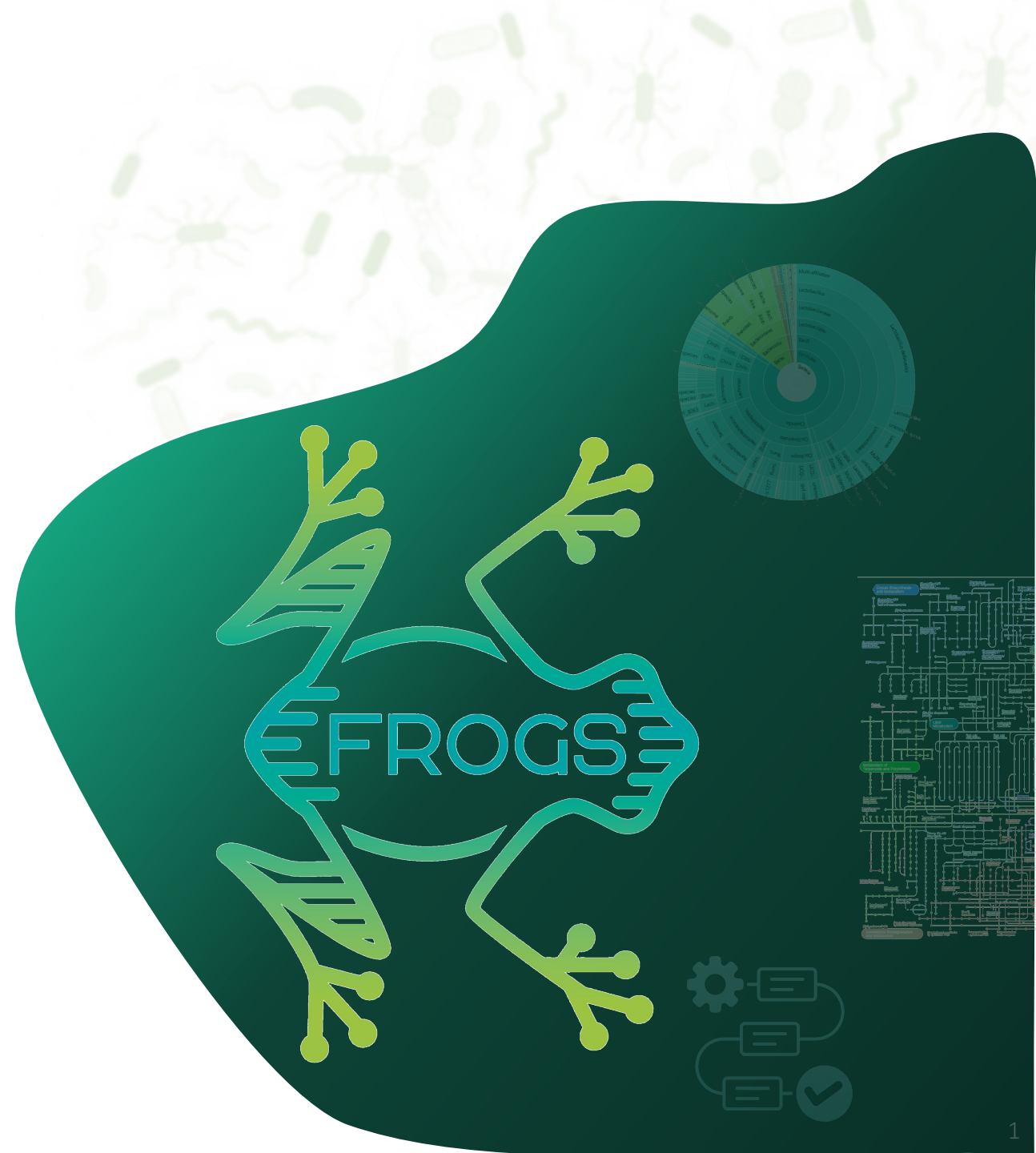


Using FROGS by itself


on long-reads data

Lucas AUER, Gabryelle Agoutin,
Maria BERNARD, Géraldine PASCAL,
Maëlle POMIÈS & Olivier RUÉ



Presentation of the dataset

Effect of live yeast supplementation in sow diet during gestation and lactation on sow and piglet fecal microbiota, health, and performance

Nathalie Le Floc'h,^{†,1}  Caroline Stéphanie Achard,[‡] Francis Amann Eugenio,[†]
Emmanuelle Apper,[‡] Sylvie Combes,^{||} and Hélène Quesnel[†]

