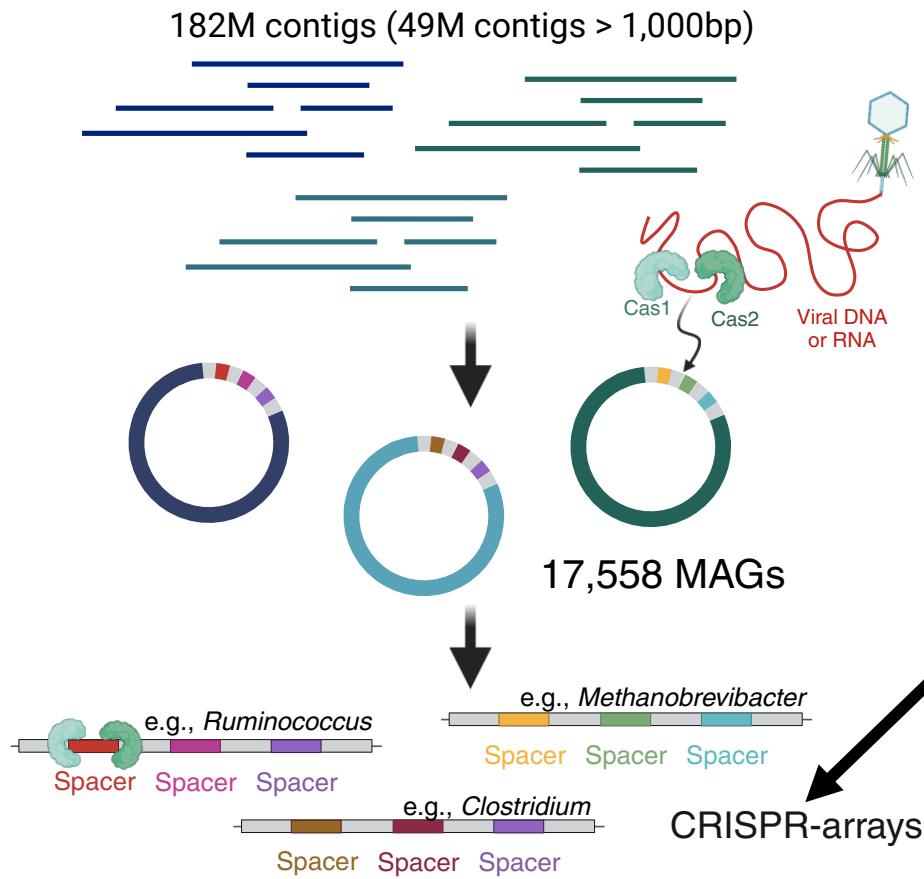
# How to identify those phage hosts?



# Parallel experiment to link phages to microbes



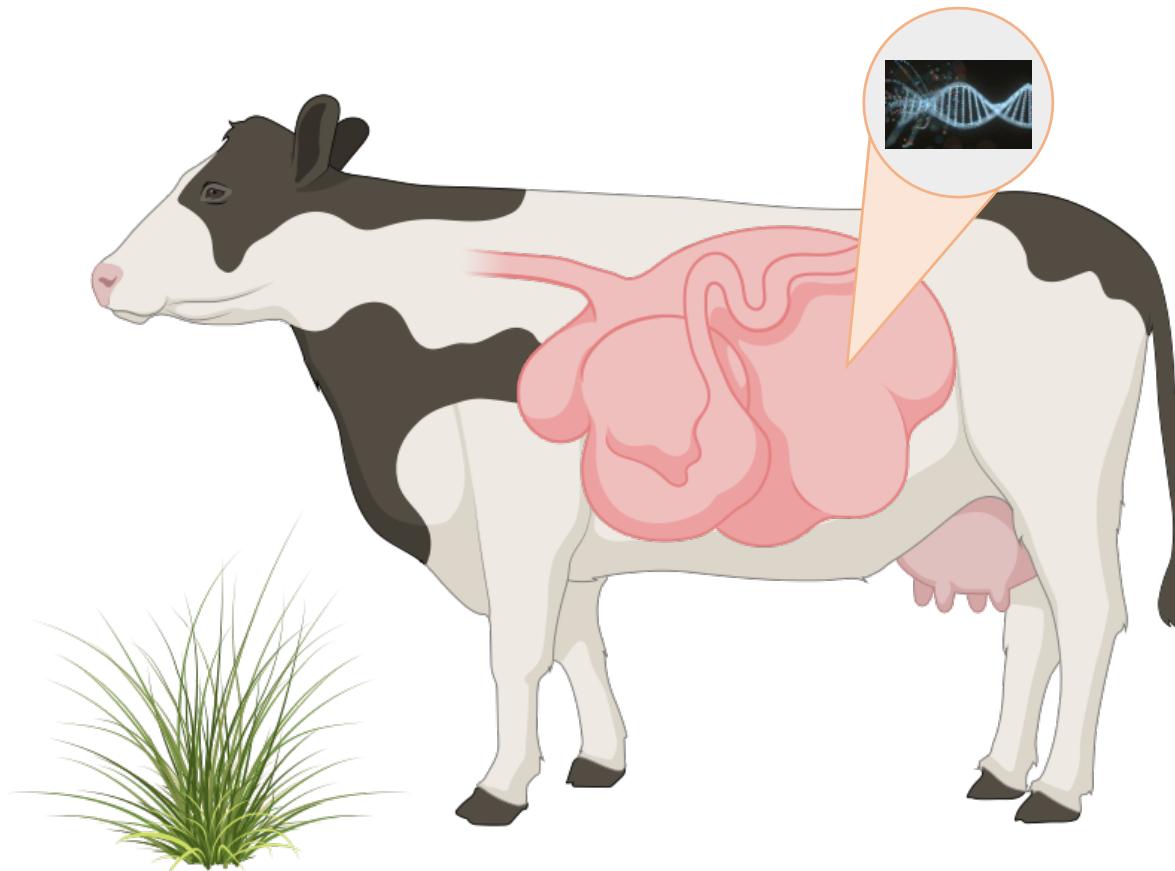
# Parallel experiment to link phages to microbes



208 microbial genomes (MAGs)

