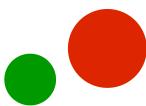
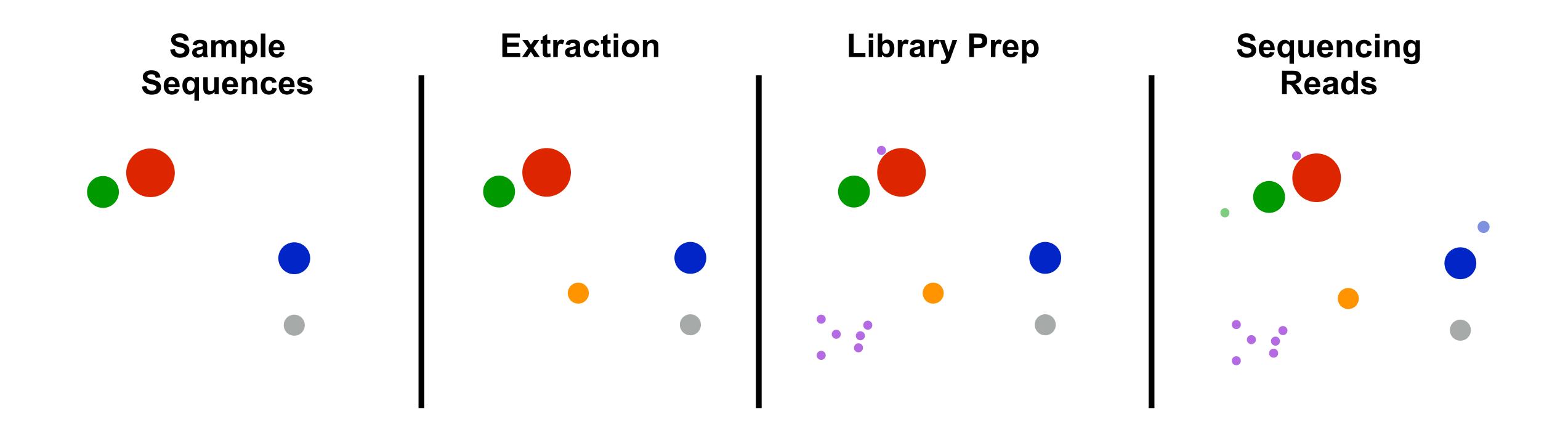
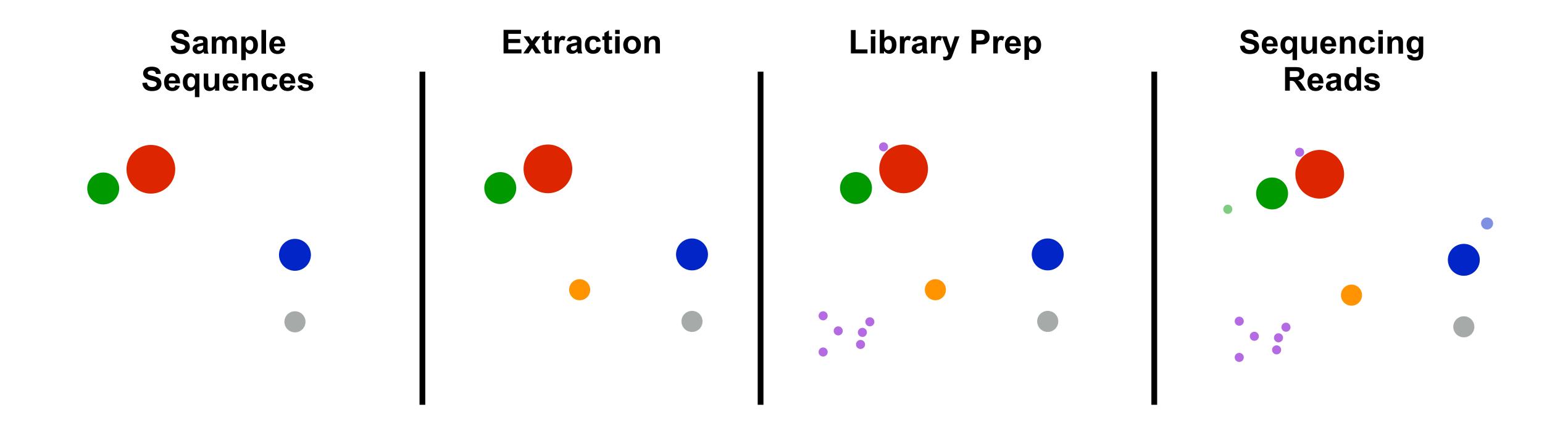
# Feline Bile and Low Biomass Microbiome Measurement