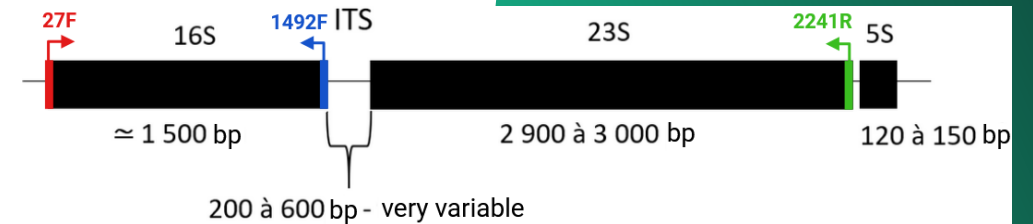
48 sows and their piglets

16S V3-V4 

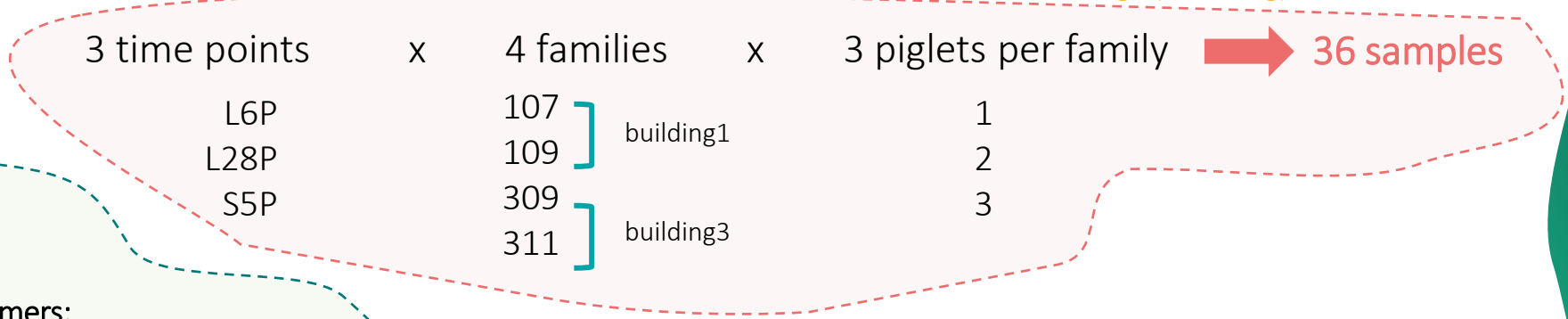
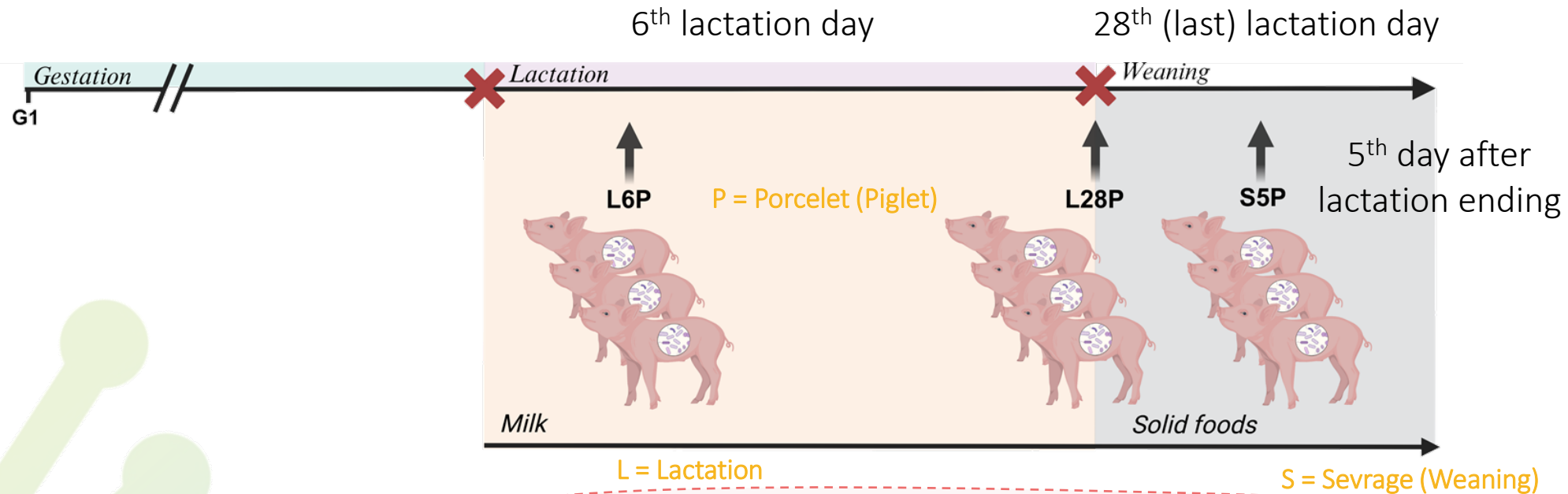


