

Global Methane Genetics initiative

Microbiome Working Group



Organisation: Birgit Gredler, Oscar Gonzalez-Recio, Aniek Bouwman

November 27, 2025



Welcome!

■ Aim

- Update what's happening in Global Methane Genetics
- Network building
- Share: knowledge & hurdles
- Discussion: gaps & needs

Agenda

- Update from GMG (Roel)
 - data sharing & data base development
- Update ICAR Feed&Gas wikipage (Birgit)
- Update from the GMG microHub project (Oscar)
- Update projects outside GMG (all)
- Open discussion about research gaps (all)
- AOB

Global Methane Genetics initiative

Investment of 27M US\$
25 countries, 50 partners, 25 breeds
Methane pheno- & genotypes ~110k cattle & sheep, ~20k microbiome

Dairy:

Holstein (~42k)
Jersey (~8k)
(Nordic) Red Breeds
(~7.3k)
Brown Swiss (~3.3k)

Beef:

North America (~6k)
Australia, Ireland,
UK, NZ (~18.5k)

Develop protocols
Phenotyping
World-wide sharing
Genetic evaluation

**Sheep: world-wide
reference population**
Australia & New Zealand
UK & Ireland
Uruguay (~17k)

Africa

Local breeds & crosses (~4k)

Latin America

Beef (~7k)

Microbiome:

World-wide reference
population
(~20k samples)

Update GMG

- Startup meeting Innsbruck
- Second newsletter ¹⁾.
- Contracts
- Proposal development: small holder farms, Asia & buffalos
- Framework of adoption & incentivization system for genetic selection as methane mitigation tool

GMG data sharing & data base

- Two data sharing agreements: 1) GMG paid data and 2) exchange for record contribution. Will be sent for signature to partners
 - Database
 - Business requirement and review existing database
 - June three offers to build August contracted, weekly meetings, tomorrow consultation meeting
- planned delivery February

Architecture & Data Flow

➡ User upload Via SharePoint: 1 → 2

➡ User upload Via Web: 7 → 8

➡ Data processing: 3 → 4 → 5 → 6 → 9



Landing DB

- ✓ Storing a copy of imported files
- ✓ Validate & cleanse data



Data Warehouse

- ✓ Centralized methane data store
- ✓ Keeping historical version of data records
- ✓ MiXBLUP integration
- ✓ Contribution score

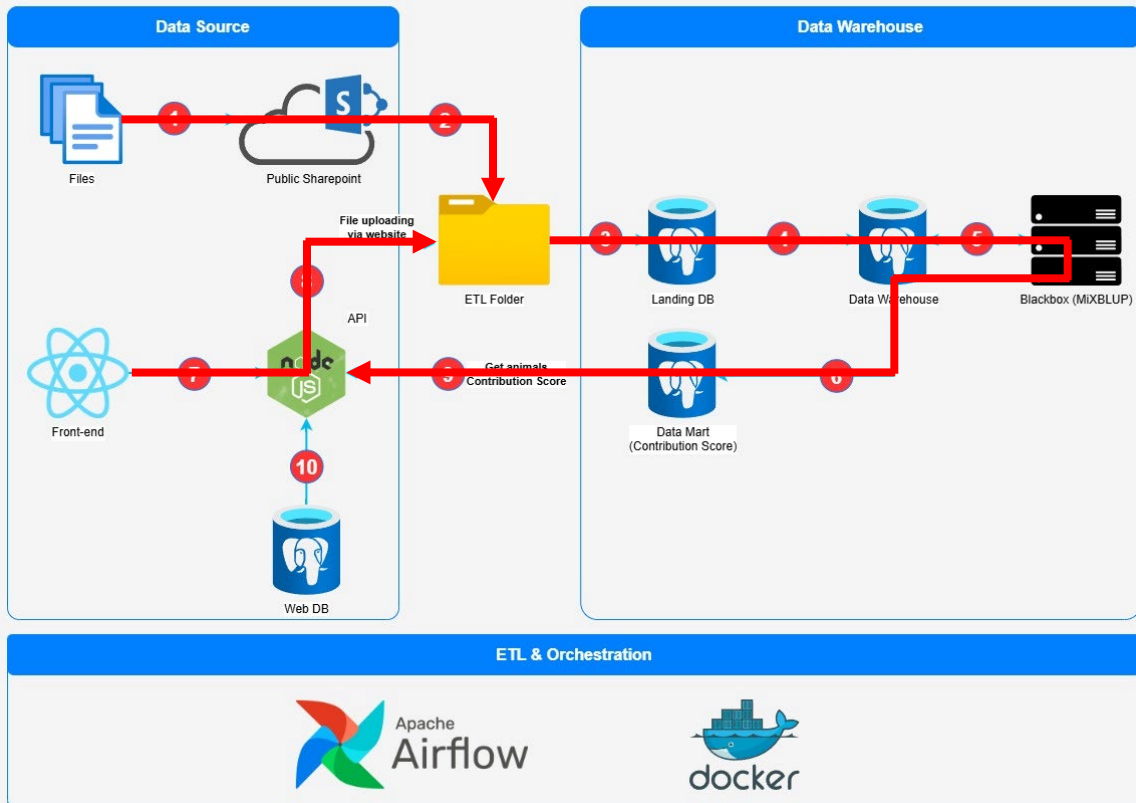


Data Mart (Contribution Score)

- ✓ Dedicated database for data extraction

WAGENINGEN
UNIVERSITY & RESEARCH

Methane Global Hub Architecture



ICAR Feed&Gas wikipage



- [https://wiki.icar.org/index.php/Section 20 %E2%80%93 Methane Emission for Genetic Evaluation](https://wiki.icar.org/index.php/Section_20_%E2%80%93_Methane_Emission_for_Genetic_Evaluation)
- GreenFeed & Wearables
- Methane device validation center:
- [https://wiki.icar.org/index.php/ICAR validation methane recording devices](https://wiki.icar.org/index.php/ICAR_validation_methane_recording_devices)
- Microbiome protocols coming soon
- [Activities: https://wiki.icar.org/index.php/Section 20: Activities](https://wiki.icar.org/index.php/Section_20:_Activities)
- Activities – send us information about projects & events to be included here!

What's happening in other rumen microbiome/methane projects/initiatives

Global Methane Genetics initiative

Led by



WAGENINGEN
UNIVERSITY & RESEARCH

In Partnership with



BEZOS
EARTH
FUND



Global
Methane
Hub

Micro HUB

November 27th
GMG presentation meeting

TABLE OF CONTENTS

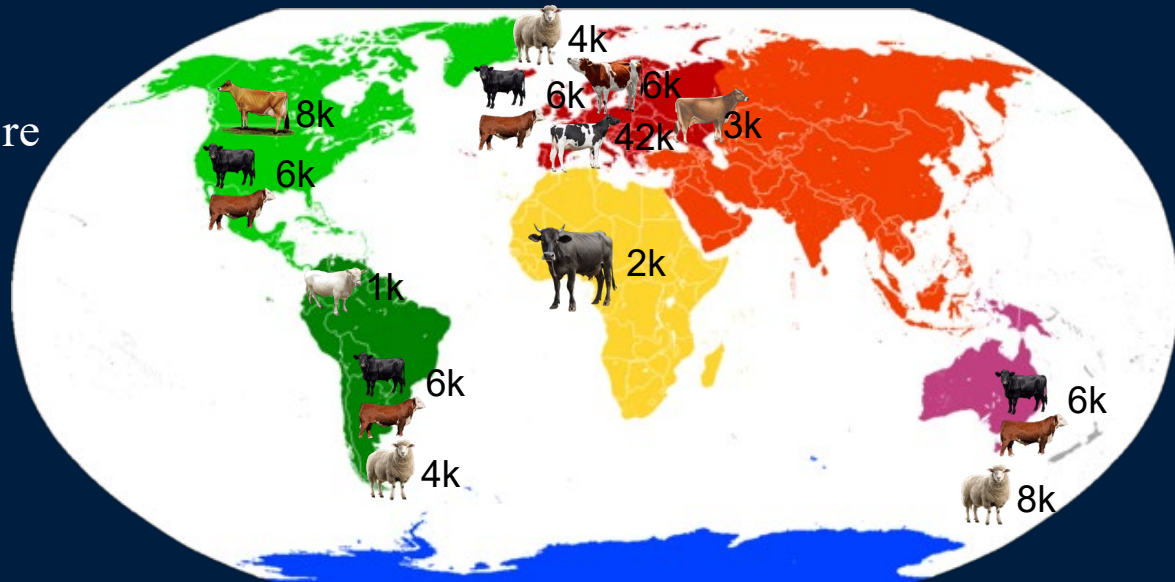


- microHub overview
- Update on the progress
- Bioinformatics
- Other projects
- Questions and discusión, research gaps, what's next

Global Methane Genetics Initiative



- 110,000 CH₄ phenotypes from different breeds and productive systems
 - 20% microbiome samples
 - 20,000 microbiomes
- +
4,000 from microHub core



Why microbiome?