Sample Sequences

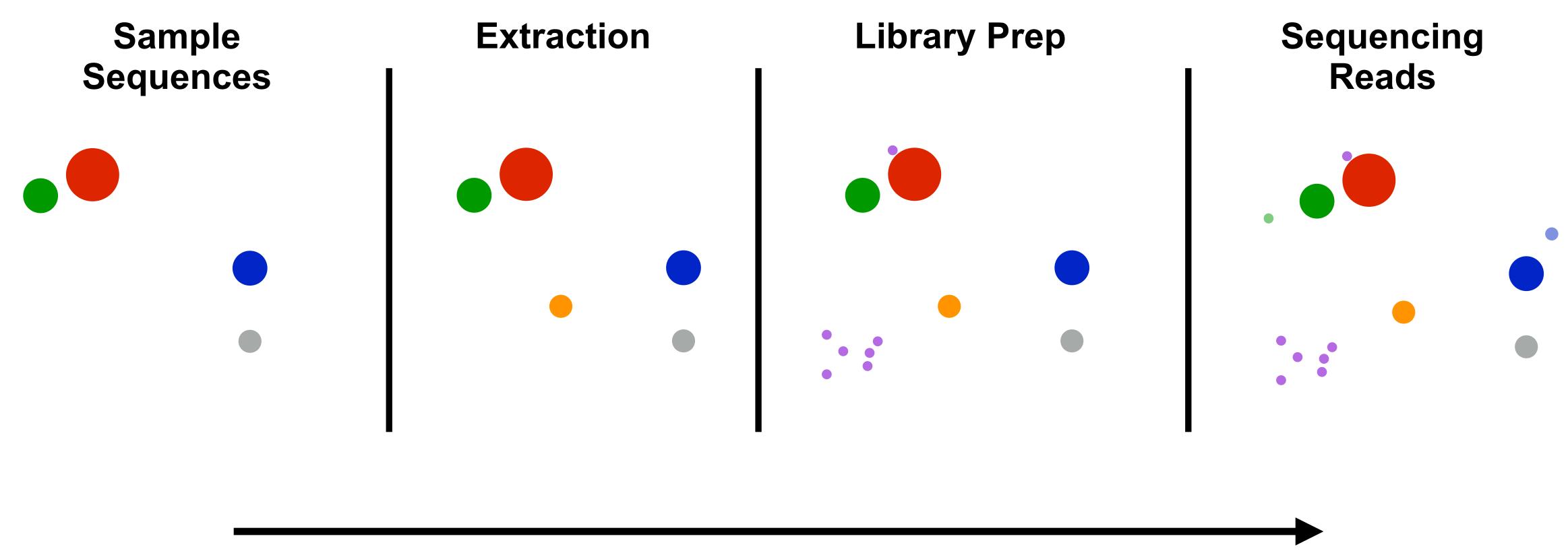






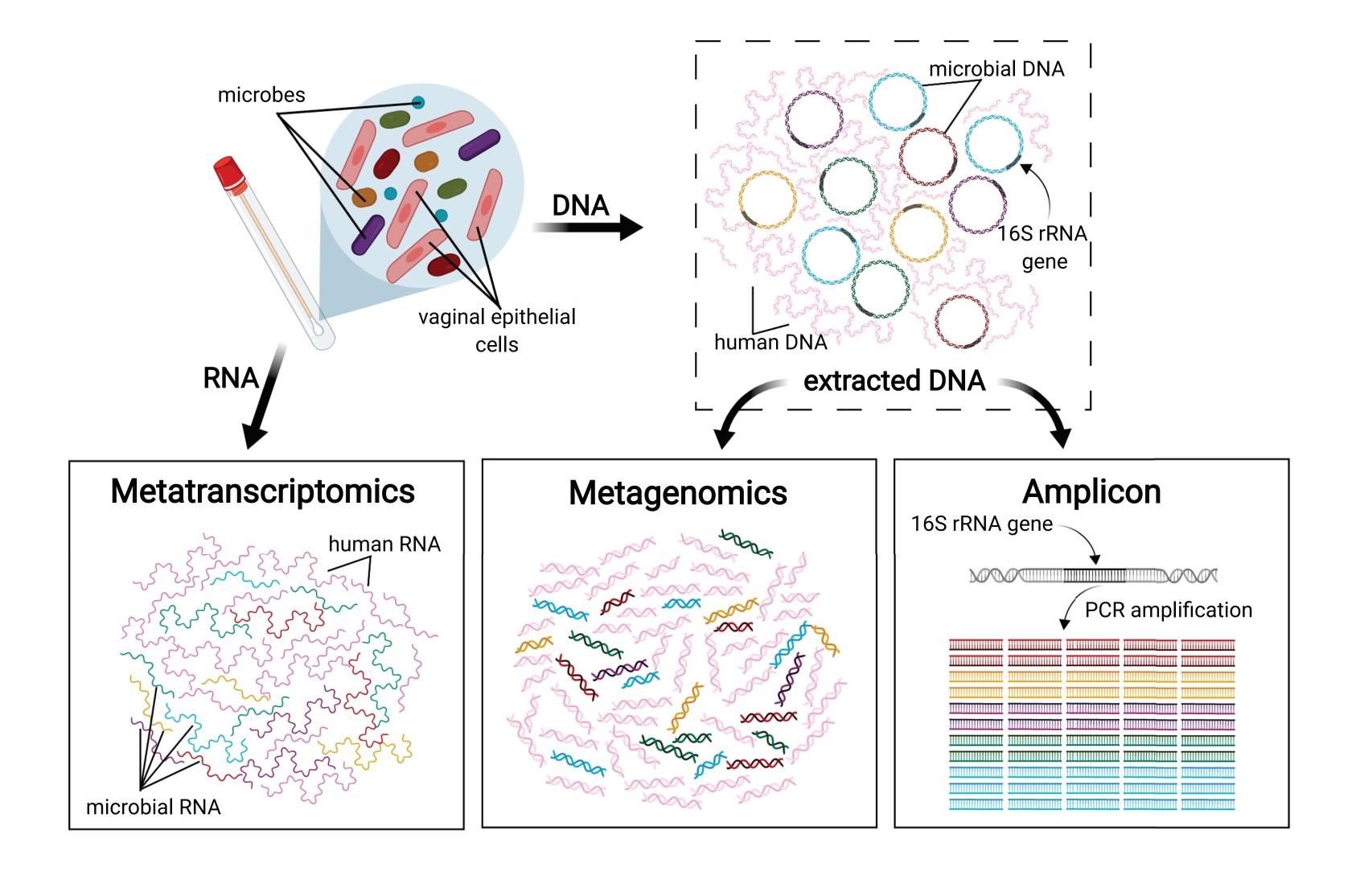


Contaminants introduced throughout the measurement process
High input biomass "crowds out" contaminants.