15 Caudoviricetes (dsDNA)
1 Leviviricetes (ssRNA)

# How about the influence of the host on feed efficiency?

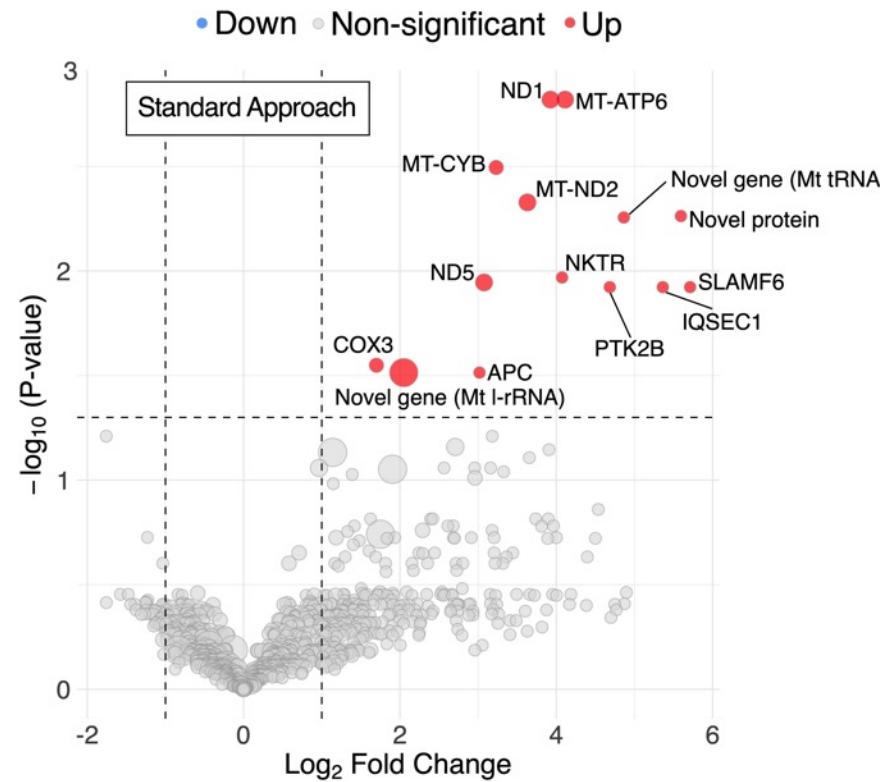


**Approaches to capture cow transcripts in a gut sample:**

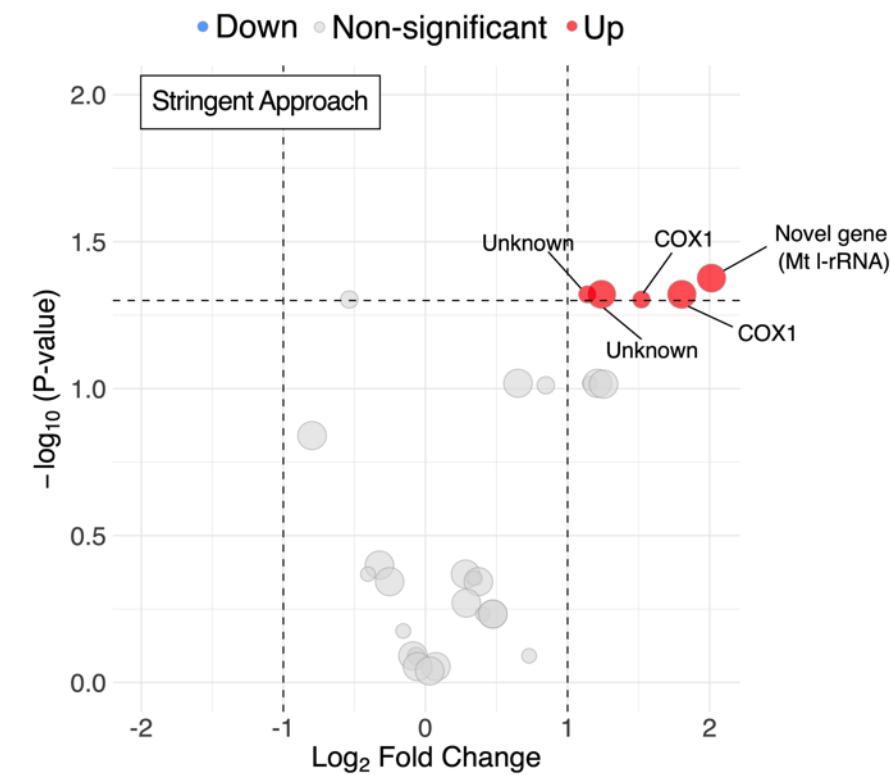
**STANDARD APPROACH:**  
Alignment to ref genome