- Microbiome information can accelerate genetic progress on reducing methane emissions



ROLE OF MICROBIOME IN

- *Methane emissions*
- *Digestion*
- *Overall health*

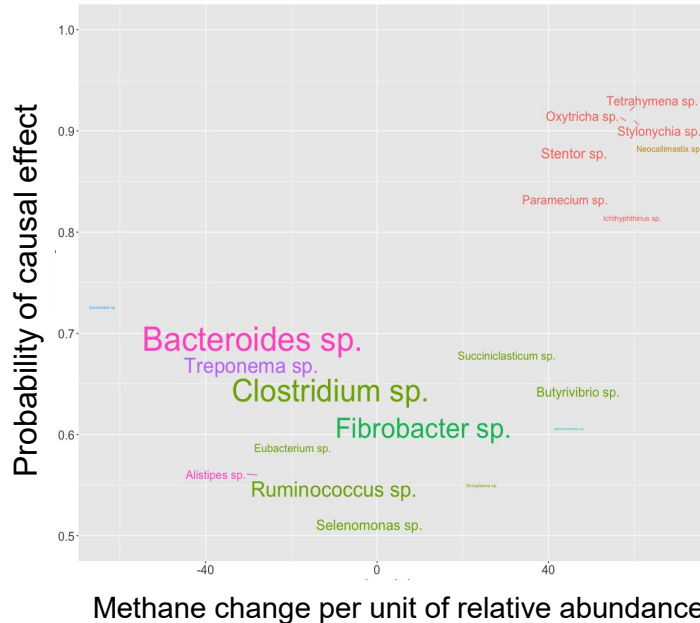
ROLE OF HOST

- *Symbiosis*
- *Microbiome composition ($h^2=0.30$)*
- *Genetically correlated with methane*



Why microbiome?

- There is causal relationship between microbiome-host-methane (according to structural equations models).



Eukaryotes provide H₂ to archaeas
Some groups of bacteria are H₂ sinks

ORIGINAL ARTICLE

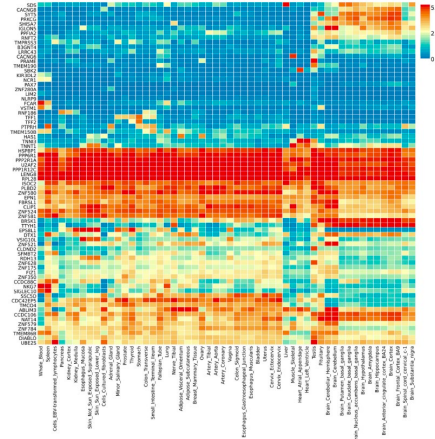
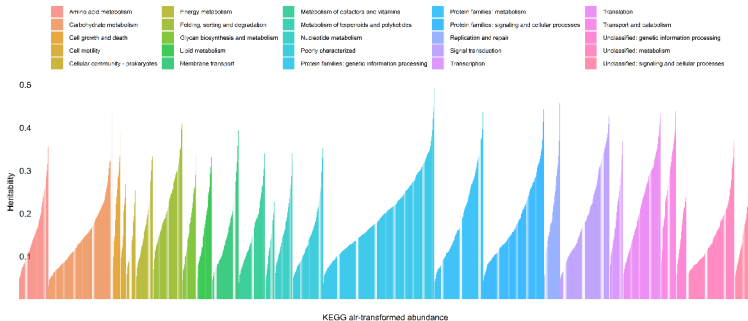
Peer-Reviewed Journal of Microbiology and Biotechnology | WILEY

Structural equation models to disentangle the biological relationship between microbiota and complex traits: Methane production in dairy cattle as a case of study

Alejandro Saborío-Montero^{1,2} | Mónica Gutiérrez-Rivas¹ | Aser García-Rodríguez³ | Raquel Atxaerandio³ | Idoia Goiri³ | Evangelina López de Maturana⁴ | José Antonio Jiménez-Montero⁵ | Rafael Alenda⁶ | Oscar González-Recio^{1,6}

Why microbiome?

- Rumen microbiome composition is heritable, due to genetic factors that affect rumen environment. (larger h^2 estimates for important features compared to methane)



Animal 17 (2023) 100780

Contents lists available at [ScienceDirect](#)

Animal
The international journal of animal biosciences

ELSEVIER

Review: Diving into the cow hologenome to reduce methane emissions and increase sustainability

Oscar Gonzalez-Recio^{a,*}, Natalia Scrobota^{a,b}, Javier López-Paredes^c, Alejandro Saborío-Montero^{d,e}, Almudena Fernández^a, Evangelina López de Maturana^{b,f,g}, Beatriz Villanueva^a, Idoia Goñi^h, Raquel Abxaerandio^h, Aser García-Rodríguez^h

How to account for the complexity of the microbiome

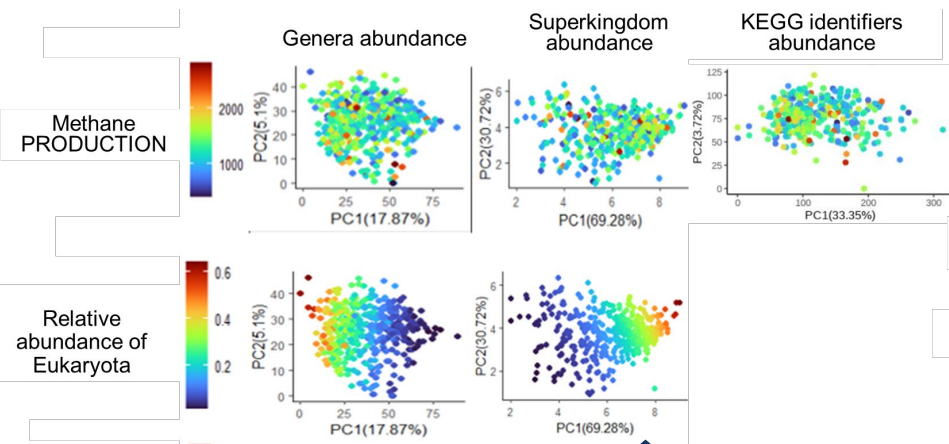
- Aggregated variables based on principal components



J. Dairy Sci. 104:8135–8151
<https://doi.org/10.3183/jds.2020-20005>
 © 2021 American Dairy Science Association®. Published by Elsevier Inc. and FASS Inc. All rights reserved.

A dimensional reduction approach to modulate the core ruminal microbiome associated with methane emissions via selective breeding

Alejandro Saborio-Montero,^{1,2,3} Adrian López-García,¹ Mónica Gutiérrez-Rivas,¹ Raquel Atxaerandio,⁴ Iñaki Goñi,⁵ Aser García-Rodríguez,⁶ José A. Jiménez-Montero,⁴ Carmen González,⁴ Javier Tamames,⁴ Fernando Puente-Sánchez,⁴ Luis Varona,⁴ Magdalena Serrano,⁴ Cristina Ovilio,⁷ and Oscar González-Reico^{1,2,3}