Not true for low input biomass.



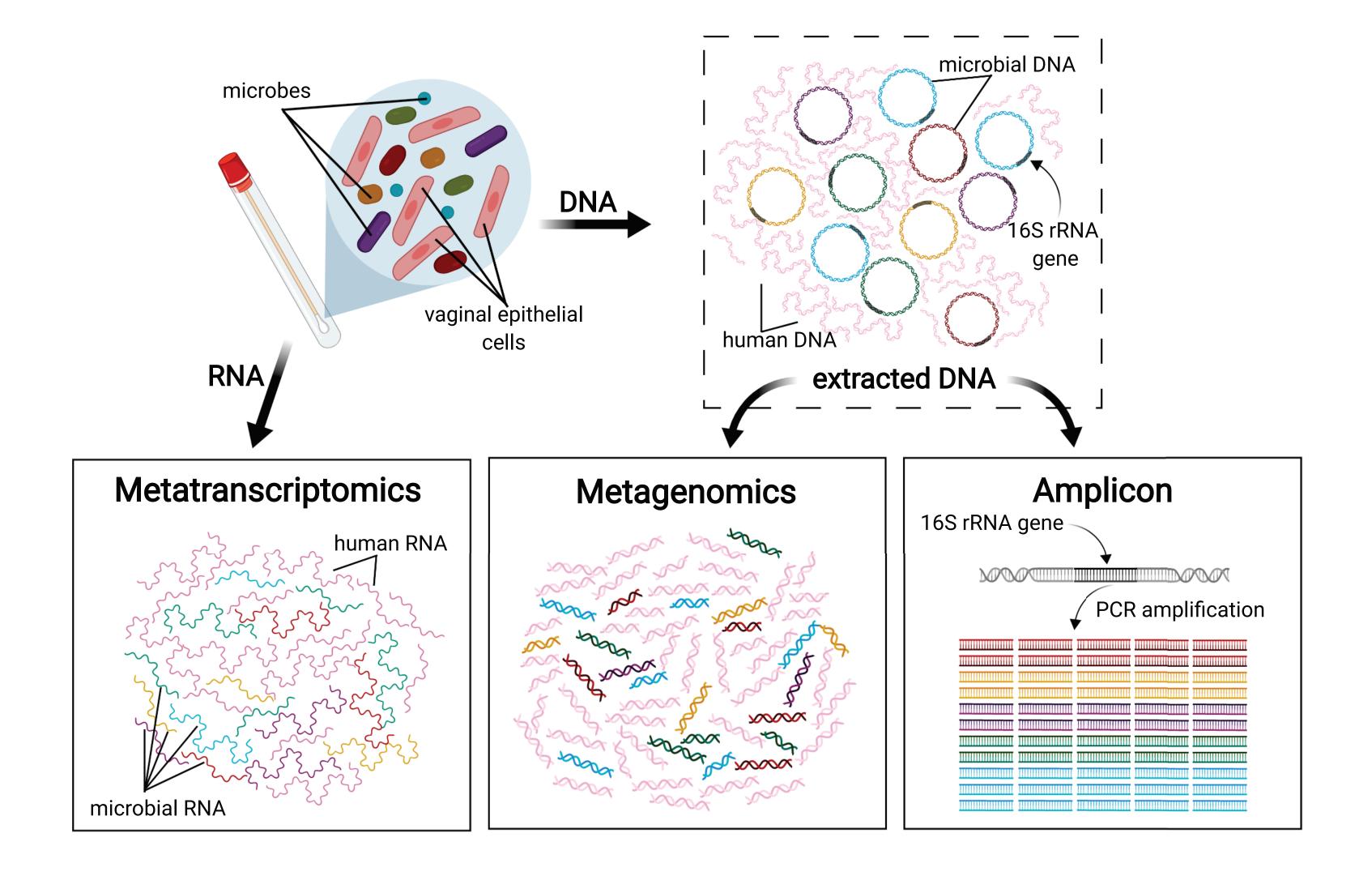
Contaminants introduced throughout the measurement process
High input biomass "crowds out" contaminants.
Not true for low input biomass.

