Global Methane Genetics initiative

Led by WAGENINGEN UNIVERSITY & RESEARCH

In Partnership with BEZOS EARTH FUND · Global Methane Hub

MicroHUB

Rumen sampling Q&A session

December 2nd, 2025

TABLE OF CONTENTS

- Ethics approval
- Detail sampling Protocol
- Oral sampling example (optional)
- Data sharing
- Recognition to GMG partners
- Additional Q & A

Ethics approval

- Number of animals:
  - Hsien, W. Y., et al. (2024). Machine learning for prediction of methane emissions from dairy cattle using multiomics data. Australasian Dairy Science Symposium, Christchurch, New Zealand.
  - Sepulveda, B. J. (2024). Exploring genetic solutions to minimise enteric methane emissions via feeding behaviour traits and rumen microbiome. Thesis Doctor of Philosophy, La Trobe University.
  - Gonzalez-Recio, O. et al. (2014): On the value of the phenotypes in the genomic era. JDS, 97:7905-7915.
- Updated information can be provided

SOP Rumen sampling

- Documentation shared among GMG partmers

Standard Operating Procedure (SOP)

Title: Rumen Fluid Collection in Cattle

SOP Number: RFCC-001

Effective Date: June 1st, 2025

Prepared by: Oscar Gonzalez-Recio, Aser García Rodriguez, David Flossdorf, Mirjam Spoelstra, Aniek Bouwman, Suzanne Rowe, Goutam Sahana, (others)

Approved by: microHub GMG project

Version: 1.0

1. Purpose

The micro-HUB project will establish a reference population with phenotype, genotype and metagenome information, that will serve to create a genomic evaluation system that can be used to select the parents of the next generation with microbiome profiles that produce less enteric methane while maintaining profit and health genetic progress.

The Global Methane Genetics methane phenotyping program provides a unique opportunity to create the largest reference metagenome representing a large diversity of breeds, productive systems and geographical regions. This will allow estimating genomic breeding values for microbiome composition and shift the rumen microbial composition towards more efficient pathways accelerating the reduction of methane emissions using selective breeding.

The micro-Hub project will share the expertise and experience on the collection of rumen samples, DNA extraction, sequencing, bioinformatic analyses and strategies to include microbiome composition in breeding programs.

The purpose of this document is to outline a standardized procedure for the safe, hygienic, and effective collection of rumen fluid samples in cattle for metagenomics analysis, ensuring high sample quality and animal welfare.

Please help us obtain good quality samples by following the SOP as instructed.

2. Scope

This SOP applies to all personnel involved in the collection, handling, and processing of ruminal fluid samples from live cattle in the GMG project.

3. Responsibilities

- Personnel: Must be trained in cattle handling and sampling techniques.
- Team Leader/PI: Ensures adherence to SOP, proper documentation, and compliance with

Rumen sampling sheep

1. Purpose of Manipulation

This procedure is used to collect rumen samples from mature sheep and lambs that do not have rumen fistulae. The samples are used for microflora measurements, measurement of rumen pH and fatty acid concentrations, including volatile fatty acids, and measurement of general rumen function.

2. Equipment List

- PPE gear: Gloves, overalls and steel capped or solid shoes
- PVC stomach tube:11mm adult sheep and 9.5mm young sheep. (See **Tube selection** section for more detail)
- Syringe (140mL or 400mL)
- Container for sample collection (urine vials,70ml)
- Suitable facilities to catch and restrain the animal – small pen, or a race, or sheep handler
- Recording sheet
- Permanent marker (optional)
- Pipette
- 8ml tubes prelabelled containing TNX2 solution

3. Tube Selection/Creation

Length: 115cm length is recommended for large (80kg) adults, while 90-100cm is sufficient for smaller sheep.
A commercial stomach tube may be available, depending on size required, which will have a rounded end. If you are making your own tube from soft PVC tubing, both ends will be open. For stalky/thick rumen contents, an open end may be required for samples.
If not already present, make 3 holes in the side of the tube, approximately 3-10cm from the end. Create these by folding the tube and sniping off the corner. A longer (15mm) oval shape allows maximum volume for particulates to pass through, seen in figure 2, while allowing the tube to retain its structure well and not fold over while passing. Ensure the tube does not have any rough edges or sharp ends. Smooth the edges my cutting around the opening then heating tubing up to smooth over.
Attach the tube to the syringe then use clamp to ensure it is sealed (Figure 3). Draw water up the tube to ensure the seal is tight and the suction is sufficient.
Before sampling apply lubricant around the edge of the plunger to ensure it is sliding smoothly. See

SAMPLING

- Head restraint

SAMPLING

- material

- Esophageal probe (18 mm × 160 cm) (Fig 1 shows probe used in Spain, and Fig 2 shows probe used in The Netherlands). Should have holes of small size at the tip, to avoid large particles going through the probe.

Fig 1.