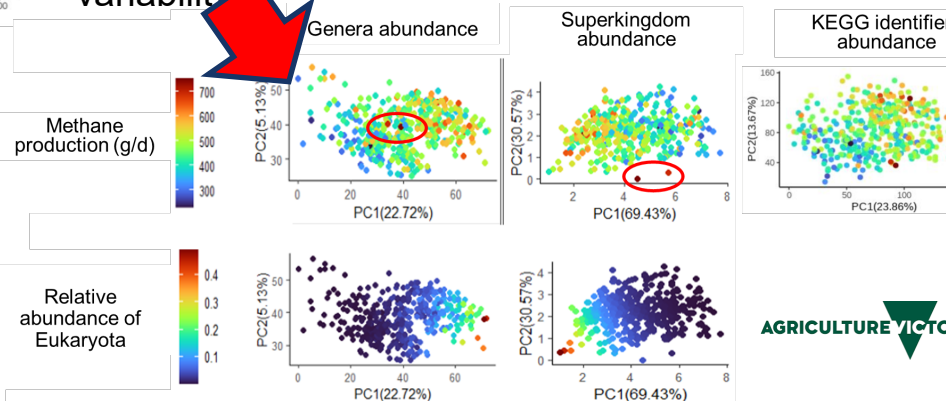
Spain



33% total microbiome variability

45% total microbiome variability

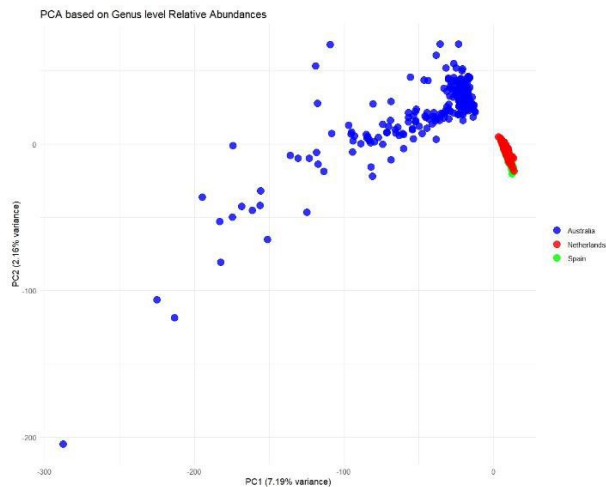
Australia



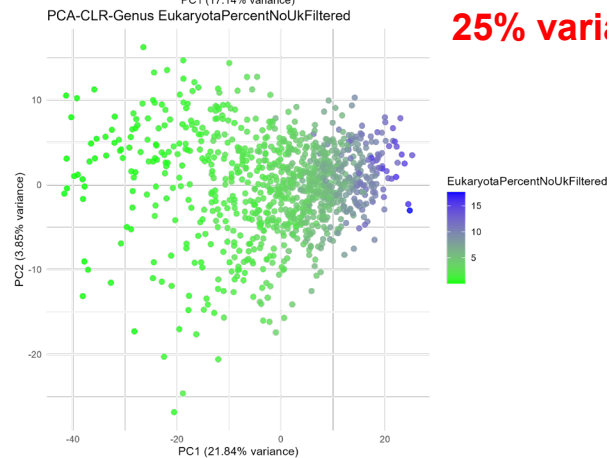
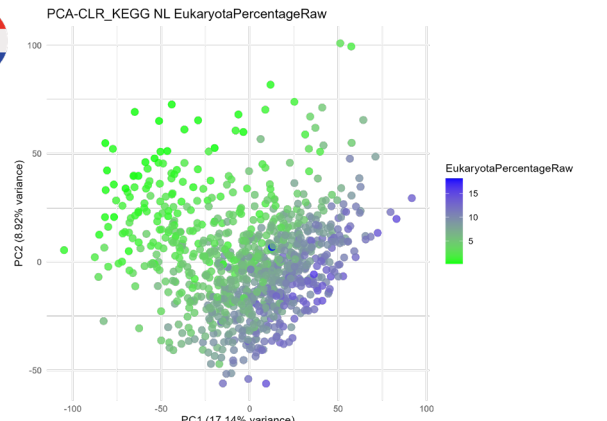
AGRICULTURE VICTORIA

How to account for the complexity of the microbiome

- Aggregated variables based on principal components



The Netherlands
Spain
Australia



25% variability



David Flossdorf

Microbiome-driven breeding for lower emissions

- Heritabilities of the PC and genetic correlation with methane and other relevant microbial features



J. Dairy Sci. TBC:1–19
<https://doi.org/10.3168/jds.2024-25436>
 © TBC, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
 This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>).

JDS25436

Rumen metagenome as a genomic selection target
 to reduce enteric methane emissions

B. J. Sepúlveda,^{1,3*} O. González-Reco,¹ A. J. Chamberlain,^{1,2} M. M. Khansefid,¹ B. G. Cocks,²
 J. Wang,¹ C. P. Prowse-Wilkins,^{1,4} L. C. Marett,² S. R. O. Williams,² J. L. Jacobs,^{2,5}
 A. García-Rodríguez,² J. A. Jiménez-Montero,² and J. E. Pryce^{1,2}



Spain



$h^2=0.17$



$h^2=0.28$

Australia



Microbial aggregate variables	h^2	↔	Genetic correlation w/ CH ₄
PC1 Genus	0.44		0.62
PC1 KO	0.35		0.74
PC2 Genus	0.42		0.62
PC2 KO	0.35		0.68

Microbial aggregate variables	h^2	↔	Genetic correlation w/ CH ₄
PC1 Genus	0.11		0.77
PC1 KO	0.17		0.73
PC2 Genus	0.11		0.86
PC2 KO	0.10		0.65

High genetic correlation of PC with the relative abundance of Eukaryotes and Archaea

Microbiome-driven breeding for lower emissions

- Rumen metagenome as a genomic selection target to reduce enteric methane emissions
- Correlated response
- Different strategies to select the “core” microbiome.

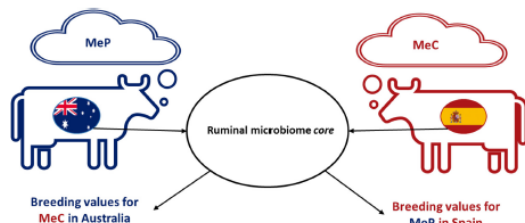


Figure 6. Sharing reference populations of the ruminal metagenome core facilitates the prediction of enteric methane production (MeP) recorded as grams per day (g/d) with sulfur hexafluoride in a dairy cattle population in Australia, and enteric methane concentration (MeC) recorded as parts per million (ppm) with sniffers in Spain.



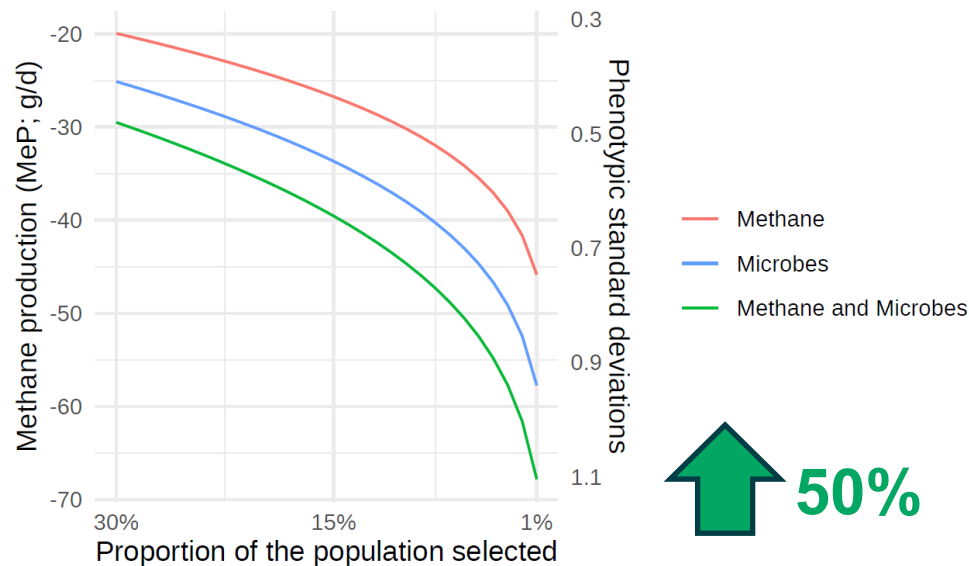
J. Dairy Sci. TBC-1-19
<https://doi.org/10.3168/jds.2024-25436>

JDS25436

© TBC, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
 This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0>).