Input biomass can also be "low" in a different way -- low relative to the non-target background.



Host-associated microbiomes are often embedded in vastly larger amounts of host cells/biomass/DNA!

Figure: Berman, McLaren & Callahan. BJOG, 2020.



Host-associated microbiomes are often embedded in vastly larger amounts of host cells/biomass/DNA!

This can lead to another type of "contamination" in the measurements. Background DNA that through off-target amplification or mistaken assignment that is misinterpreted as microbial.

Figure: Berman, McLaren & Callahan. BJOG, 2020.

Salter et al. BMC Biology 2014, **12**:87 http://www.biomedcentral.com/1741-7007/12/87



#### RESEARCH ARTICLE

**Open Access** 

Reagent and laboratory contamination can critically impact sequence-based microbiome analyses

Susannah J Salter<sup>1\*</sup>, Michael J Cox<sup>2</sup>, Elena M Turek<sup>2</sup>, Szymon T Calus<sup>3</sup>, William O Cookson<sup>2</sup>, Miriam F Moffatt<sup>2</sup>, Paul Turner<sup>4,5</sup>, Julian Parkhill<sup>1</sup>, Nicholas J Loman<sup>3</sup> and Alan W Walker<sup>1,6\*</sup>

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#### nature

# Human placenta has no microbiome but can contain potential pathogens

 $Marcus\ C.\ de\ Goffau^{1,2,8},\ Susanne\ Lager^{3,4,5,8},\ Ulla\ Sovio^{3,4},\ Francesca\ Gaccioli^{3,4},\ Emma\ Cook^3,\ Sharon\ J.\ Peacock^{1,6,7},\ Julian\ Parkhill^{1,2*},\ D.\ Stephen\ Charnock-Jones^{3,4,9}\ \&\ Gordon\ C.\ S.\ Smith^{3,4,9*}$ 

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#### **RESEARCH ARTICLE**

September/October 2023 Volume 14 Issue 5 e01607-23 https://doi.org/10.1128/mbio.01607-23

# Major data analysis errors invalidate cancer microbiome findings

Abraham Gihawi<sup>1</sup>, Yuchen Ge<sup>2,3</sup>, Jennifer Lu<sup>2,3</sup>, Daniela Puiu<sup>2,3</sup>, Amanda Xu<sup>2</sup>, Colin S. Cooper<sup>1</sup>, Daniel S. Brewer<sup>1,4</sup>, Mihaela Pertea<sup>2,3,5</sup>, Steven L. Salzberg (D) <sup>2,3,5,6</sup>

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And blood microbiome, and brain microbiome, and aerobiome, and...