**CLASSIFICATION APPROACH:**  
Read extraction + assembly

# Differential host gene expression in rumen content

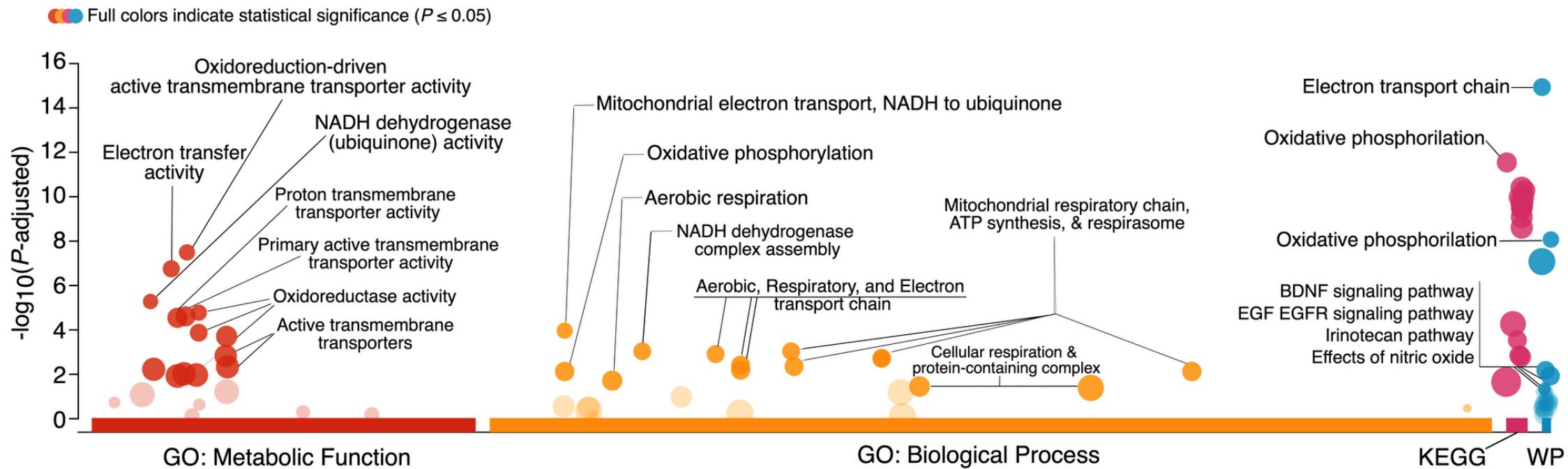


Alignment based

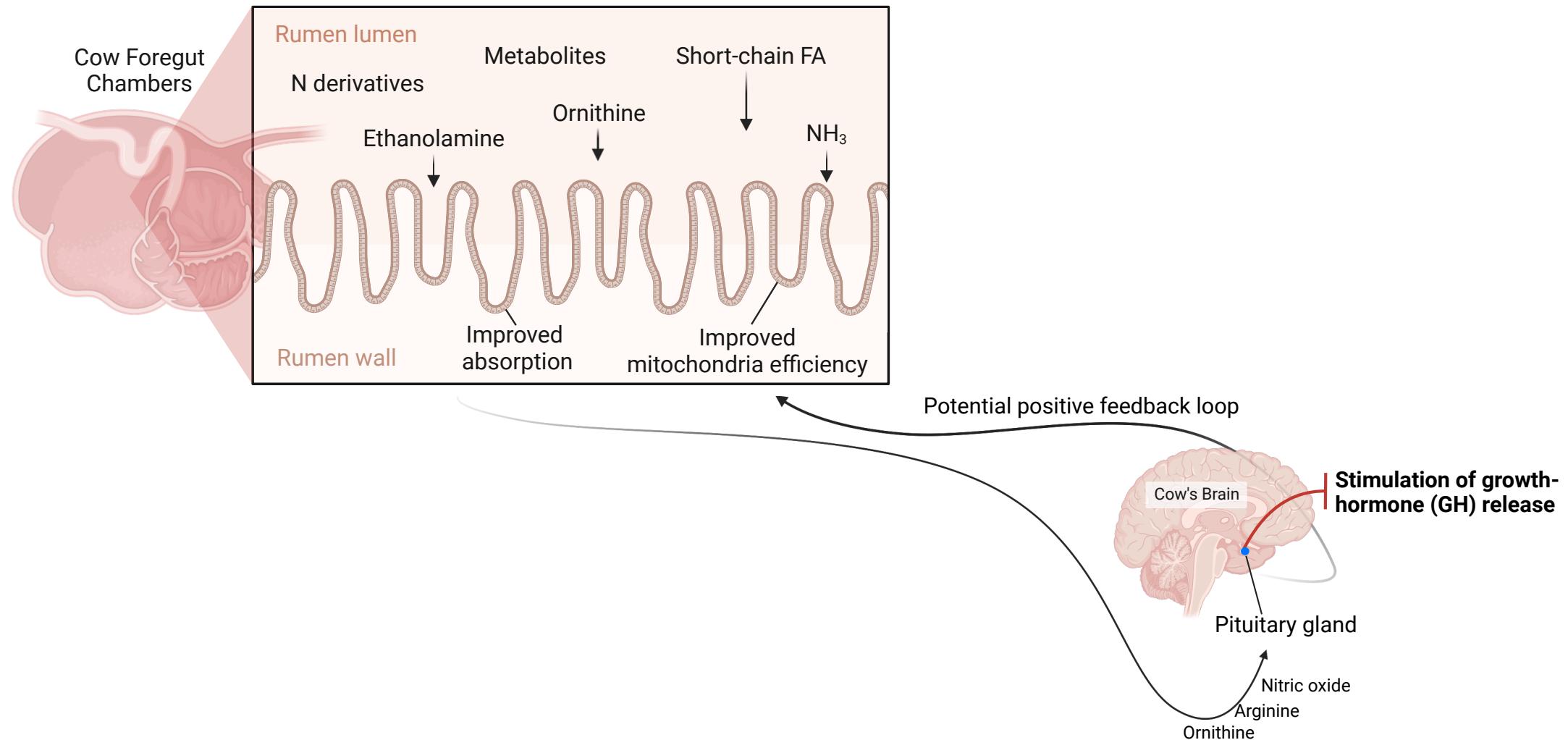


Classification based

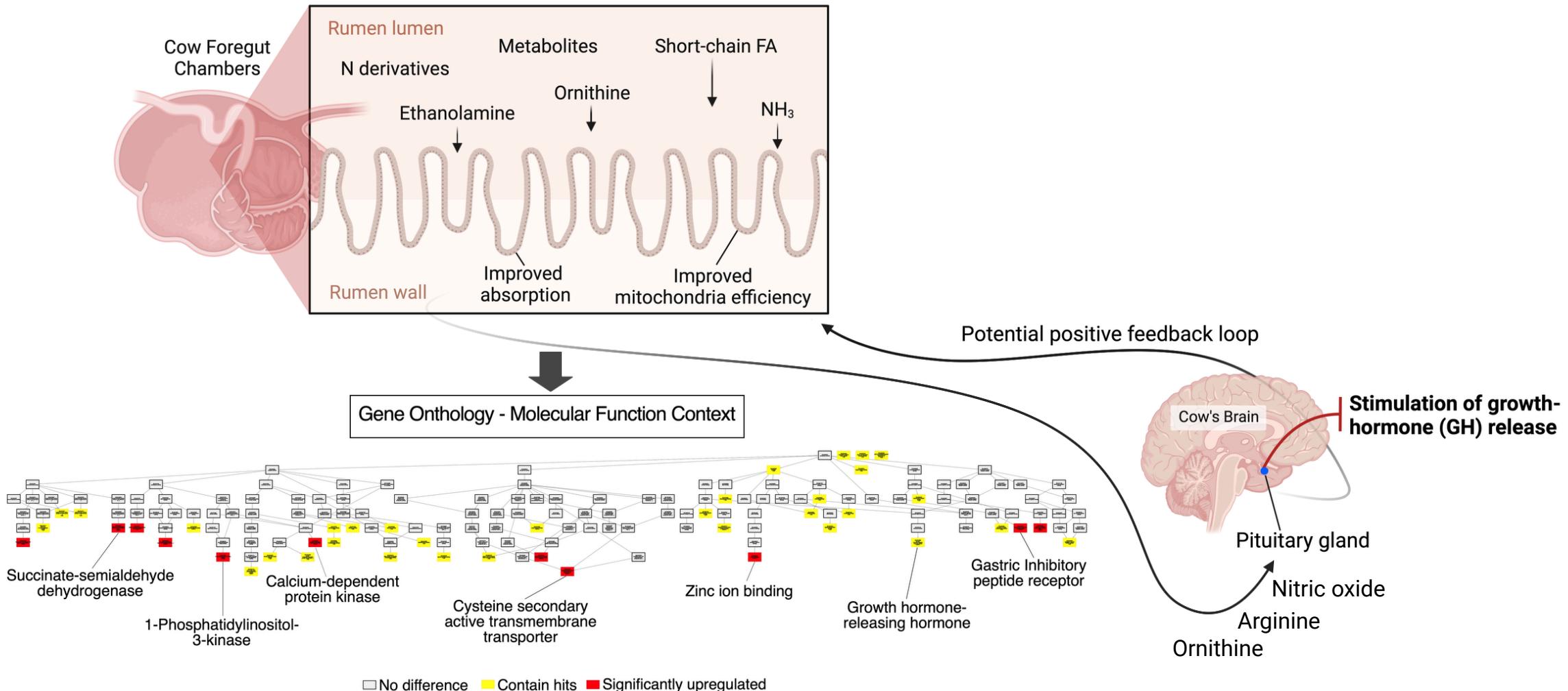
# Differential Host gene expression in rumen content



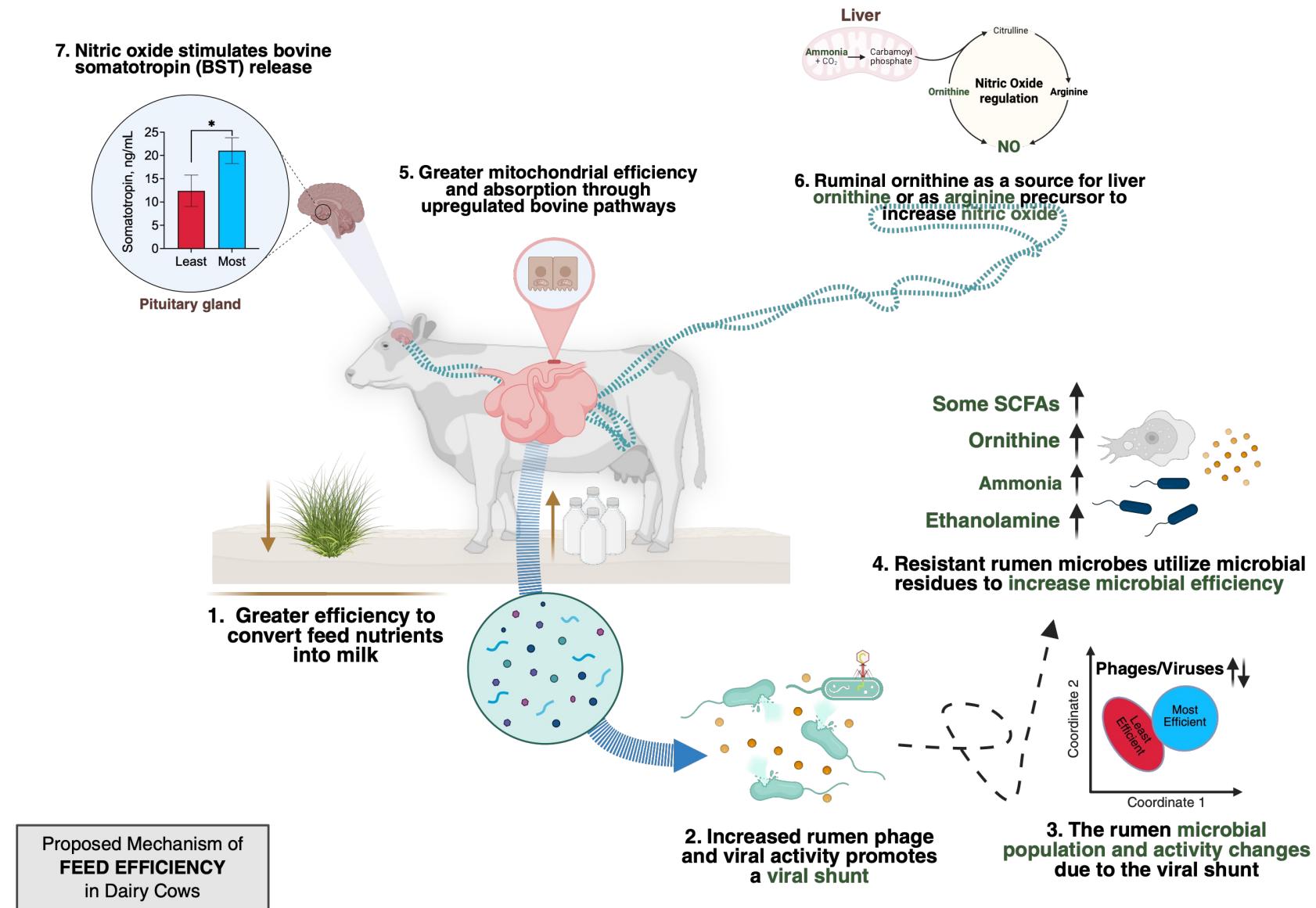
# Differential Host gene expression in rumen content