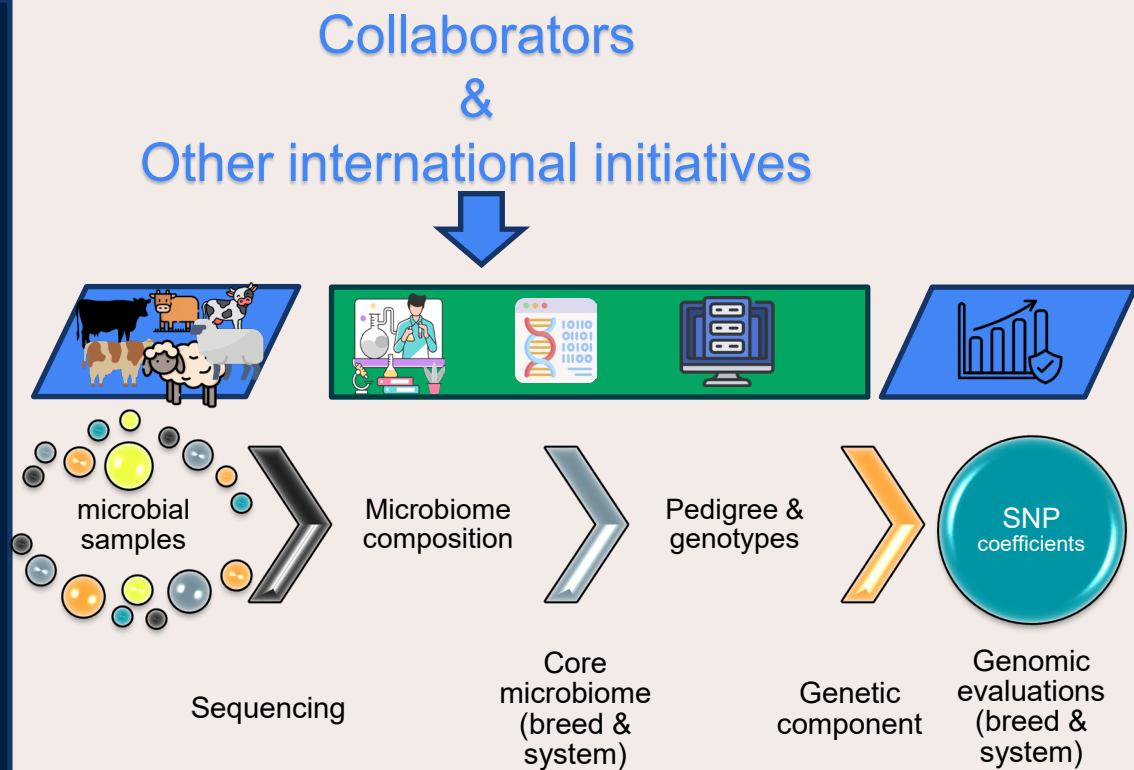
Rumen metagenome as a genomic selection target
 to reduce enteric methane emissions

B. J. Sepulveda,^{1,2} O. González-Recio,¹ A. J. Chamberlain,^{1,2} M. Khansefidi,¹ B. G. Cocks,²
 J. Wang,¹ C. P. Prowse-Wilkins,^{1,4} L. C. Maretti,² S. R. O. Williams,² J. L. Jacobs,^{2,5}
 A. García-Rodríguez,² J. A. Jiménez-Montero,¹ and J. E. Pryce^{1,2}



SAMPLING AND SEQUENCING STRATEGY

- Sheep (4000)
- Dairy (7000)
- Beef (8000)
- African breeds & crosses (1200)

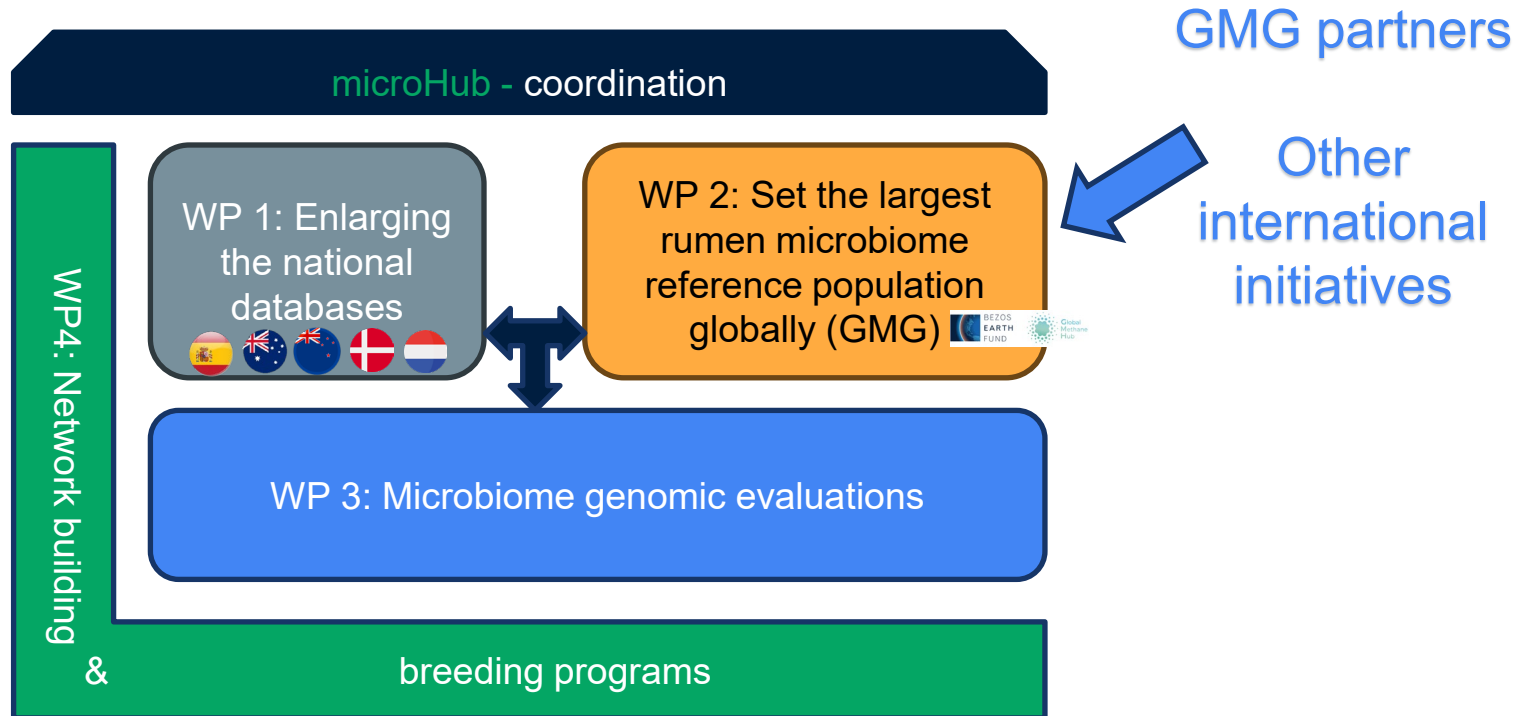




Key objectives

- The microHub project will establish a **reference population** with metagenomic and genomic data covering different **breeds** and **systems** (GMG partners & external)
- Find **core microbiome** composition/ characteristics associated to methane emissions and rumen health (specific of production system?)
- Define microbiome **phenotypes** of interest for breeding purposes
- Create a **genomic evaluation** system that can be used to select the parents of the next generation with microbiome profiles that reduce enteric methane emission
- Investigate the microbiome profile as a **correlated trait** for reducing methane emissions
- Lower the global environmental impact of cattle through microbiome driven selection while maintaining **rumen health**

- WPs presentation





GMG-microHub

[▶ Watch How to Make a Gantt Chart in Excel](#)

Project Start: Tue, 7/1/2025
Today: Thu, 9/18/2025
Display Week: 1

Jun 30, 2025							Jan 26, 2026							Aug 24, 2026							Mar 22, 2027							Oct 18, 2027							May 15, 2028							Dec 11, 2028							Jul 9, 2029							
30	30	29	28	28	27	27	26	25	27	26	26	26	25	25	24	23	23	22	22	21	20	22	21	21	20	20	19	18	18	17	17	16	15	16	15	15	14	14	13	12	12	11	11	10	9	11	10	10	9	9	8	7	7	6	6	5
M	W	F	S	T	T	S	M	W	F	S	T	T	S	M	W	F	S	T	T	S	M	W	F	S	T	T	S	M	W	F	S	T	T	S	M	W	F	S	T	T	S	M	W	F	S	T	T	S	M	W	F	S	T	T		

TASK	ASSIGNED TO	PROGRESS	START	END
------	-------------	----------	-------	-----

WP1

Task 1.1. Process existing database with pipelines	10%	7/1/25	12/31/26
Milestone 1.1. Countries share >3k metagenome samples		12/11/27	1/1/28
Task 1.2. Expand reference populations	0%	7/1/25	12/31/27
Task 1.3. Coordination of rumen sampling within GMG initiative	20%	7/1/25	12/31/26

WP 2

Task 2.1. SOP: Sample Collection	80%	1/1/26	12/31/26
Task 2.2. SOP: sequencing	1%	12/1/25	7/1/27
Task 2.3. SOP: Bioinformatics	10%	10/1/25	7/1/27
Milestone 2.1. Shared SOP & reference metagenome		6/1/27	7/1/27
Milestone 2.2. Public SOP release on GitHub		6/1/29	7/1/29
Milestone 2.3. Core metagenome defined		12/1/27	1/1/28
Task 2.4. International reference metagenome		7/1/26	1/31/27
Task 2.5. Determine global core metagenome		1/1/27	12/1/27