#### Multiple overlapping challenges

Experimental: e.g. Clean isolation of samples and DNA

Bioinformatic: e.g. Mismapping of host DNA to microbial genomes

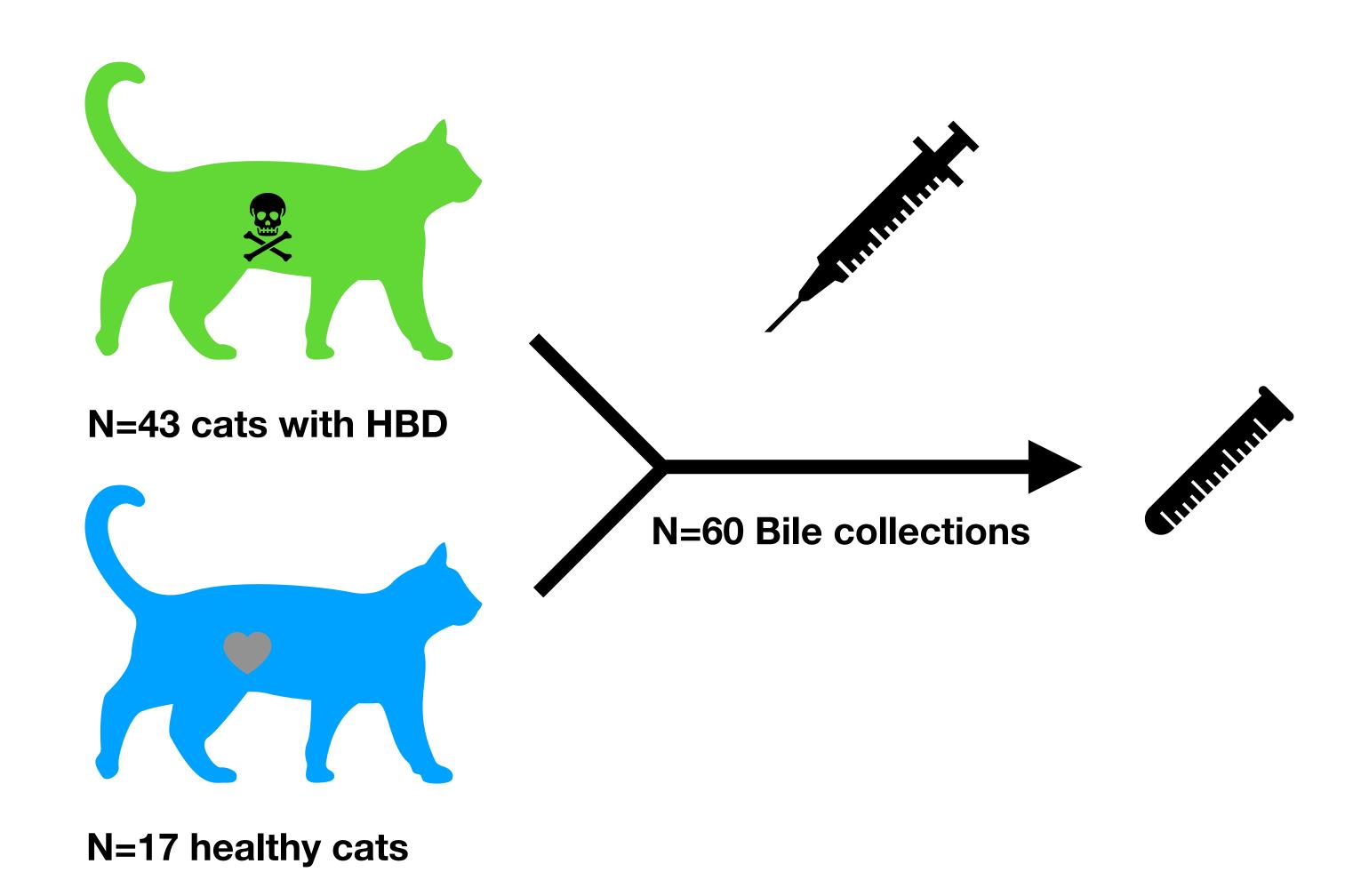
Interpretation: e.g. Live or dead? Resident or transient?