SeqOcln

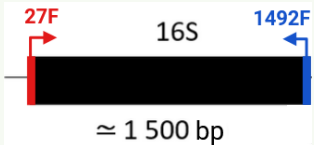
16S-ITS-23S 



Presentation of the training subset



Marker is the full 16S:



PCR primers:
 27F: AGRGTTYGATYMTGGCTCAG
 1492F: AAGTCGTAACAAGGTARCY
 (both in 5' 3')

Practice session



Create a new history, you may entitle it “Piglet_16S_longread” for exemple


Please, upload the data and metadata files that you find here :

https://web-genobioinfo.toulouse.inrae.fr/~formation/15_FROGS/30_March2026/Piglets_16S_long.tar.gz
https://web-genobioinfo.toulouse.inrae.fr/~formation/15_FROGS/30_March2026/Piglets_metadata.tsv

Choose FROGS Core Main 1b Reads processing of long reads,
use swarm to create ASVs.

Modify parameters and run the process !

Choose FROGS Core Main 1b Reads processing of long reads

 **FROGS Core 1-Main** ASV reconstruction and taxonomic affiliation (Galaxy Version 5.1.0+galaxy0)

Tool Parameters

Select a tool from the FROGS Core suite to run your analysis.

- Please select a tool --
- 1.a. Reads processing of short reads
- 1.b. Reads processing of long reads
- 1.c. Reads processing of 454 reads
- 2. Remove chimera
- 3. Cluster/ASV filters
- 4. Taxonomic affiliation
- 5. Phylogenetic tree building
- ITSx

Practice session

Modify parameters and run the process !

Input type

TAR Archive

Sample files can be provided either as a single TAR archive or as separate files per sample (one or two files each).

TAR archive file *

 2: (hidden) Pig_16S_long.tar uncompressed

accepted formats ▼

The TAR file containing the long reads (.fastq.gz) for each sample. Supported sequencers: PacBio and Oxford Nanopore. (--input-archive)

Minimum amplicon length *

1000

The minimum length of the amplicons (including primers) (--min-amplicon-size)

Maximum amplicon length *

2000

The maximum length of the amplicons (including primers) (--max-amplicon-size)

Practice session

Do the sequences include PCR primers?

Yes

No

Indicate whether the sequences still include PCR primers. Select "Yes" if primers are present, "No" if they have already been removed.

5' primer *

AGRGTTYGATYMTGGCTCAG

Enter the 5' primer sequence. Wildcards are allowed. The sequence must be provided in 5' → 3' orientation. (--five-prim-primer)

3' primer *

AAGTCGTAACAAGGTARCY

Enter the 3' primer sequence. Wildcards are allowed. The sequence must be provided in 5' → 3' orientation. (--three-prim-primer)

Practice session

Process type

- Preprocessing only
- Preprocessing and clustering with Swarm
- Preprocessing and denoising with DADA2. !\ Only Pacbio sequencers are currently supported !\.

Select the type of process to run (`--process`)

Swarm distance threshold *

Distance threshold used by Swarm for clustering. (`--distance`)

Clustering refinement

- With `--distance = 1`, refine clusters with Swarm `--fastidious` option (recommended since FROGS 3.2)
- With `--distance > 1`, perform a pre-clustering step with FROGS `--pre-clustering` option
- No clustering refinement

(i) With `--distance = 1`, use the Swarm `--fastidious` option to refine clustering (recommended since FROGS 3.2). (ii) With `--distance > 1`, enable pre-clustering to reduce redundancy before final clustering step. (iii) Select this option to apply neither refinement nor pre-clustering.



Continue to process the data by run each tool step by step.