WP 3

Task 3.1. Microbiome genetic evaluations		7/1/27	6/30/29
Milestone 3.1. First international genomic evaluations		3/1/29	4/1/29
Task 3.2. Validate in sheep		1/1/28	6/30/29

Phase 4 Title

Task 4.1. Broad International Expansion	20%	1/7/25	9/1/29
Milestone 4.1. International initiatives informed, sampling plan set		12/1/25	1/1/26
Milestone 4.2. Metagenome databases from external partners included		5/30/27	7/1/27
Task 4.2. Propose tools and strategies for breeding programs		1/7/27	9/1/29
Milestone 4.3. Final report		6/1/29	7/1/29

Insert new rows ABOVE this one

BIOINFORMATICS

Different workflows in cattle and sheep

- Unify the protocols & quality controls as much as possible
- Improve performance of pipelines
 - SqueezeMeta
 - Used in foundational work of microHub.
 - Slow for taxonomic assignation (type of microbes)
 - Maintain assignation accuracy
- Define databases
- Define strategy for data storage

BIOINFORMATICS

STORAGE

During the project, deliver sequencing and basecalling files:

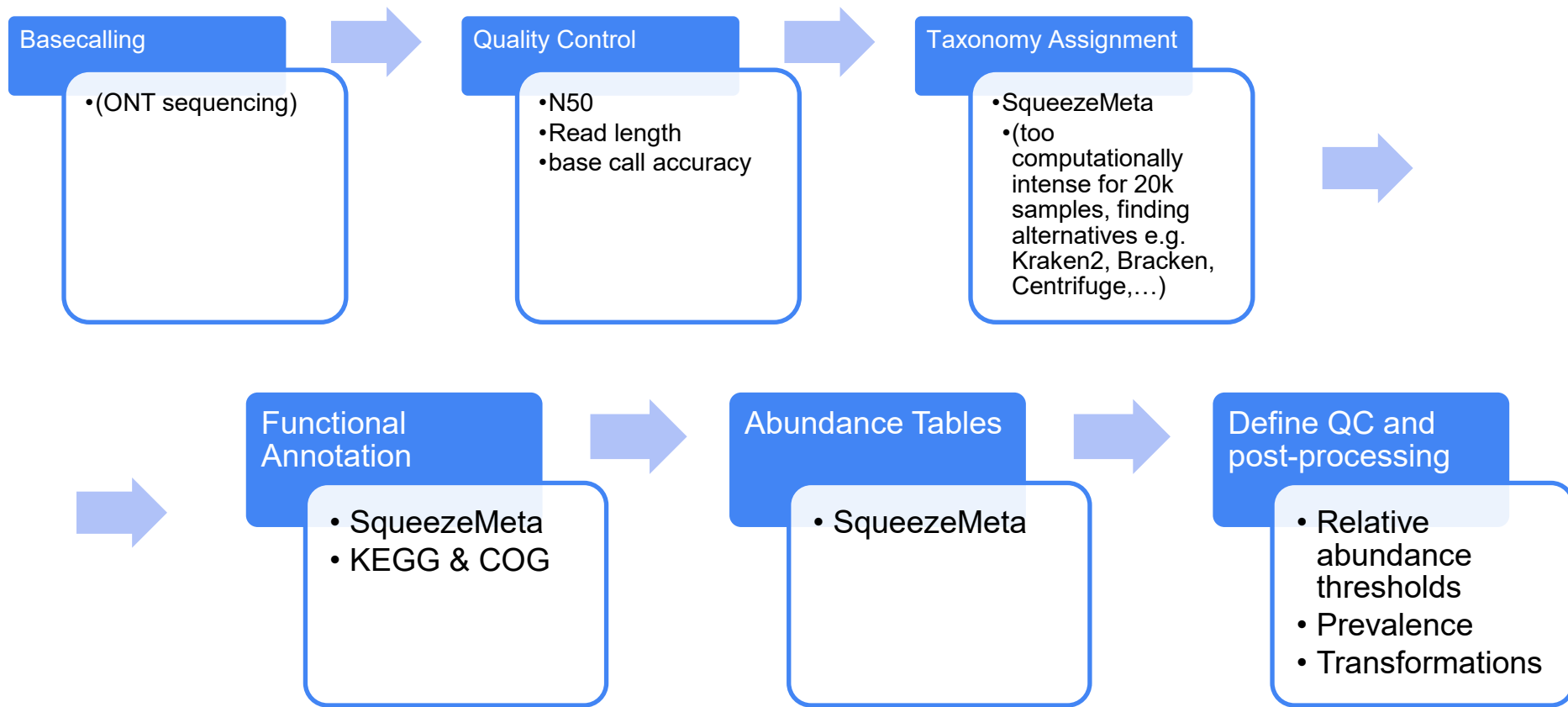
- Expect to generate > 500 TB for $\sim 25k$ samples.

BIOINFORMATICS

Proposed Metadata:

animalID	Animal ID as international codedL	character
sampleID	Sample ID	Character
Project	Name of the project	character
Country	Country of data origin	character
sample Collection Date	Date of sampling	character
Farm location	Location of farm where data was colleted	character
times after last eating	times after last eating	character
trial/treatment/specialFeed/none	Experimental condition	character
Sequencing platform	Model of the sequencing platform used	character
Basecalling method	Sotware and version	character
Sequence Quality	Q-score	float
StorageMethod	Method used to store or preserve the sample	character
Filtering method	Method used to filter or preprocess the sample before sequencing	character
N50	Length of contig covering 50% of the assembly.	integer
Yield	Amount of nucleic acid or sequence data	float
Minimum read lenght	Minimum read lenght	integer

Initial proposed bioinformatic workflow



What to provide in the GMG database?

- Per-sample QC reports
- Taxonomy
 - Absolute abundance at a lower possible taxonomic rank
- Functions KEGG
 - Absolute abundance for KEGG
 - Absolute abundance for COG

Data sharing strategy for external partners with microbiome data

What partners can provide to microHub:

- Metagenome (shotgun) raw reads (all platforms accepted: Illumina, ONT, PacBio, Others), with methane phenotypes and metadata

What microHub can provide in return:

- A table with microbial counts according to the reference metagenome established in the group
- SNP coefficients (available from the genotyping array(s) used within the GMG) to estimate GBV for microbiome traits associated to methane production.
- Sharing of results facilitated via GMG database

Ideas for broader scope research

- **Harmonization** of microbiome analyses
- Oral vs Rumen microbiome
- Combination of shotgun data and amplicons
- Metagenome function for hydrogen pathways
- Statistical analysis of metagenome data
 - Already several EoI
- **Other ideas are most welcome**

Questions?

Discussion – share your experience



Feel free to reach us out at:

gmg@wur.nl

Oral vs Rumen microbiome

32 Holstein cows



J. Dairy Sci. 107:5881–5896
<https://doi.org/10.3168/jds.2024-24014>

© 2024, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
 This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

The oral microbiome as a proxy for feed intake in dairy cattle

C. N. Marcos,^{1,2*} A. Bach,^{3†} M. Gutiérrez-Rivas,^{2,4} and O. González-Reco²

¹Departamento de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

²Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria – CSIC, 28040 Madrid, Spain

³CREA, 08007 Barcelona, Spain

⁴Bianca from the Pyrenees, Hostalets de Trast, 25795 Lleida, Spain

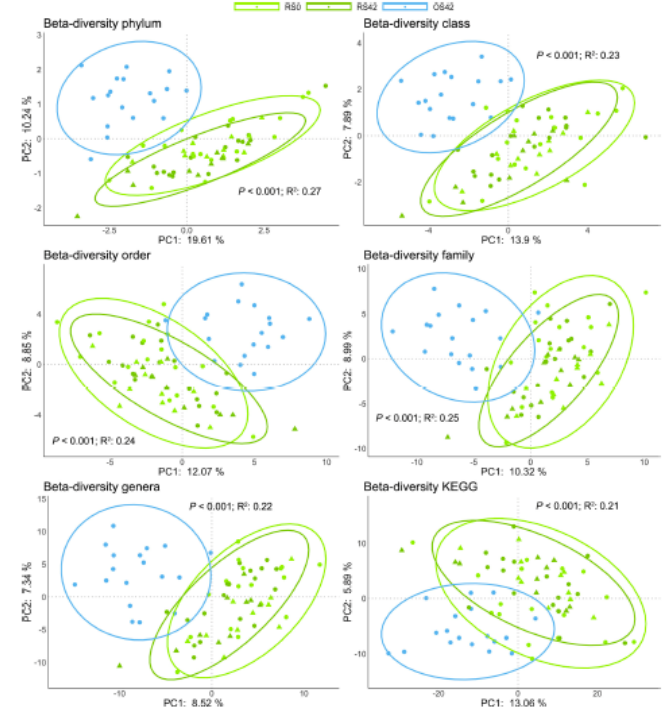


Figure 3. First 2 principal components for the microbiome composition in the rumen samples (RS) at the beginning of the trial (RS0), RS at 42 d (RS42) and oral samples at 42 d (OS42). Symbols represent the samples using Euclidean distance of centered log ratio-transformed taxa abundances or functionality colored by sample type. Cows with both OS42 and RS are represented by circles (triangles otherwise). P = significance of sample type effect in the PERMANOVA. R^2 = variability explained by sample type.