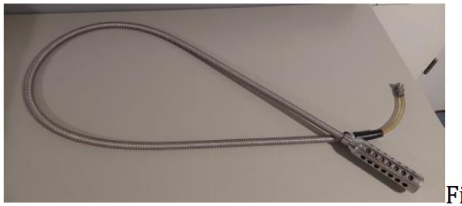


Fig 2.

- Ruminator set (tube + pump) (Fig 3). The probe is connected to a 1,000-mL Erlenmeyer flask and continued to a mechanical pump (e.g. Vacubrand ME 2SI, Wertheim, Germany). A manual hand-held pump (non-electronic) can also be used (Fig 4).

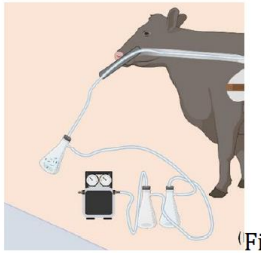


Fig 3.

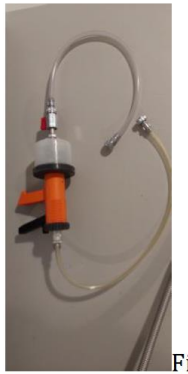
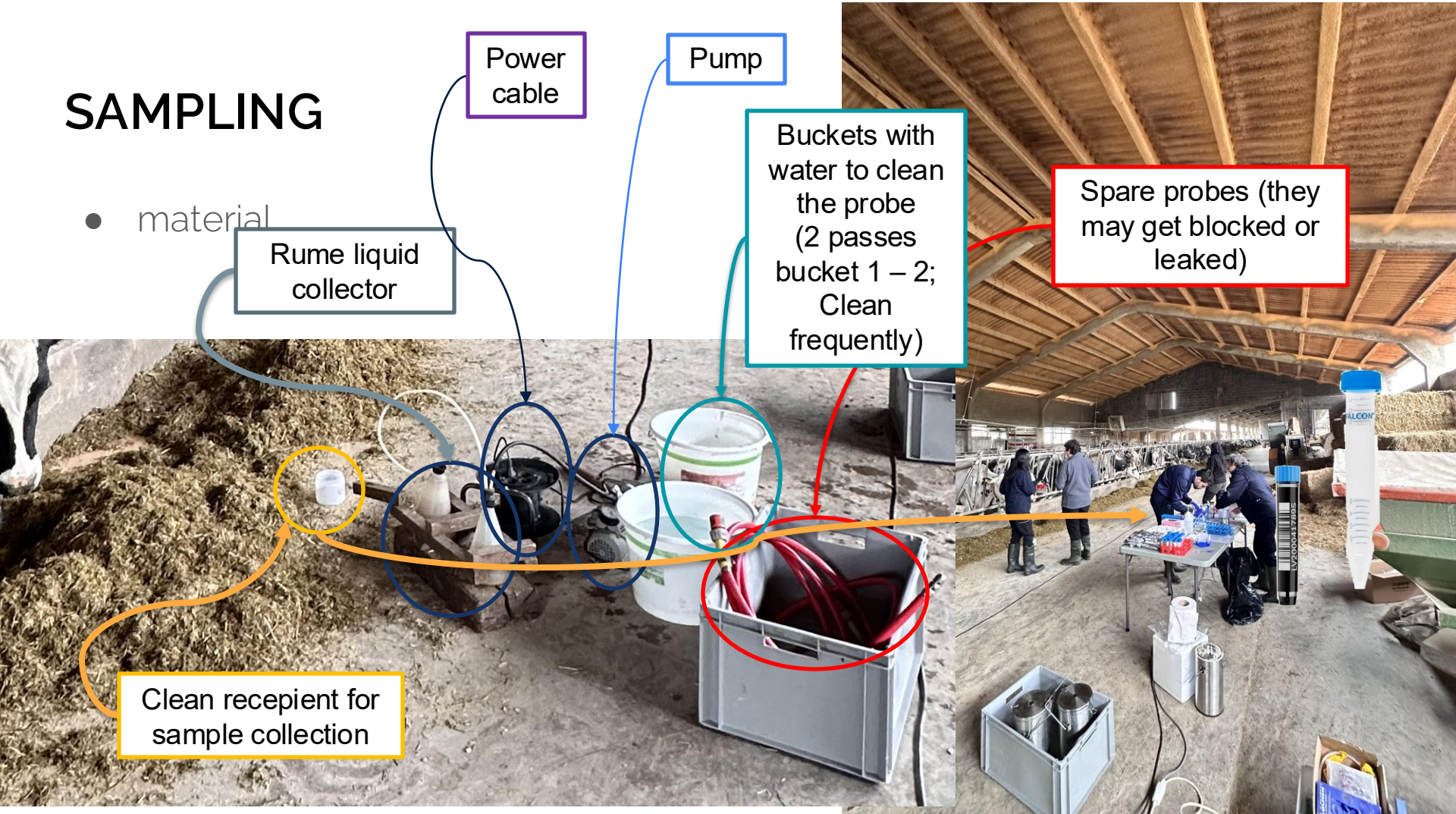


Fig 4.