# Case Study The feline bile microbiome in health and hepatobiliary disease (HBD)

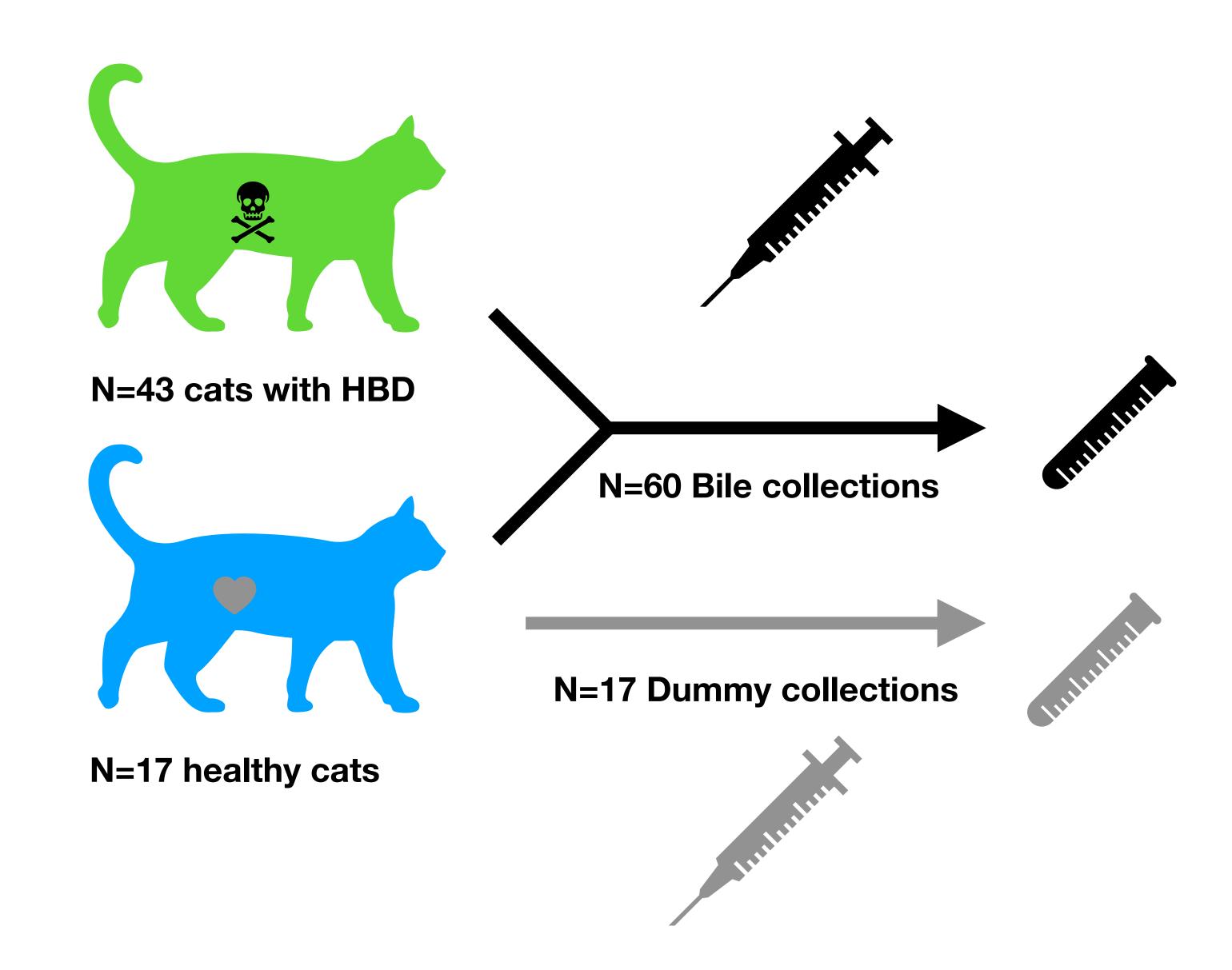
Question 1:
Is HBD sometimes caused by bacteria missed by standard clinical culture?