Global Methane Genetics microHUB Working Group Update: Sheep Bioinformatics & Background

Ben Perry*, Timothy Bilton, Hannah Henry,
John McEwan, Suzanne Rowe

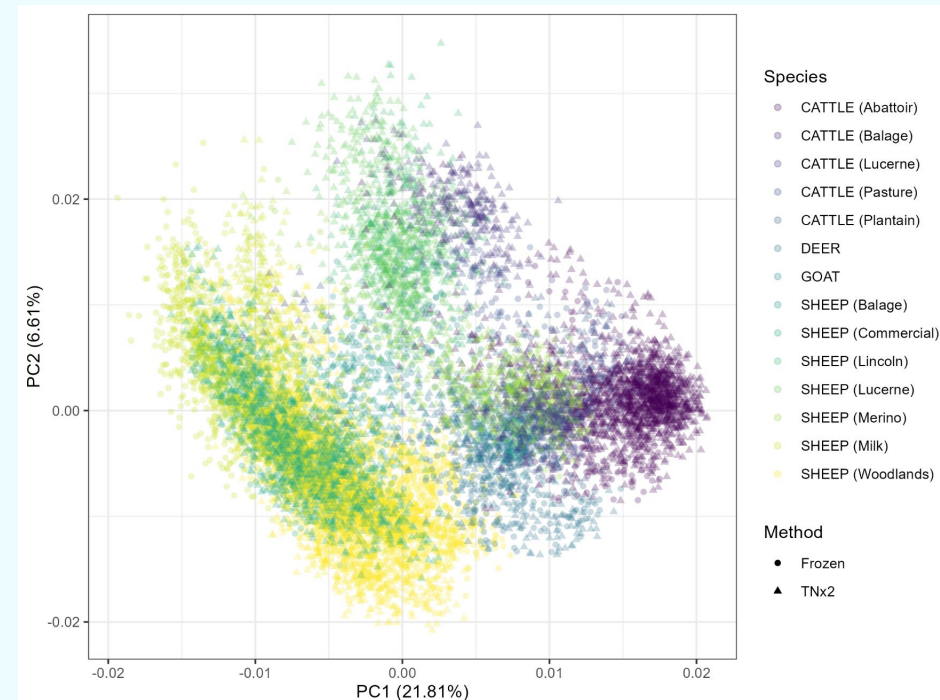
28th November 2025



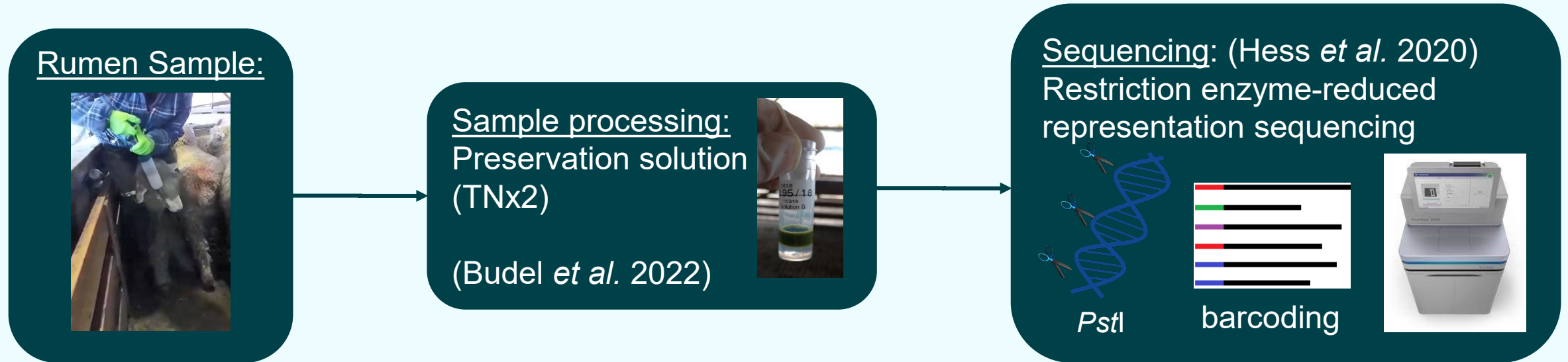
Rumen Sampling in New Zealand – Brief Context



- We've been seriously sampling since about 2015; Oldest samples ~2010
- Sheep rumens ~ 25,200; Cattle ~7600; Deer ~1400
- Develop solutions to scaling:
 - Preservant
 - Sequencing
 - Bioinformatics
- Emphasis on cost, throughput, CH4-prediction



Sample Processing and Data Generation

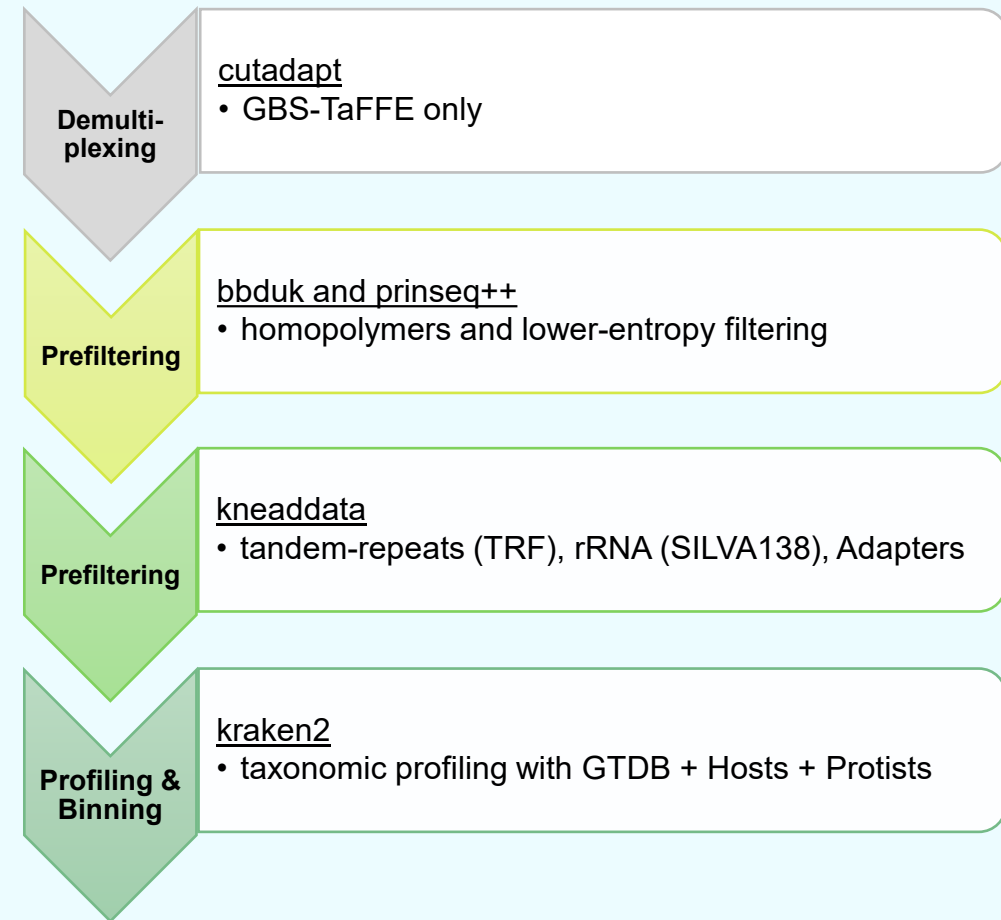


- Low cost RT preservative – TNx2 mixed 1:1 with rumen content
- In-house DNA extraction and library preparation
- Illumina sequencing - NovaSeq
- Aim for 750,00 - 500,000 reads / sample from rumen material