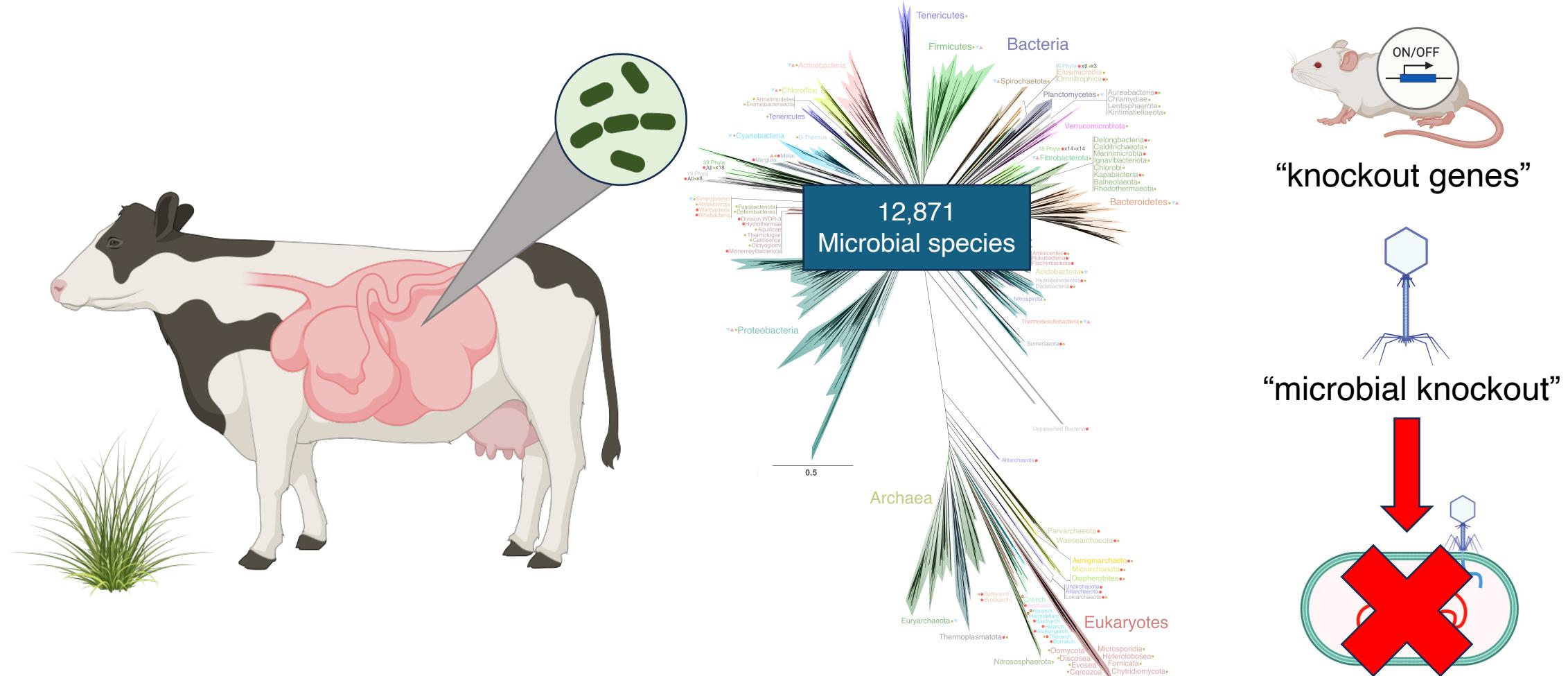
# Host transcripts association with rumen metabolites



# Summary of the findings and potential for exploration

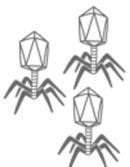


# Microbial knockout to study mechanisms

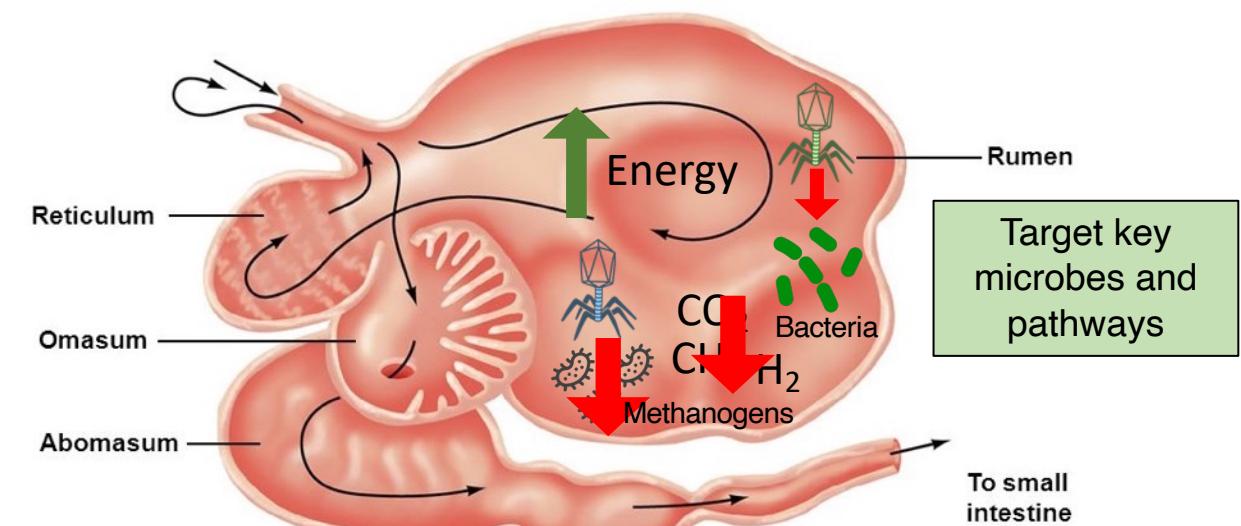
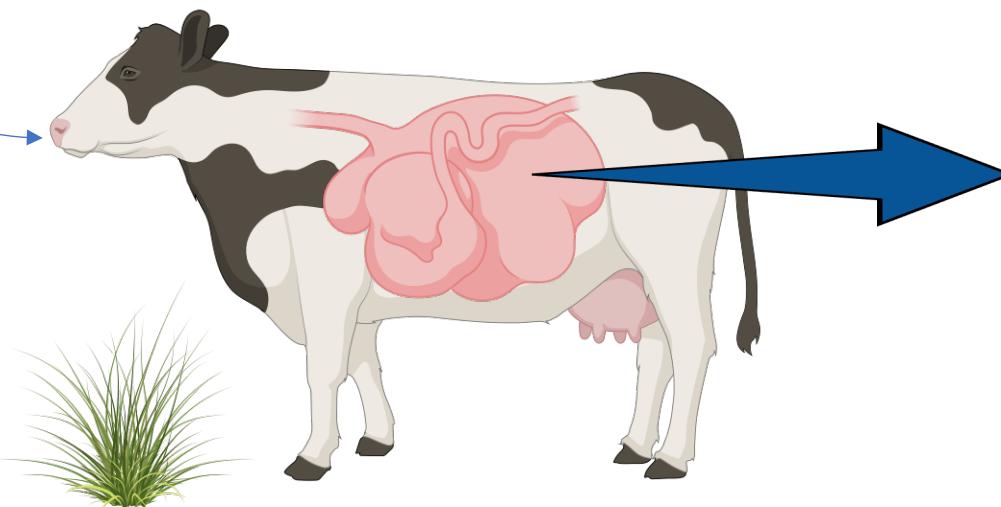


Adapted from Hessler et al., 2024. Phage-mediated microorganism depletion enables the testing of microbial interactions in microbiomes.  
Presented at the 19th International Society for Microbial Ecology (ISME19) Meeting, Cape Town, South Africa