Question 2: ls there a microbiome of bile in cats?

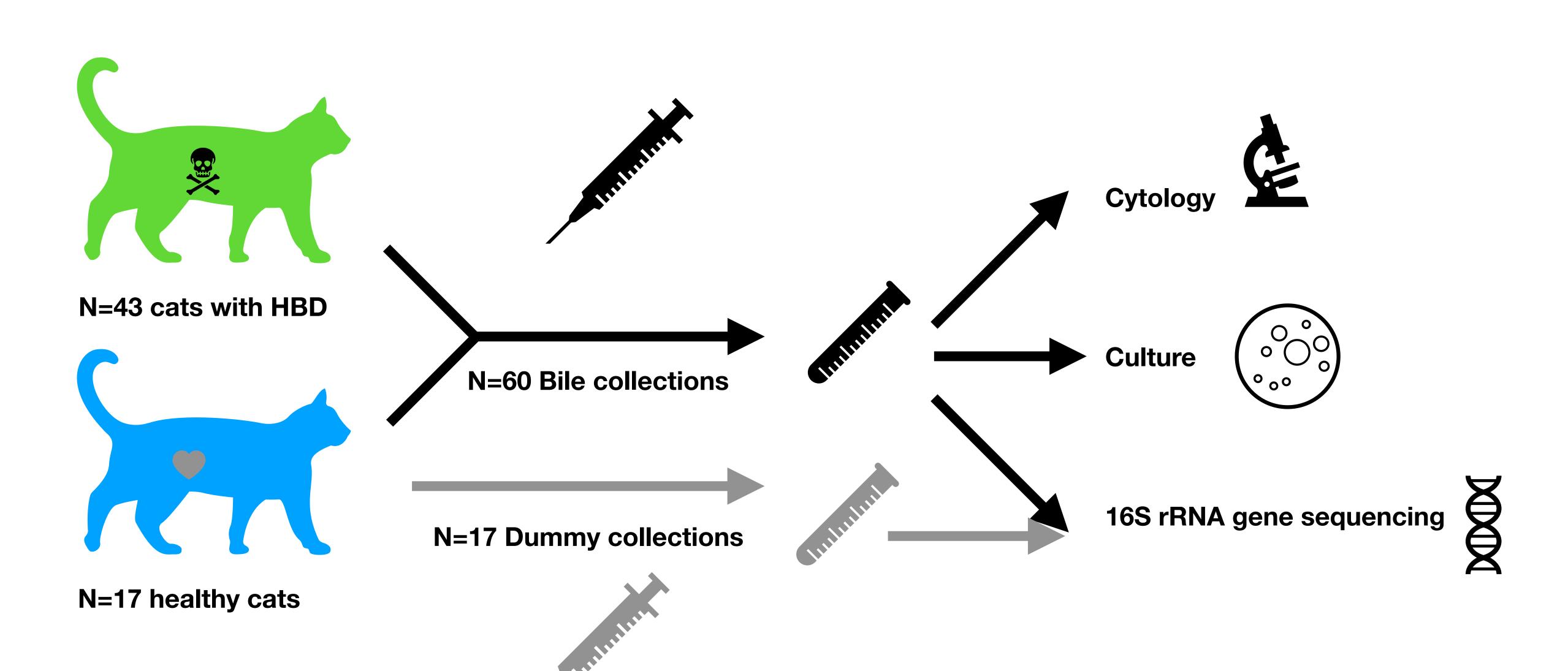
# Study Design



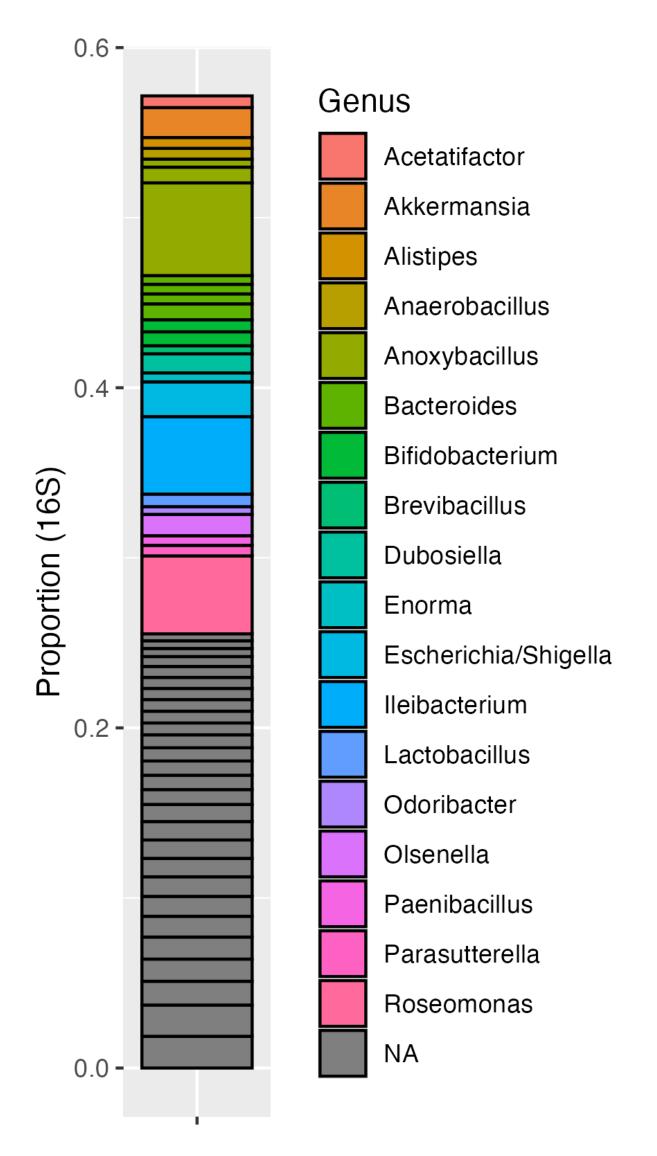
## Study Design



## Study Design



#### The Bile Microbiome

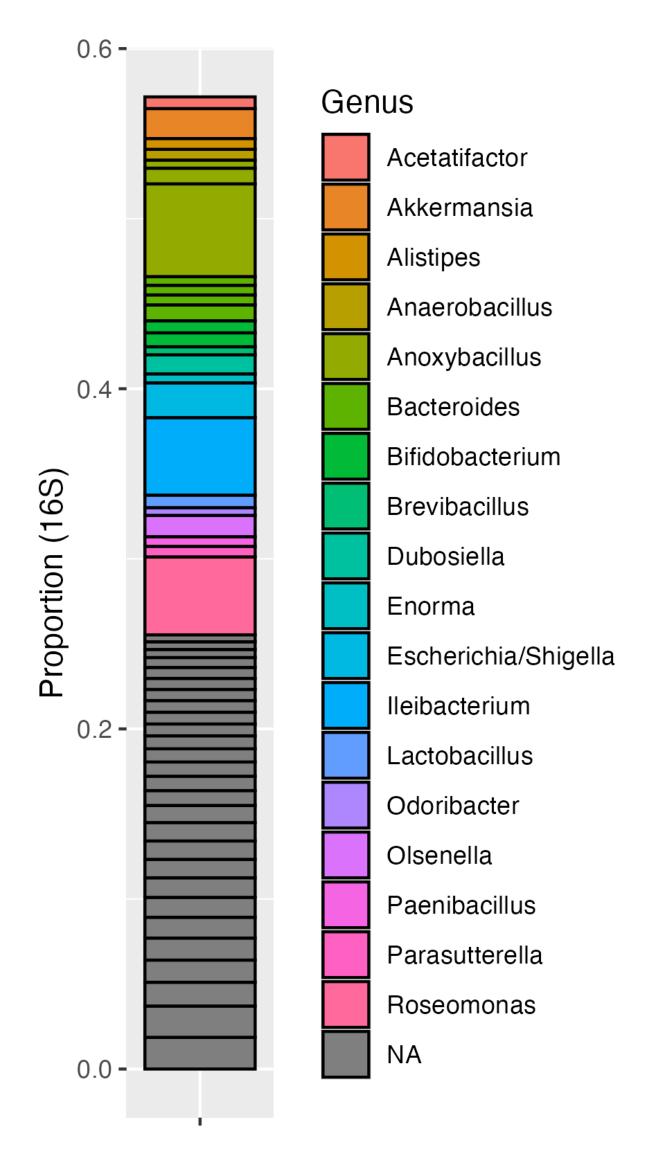