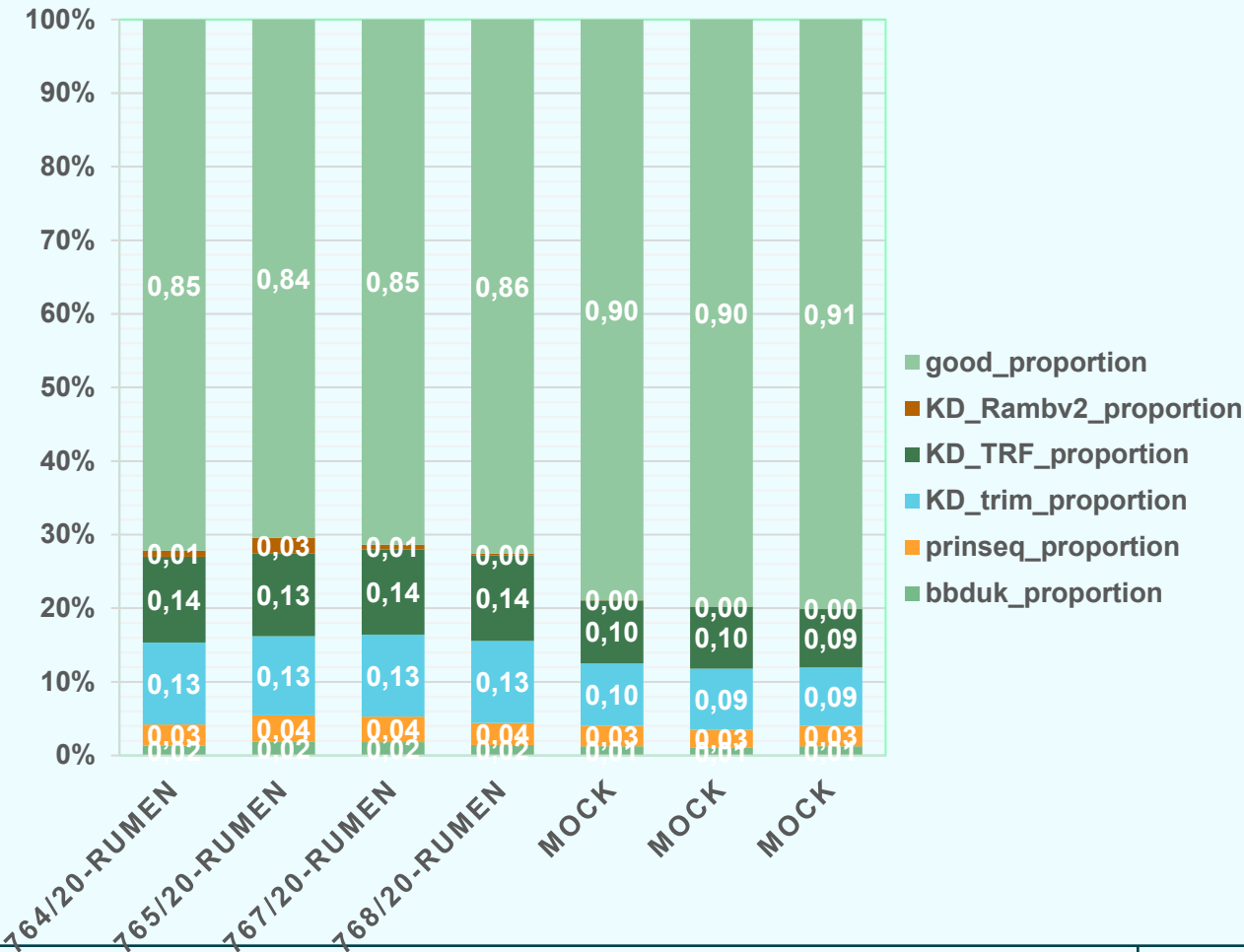
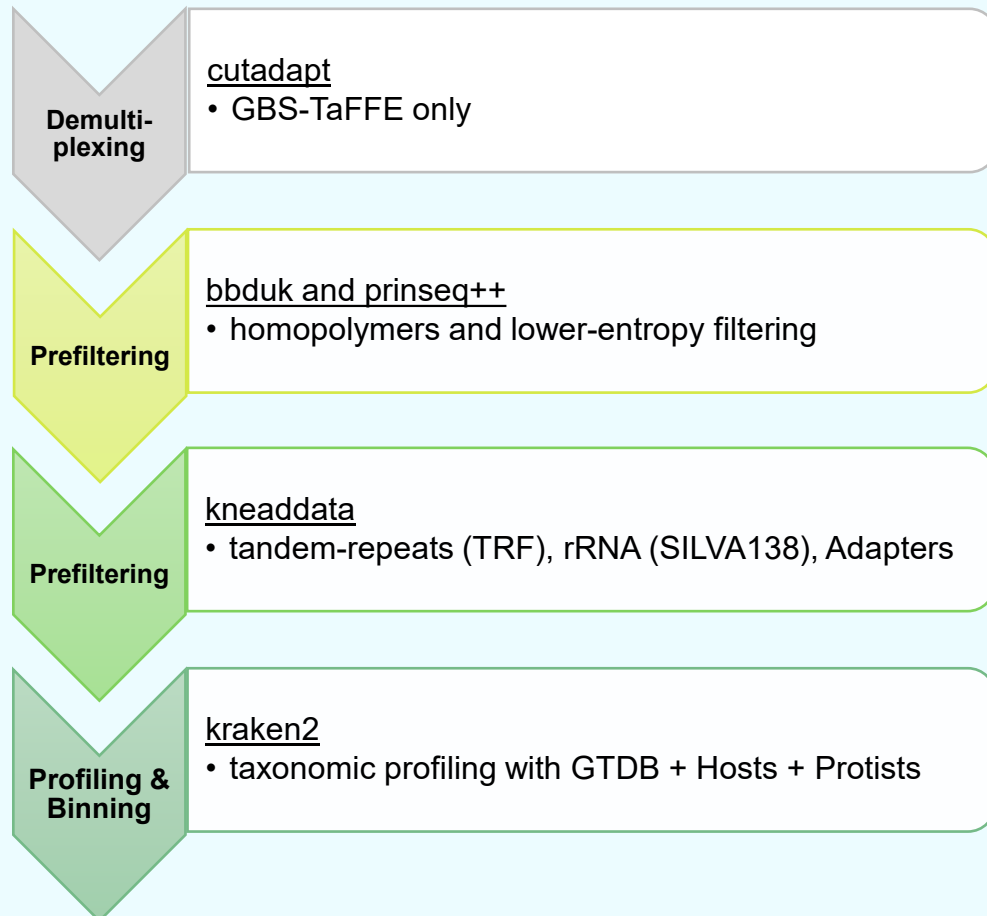
Bioinformatic Processing: Preprocessing and Reference-Based Profiling