SAMPLING

- material

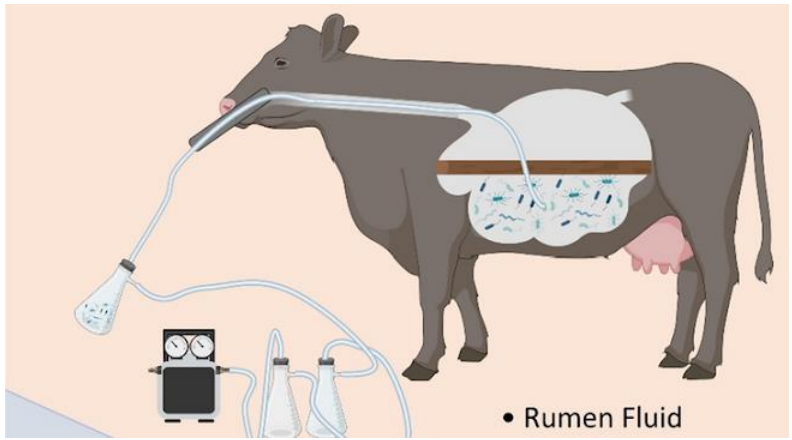


Material

Probe

Head restriction

Pumping system



Material

- Supportiing video material

Material

- Tubes
  Please contact your designated lab contact (dairy, beef sheep)

Relevant considerations

- Cryogenic
- Volume
- Label safe (missing or unidentifiable labels means lost sample)

Sampling and shipping costs have already been funded by GMG with US$100/sample (topped up budget)

SAMPLING process

Per cow

- Probe insertion
- Pumping to extract ruminal liquid
- Transfer content to collector recipient
- Mix the sample thoroughly
- Transfer to the tube (4 tubes: 2 shipped + 2 store in origin as backup)
  - 3D barcoded tubes highly recommended (contact your designated lab if not possible)
  - Label and identify the tube clearly
  - *Optional*: add preservative **if agreed** with the designated lab
- Freeze (dry iced or liquid nitrogen vapors)

Off-farm

- Store -80°
- Organize shipping with Amanda (beef), Suzanne (sheep), Oscar (dairy)

SAMPLING process – Preservative SOP

- Samples are intended to be preserved using in-house preservative (SOP also provided)

Reagent
5M NaCl
1M Tris pH 8.0
10% SDS
0.5M EDTA pH 8.0
ProClin 300
Milli-Q H2O

agresearch
āta mātai, mātai whetū

SAMPLING process – Oral swab

- Optional
- To be kept internally by each partner
- No additional funding from GMG for this

- Use PERFORMAgene PG-100 swabs (https://www.dnagenotek.com/US/products/collection-animals/performagene/PG-100)

Sampling

- The Netherlands experience

SAMPLING process – Shipping

- Ship samples to the lab of reference (conditioned on importing permits)
- Please organize this with your designated lab
- Start checking exporting/importing permits at least 2 mo in advance.

  - Dairy – UK
  - Beef – Australia
  - African crosses – Australia
  - Sheep – New Zealand
  - Control samples in all labs

THE UNIVERSITY of EDINBURGH
The Royal (Dick) School of Veterinary Studies

Contact Oscar González Recio
(oscar.gonzalezrecio@ed.ac.uk)

AGRICULTURE VICTORIA

AGRICULTURE VICTORIA

Contact Amanda Chamberlain
(Amanda.Chamberlain@agriculture.vic.gov.au)

agresearch
āta mātai, mātai whetū

Contact Suzanne Rowe
(Suzanne.Rowe@agresearch.co.nz)

Bioinformatics

- Needs for data storing raw data
  - Results: Abundance tables, QC, metadata, etc
    - GMG database
  - Fastq files: 325 Gb / 100 metagenomes. (81 Tb total). Who will store this?
    - Public: free but not private
    - Private: GMG + owner of the samples
  - Pod5 files: 2 Tb / 100 metagenomes (500 Tb total). Should we store this? GMG or partners?
    - Public: Free but not private; only 5Tb /submission
    - Privately ($56-$584 / 100 metagenomes / per year); Google Cloud, AWS

- What data should be stored? How

Data sharing within GMG

- Raw data

- Results
  - Abundance table, QC
    - Will be shared with GMG, and DTA will apply
  - SNP coefficients for genomic prediction
    - Will be shared with GMG partners

Recognition

- Scientific publication and dissemination
    - All partners need to be recognized – need to agree the terms
        - Number of authors, logos, etc.
        - Open for suggestions

Open questions

Thank you

Feel free to reach out to:

Oscar Gonzalez Recio
Gonzalez.oscar@inia.csic.es