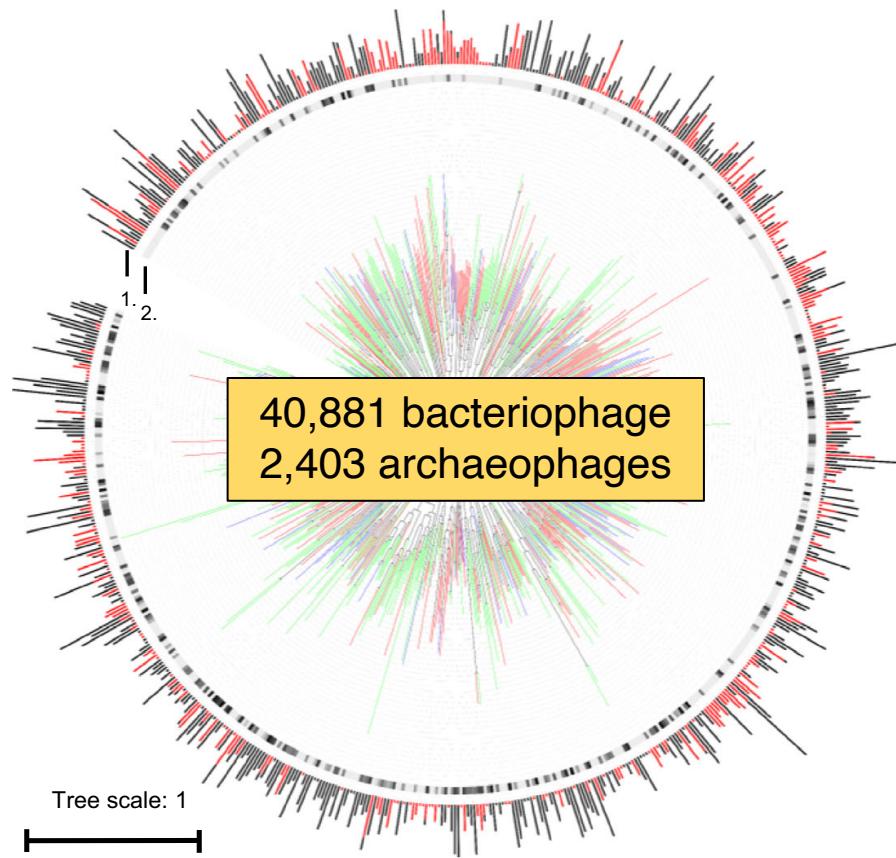
# Reengineering the rumen microbiome with phages



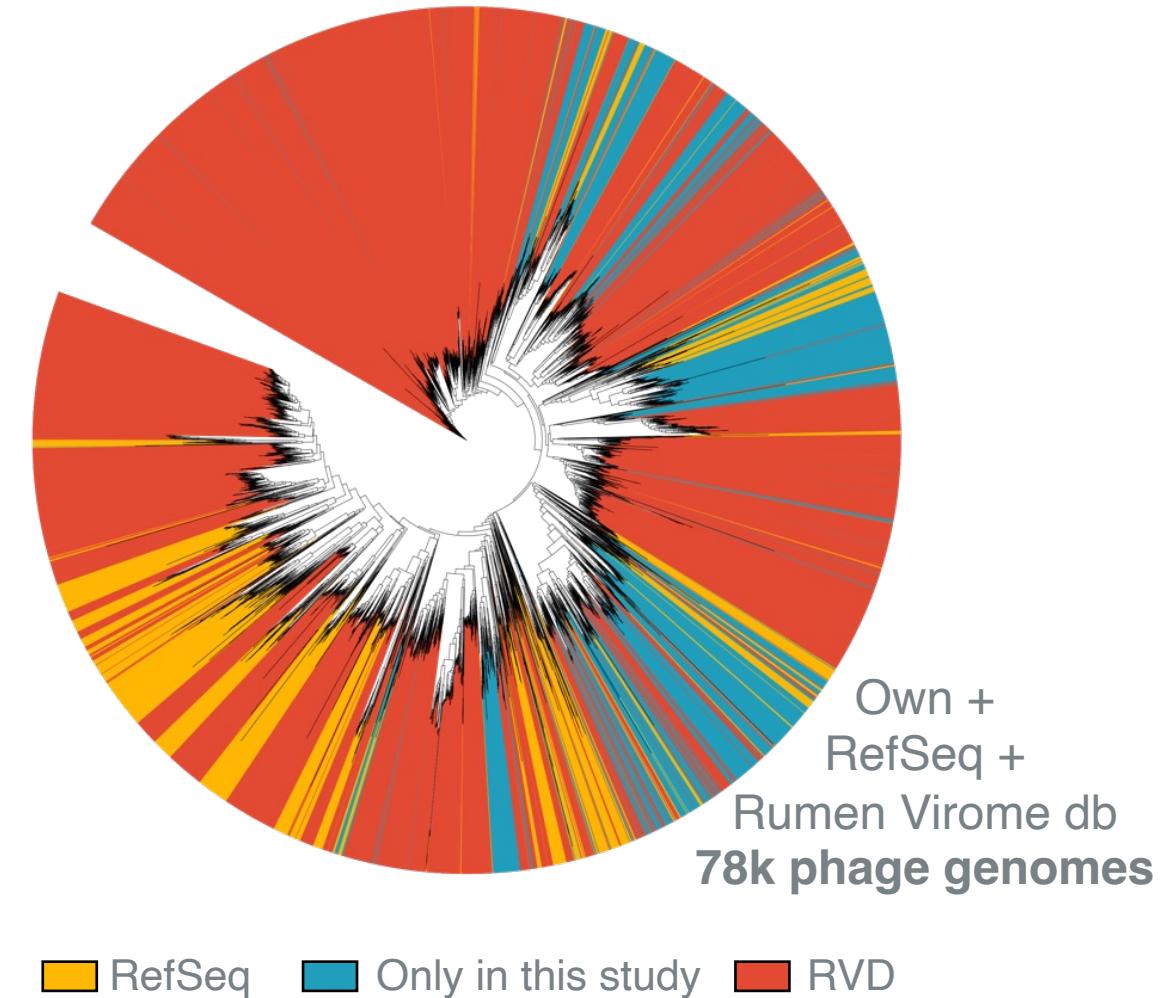
Phage therapy targeting key rumen  
microbes to improve feed efficiency  
and inhibit methanogens