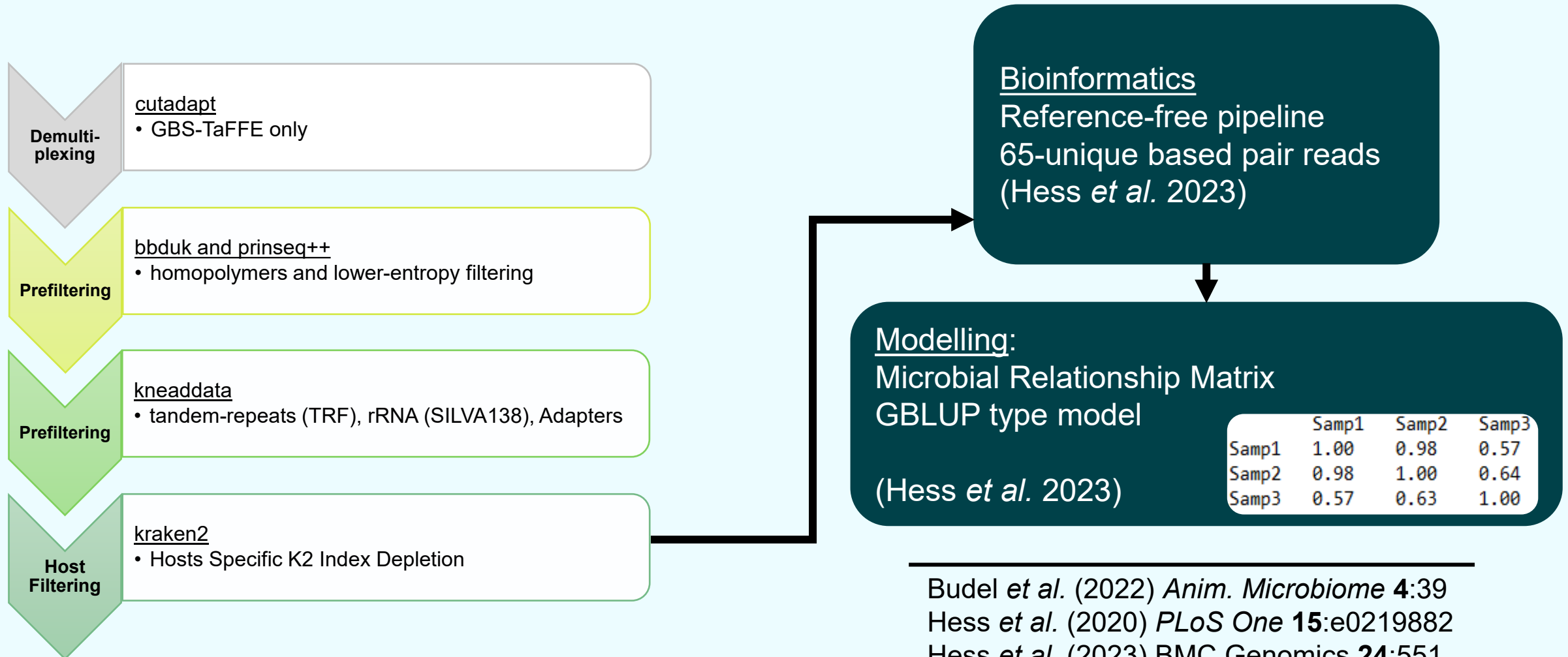
- TaFFE and GBS-TaFFE Pipelines
- Taxonomic and Functional Feature Extraction
- Emphasis on Quality and Scaling
- Sequencing data pre-processing
- Kmer-based profiling to a custom K2 index
- Generally, for reference-based analysis we work at the genera level



Bioinformatic Processing: Preprocessing and Reference-Based Profiling



Bioinformatic Processing: Reference-Free - Microbial Relationship Matrix



Budel *et al.* (2022) *Anim. Microbiome* **4**:39
Hess *et al.* (2020) *PLoS One* **15**:e0219882
Hess *et al.* (2023) *BMC Genomics* **24**:551

Bioinformatic Processing: Reference-Free - MRM



- tags are trimmed 65 bp, less than 65 bp are removed
- tags is kept if count > 0 in 25% of training set
- Resultant ~200k tags in sheep for predictions (~OTUs)
- Cohort adjustment – per tag; mean zero; variance 1 (import for predicting across farms)
- Microbial relationship matrix – GSE method MRM

Bioinformatics
Reference-free pipeline
65-unique based pair reads
(Hess *et al.* 2023)



Modelling:
Microbial Relationship Matrix
GBLUP type model
(Hess *et al.* 2023)

	Samp1	Samp2	Samp3
Samp1	1.00	0.98	0.57
Samp2	0.98	1.00	0.64
Samp3	0.57	0.63	1.00

Bilton *et al.* (2025) *Genet Sel Evol* **57**:25

Budel *et al.* (2022) *Anim. Microbiome* **4**:39

Hess *et al.* (2020) *PLoS One* **15**:e0219882

Hess *et al.* (2023) *BMC Genomics* **24**:551

Methane Prediction: Reference-Free - Microbial Relationship Matrix



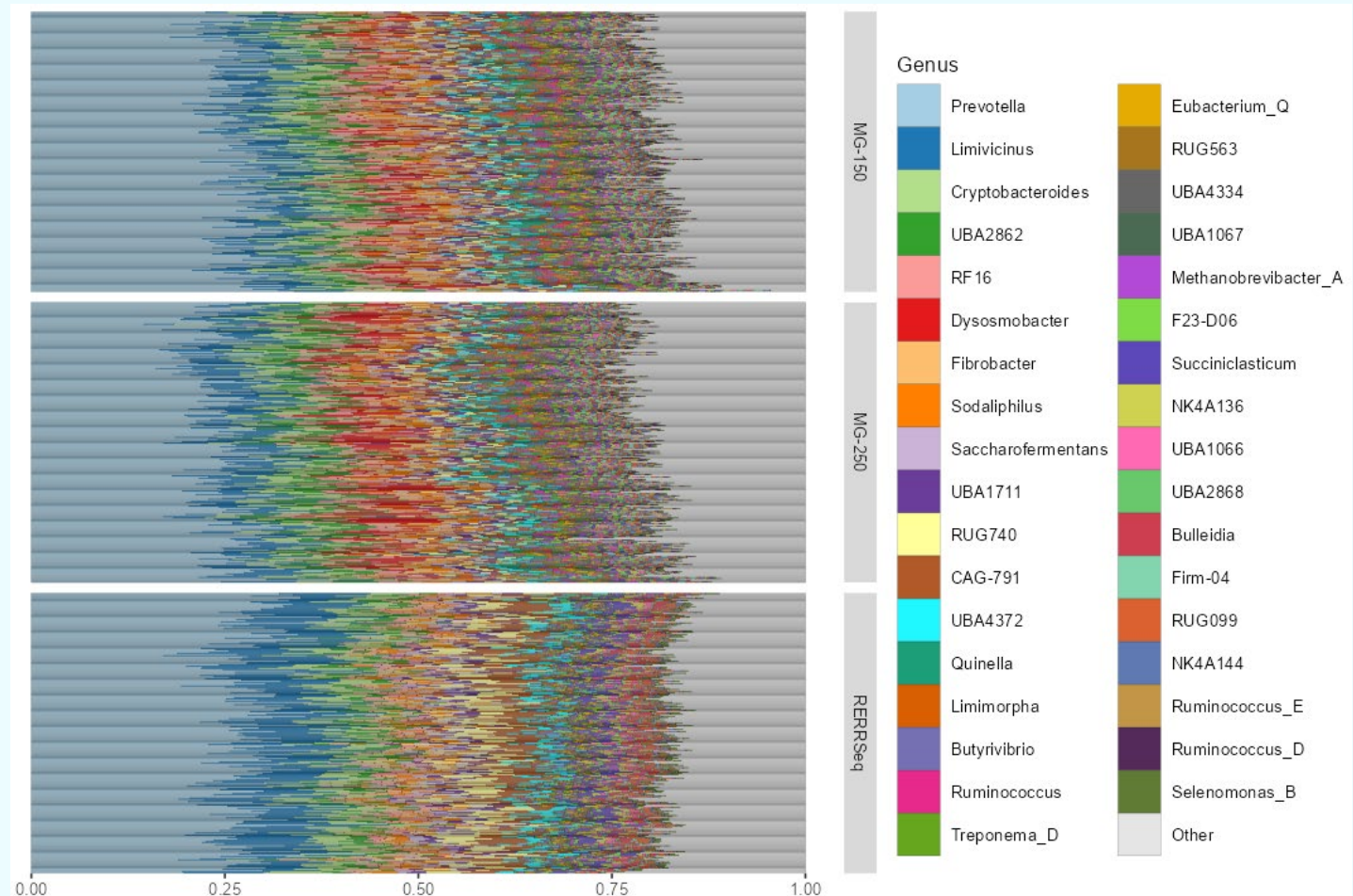
- We predict methane (g/day; g/kgDMI(feed eaten)) from MRM
- MRM is used in place of a GRM in a linear mixed model for prediction
- We are using the similarity of the microbiome (analogous to relatedness in GRM) (information from relatives used to predict for individuals)
- These prediction and shared genetics are used in the final predictions

Bilton et al. (2025) Genet Sel Evol **57**:25

Bioinformatic Processing: RERRSeq Reference-Based Profiling



- < ~35 taxa represent >0.75 of the reads assigned
- Better representation of some low abundance taxa
- All methods have intrinsic biases
- A predictable GC bias from PstI cut site



Open discussion – research gaps

Thanks for your attention & contributions!

gmg@wur.nl

Newsletter:

https://www.wur.nl/en/project/global-methane-genetics-initiative.htm?wmstepid=thank_you

**Global
Methane
Genetics
initiative**

Led by



WAGENINGEN
UNIVERSITY & RESEARCH

In Partnership with



BEZOS
EARTH
FUND



Global
Methane
Hub