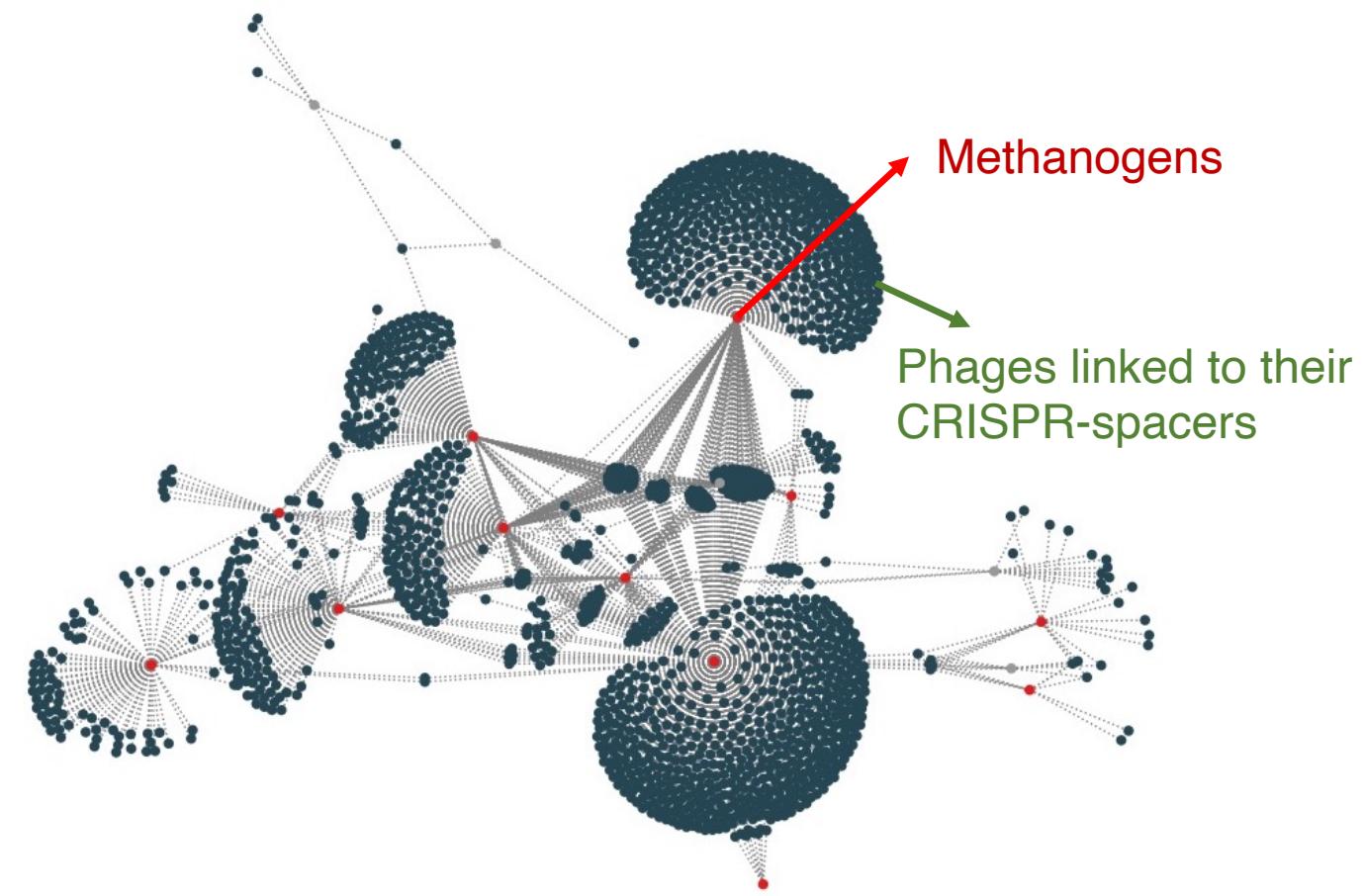
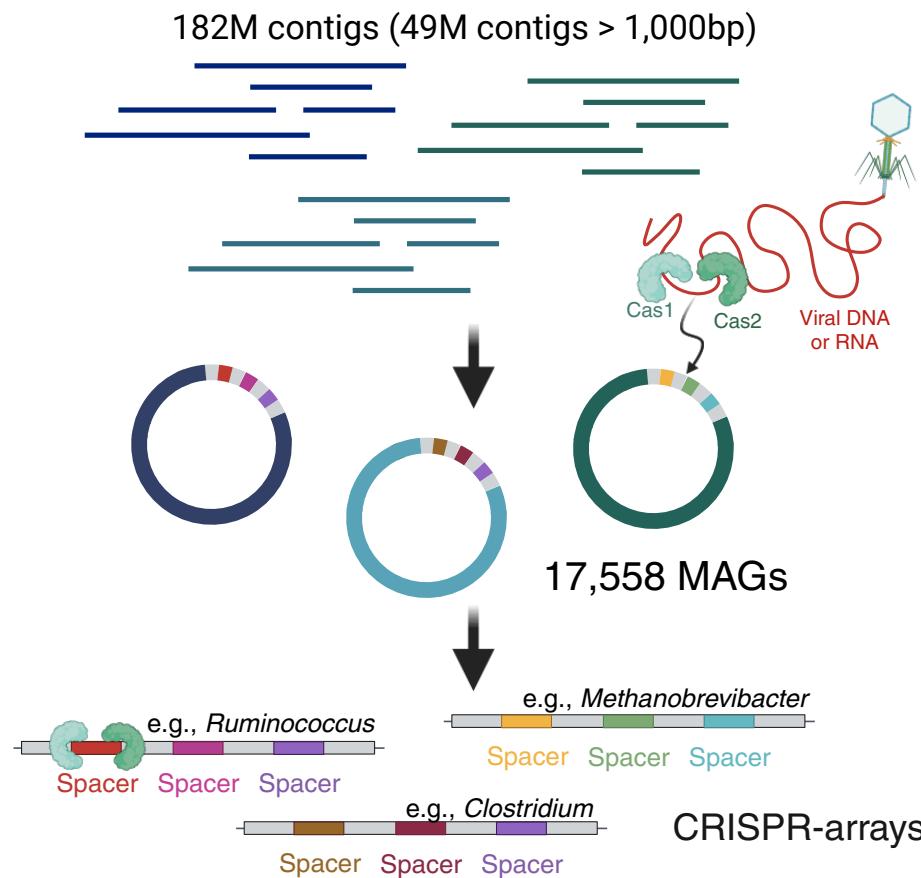
# Some archaea phages are already identified



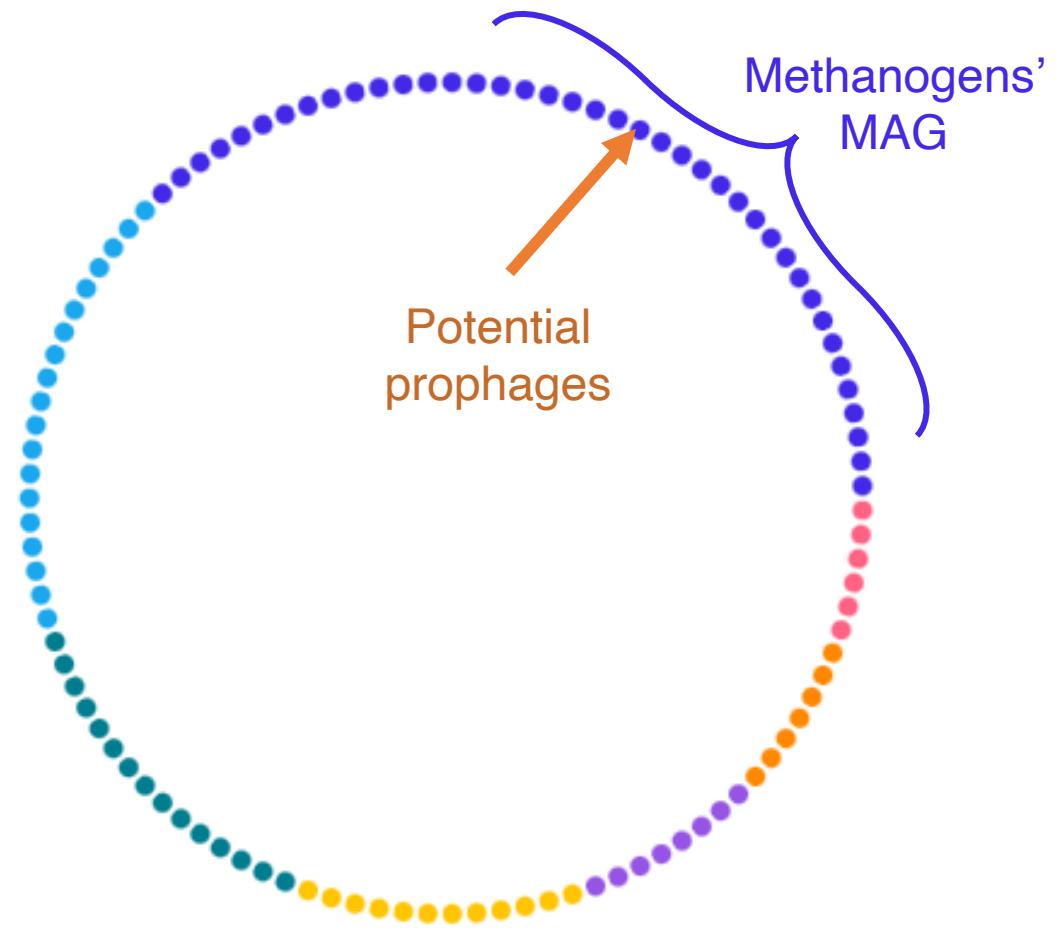
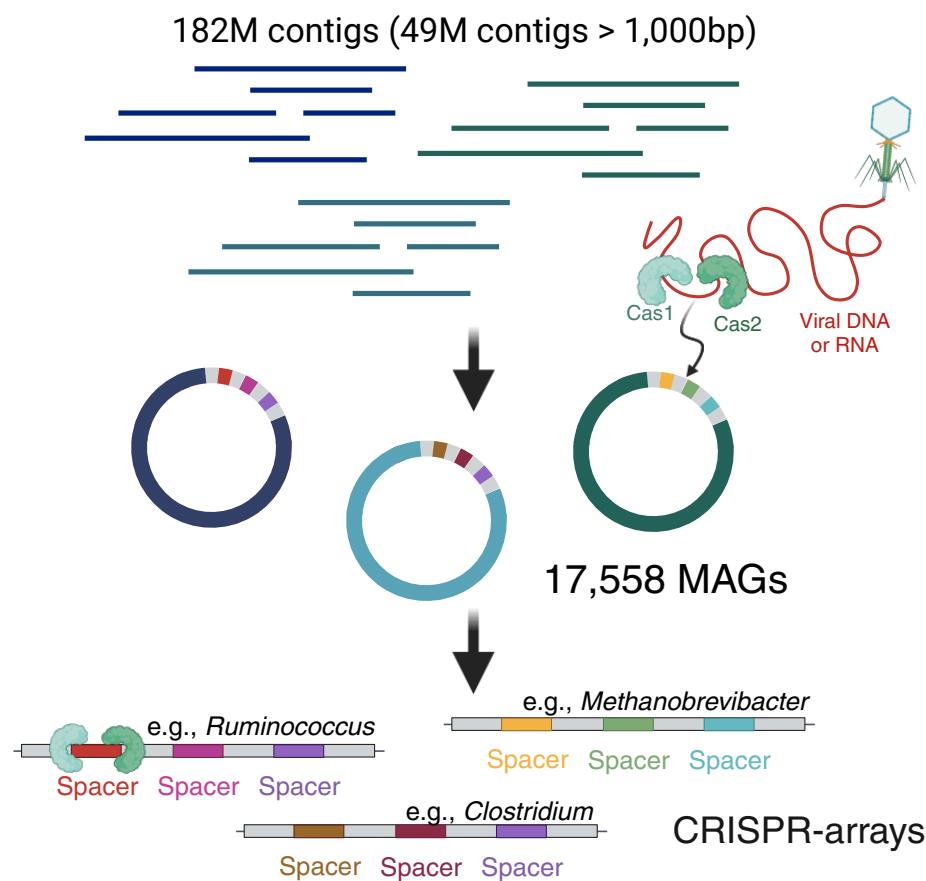
Yan et al. (2023)



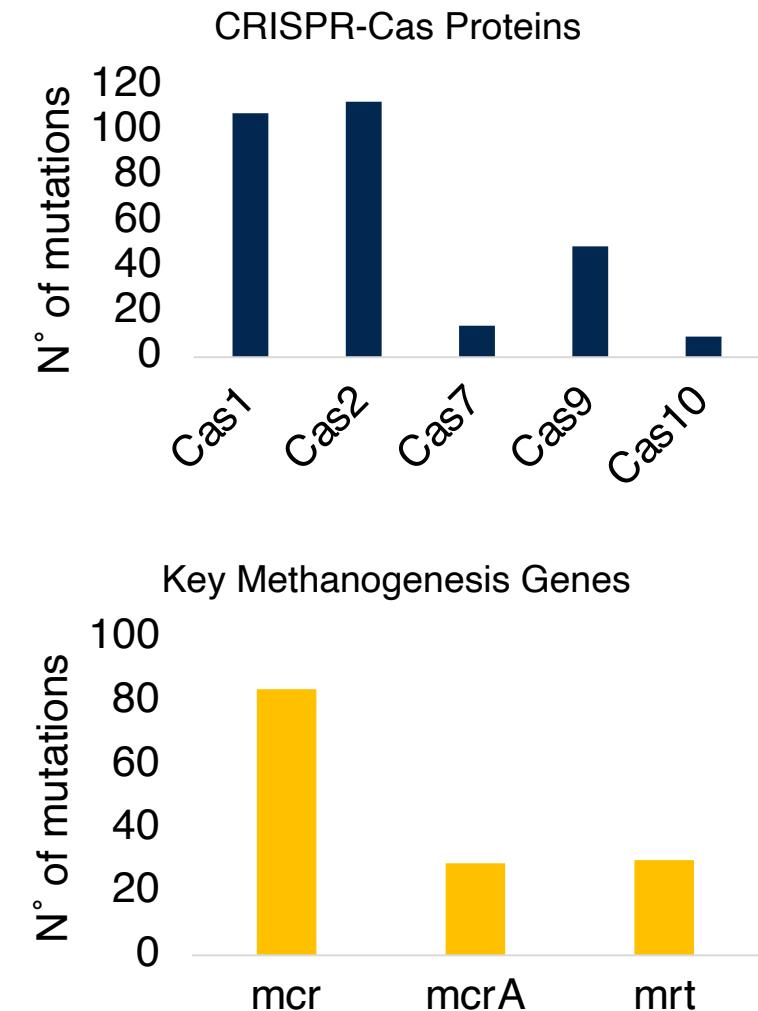
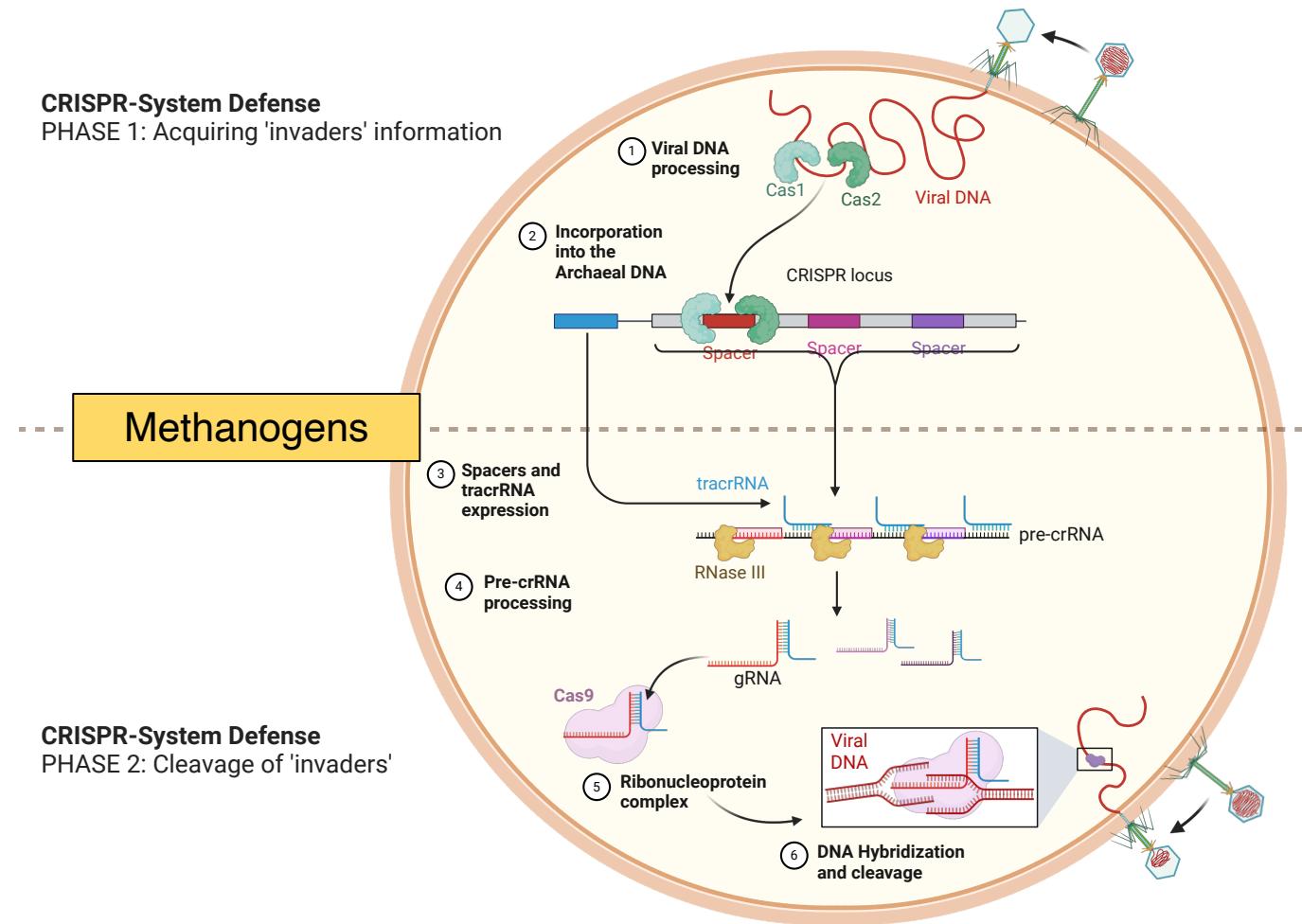
# Potentially lytic phages against methanogens



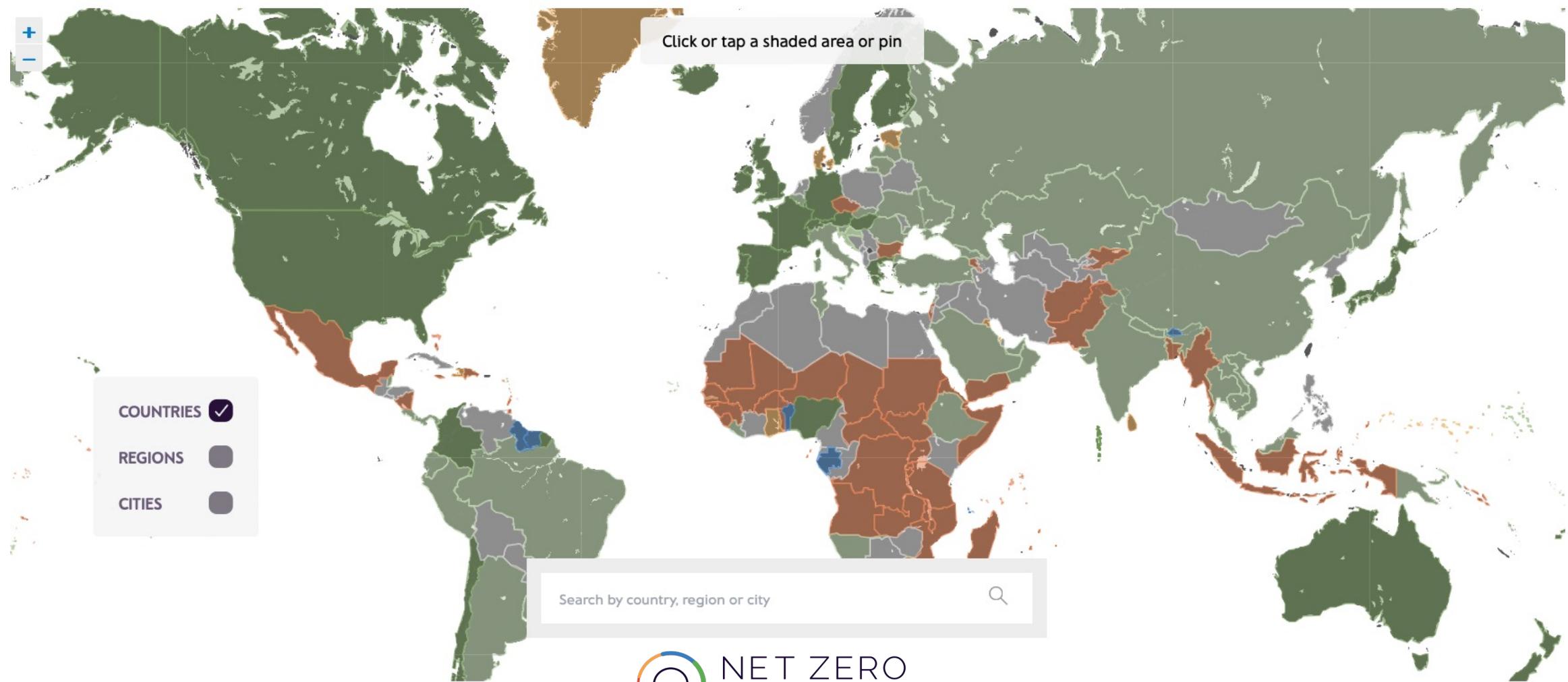
# Potentially lysogenic phages



# Characterizing the CRISPR-System of rumen methanogens

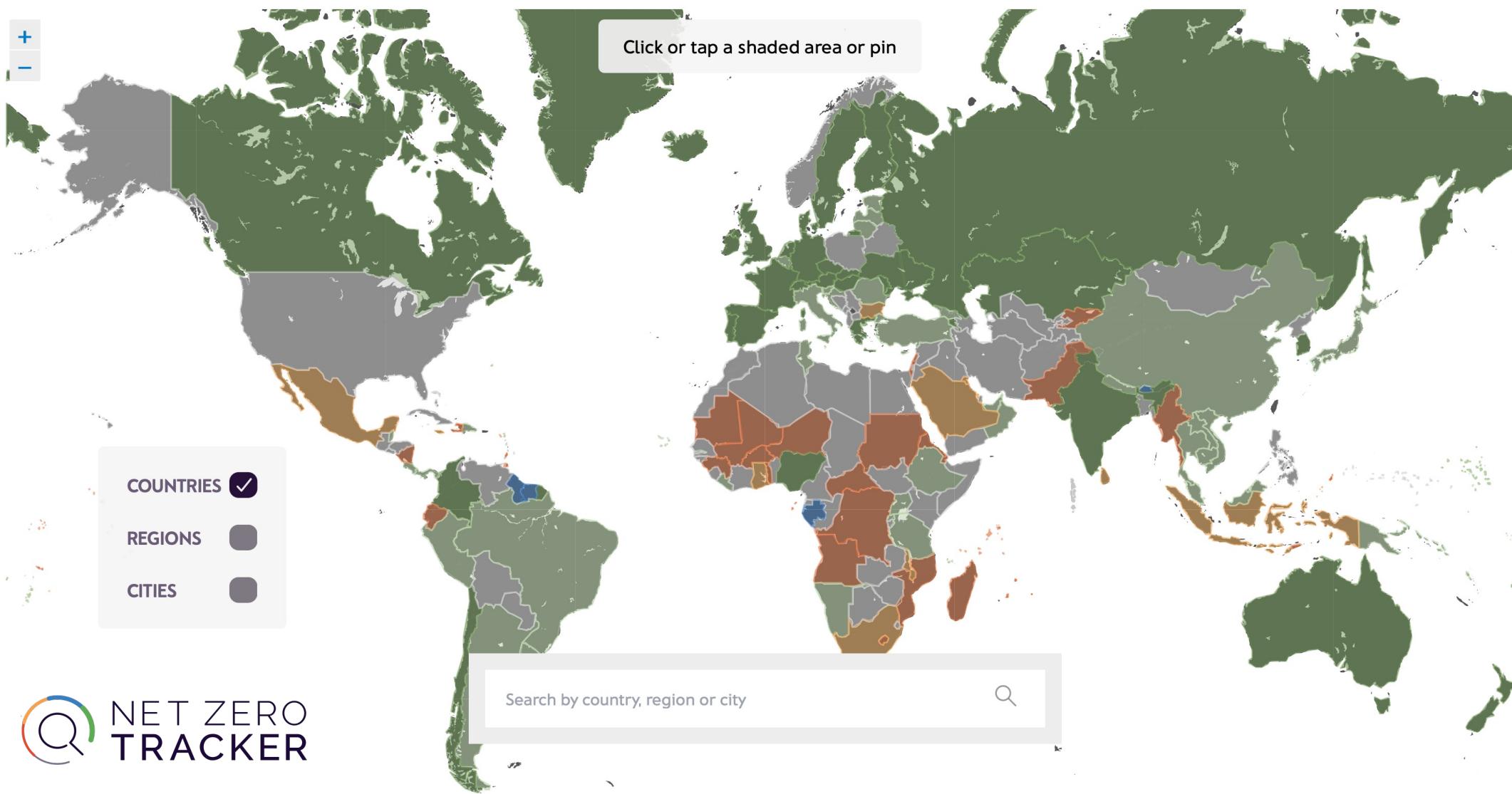


# Countries Committed to Net Zero by 2050



We analyse all countries and territories, every region in the 25 largest emitting countries and all cities with 500,000+ inhabitants.

# Countries Committed to Net Zero by 2050



# Companies Committed to Net Zero by 2050



ABInBev



Nestlé



Unilever



DSM



Heineken®



# More than 9,000 Companies Committed to Net Zero

Company	Key commitment	First announced
AB InBev	2025: 35% GHG reduction 2040: Net zero	2018
Addo Food Group	2035: Net zero	2021
Arla Foods	2030: 63% Scope 1+2 GHG emissions reduction	2021
Bacardi	2025: 50% Scope 1+2 GHG emissions reduction; 20% Scope 3 reduction	2018
Barilla	2030: 25% Scope 1+2 GHG emissions reduction; 26% Scope 3	2019
Cargill	2030: 30% reduction per ton of product sold 2050: Net zero	2019

# ACKNOWLEDGEMENTS



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U. Guelph



Caio Figueiredo  
WSU



**DAIRY HEALTH  
AND SUSTAINABILITY LAB**



Anneliek ter Horst  
UC Davis



Pouya Dini  
UC Davis



F. Penagaricano  
UW Madison



Ermias Kebreab  
UC Davis



J. E. Santos  
UF

**FACULTY AND STUDENTS  
WHO ARE DIRECTLY HELPING  
IN THE COLLECTION OF DATA:**

University of Guelph, Canada

University of Florida, USA

- Wilson Coelho Jr.
- Delaine Melo  
and others...



# Questions?

**Hugo F. Monteiro, Ph.D**

Dairy Health and Sustainability Lab

Department of Population Health and Reproduction

School of Veterinary Medicine

University of California, Davis



**UCDAVIS**