The 16S sequencing results for an example bile sample.

There appears to be a complex microbiome containing many genera and a substantial number of taxa that can't even be classified at the genus level!

**Sample 716-518 (HBD)** 

#### The Bile Microbiome?

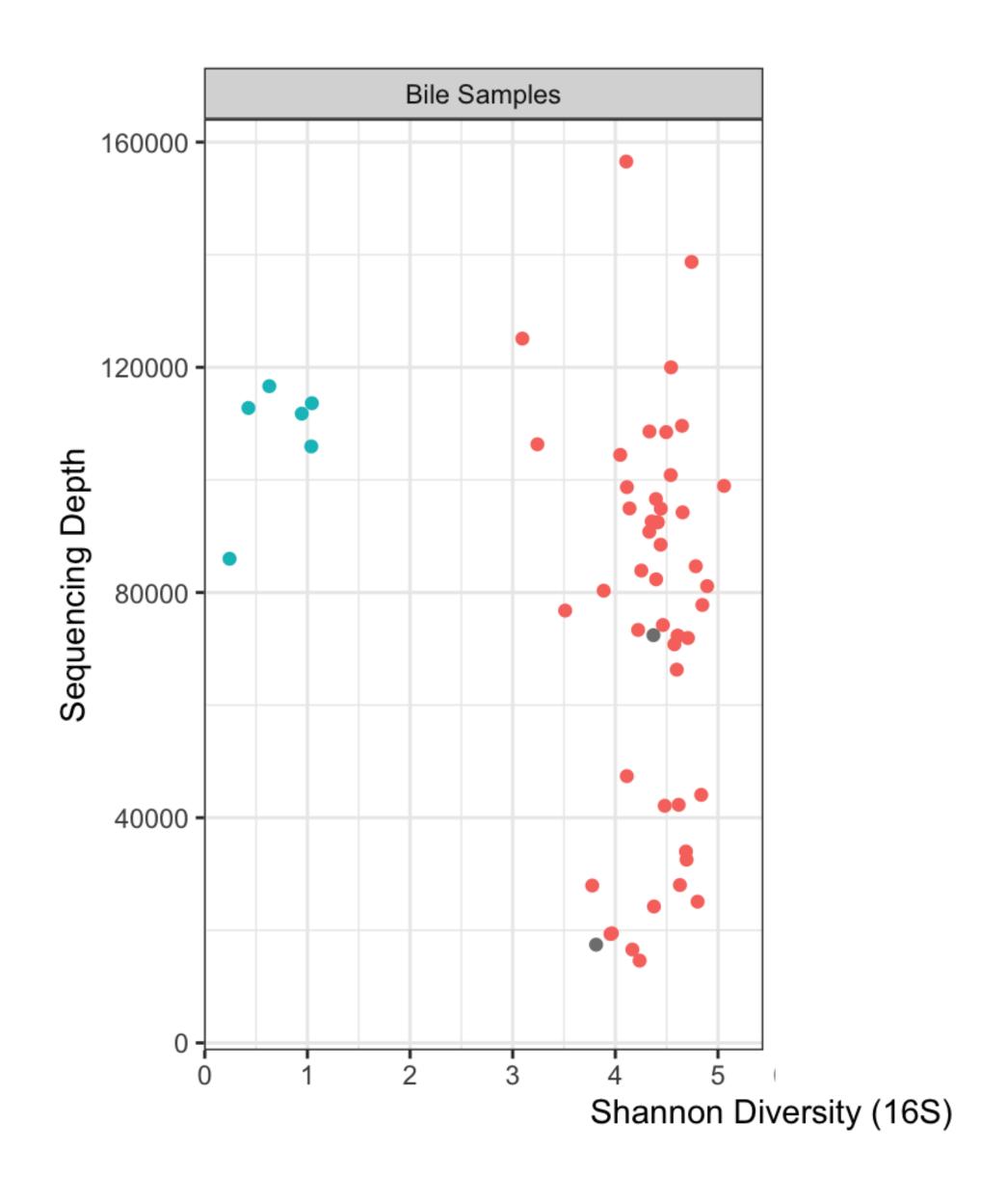


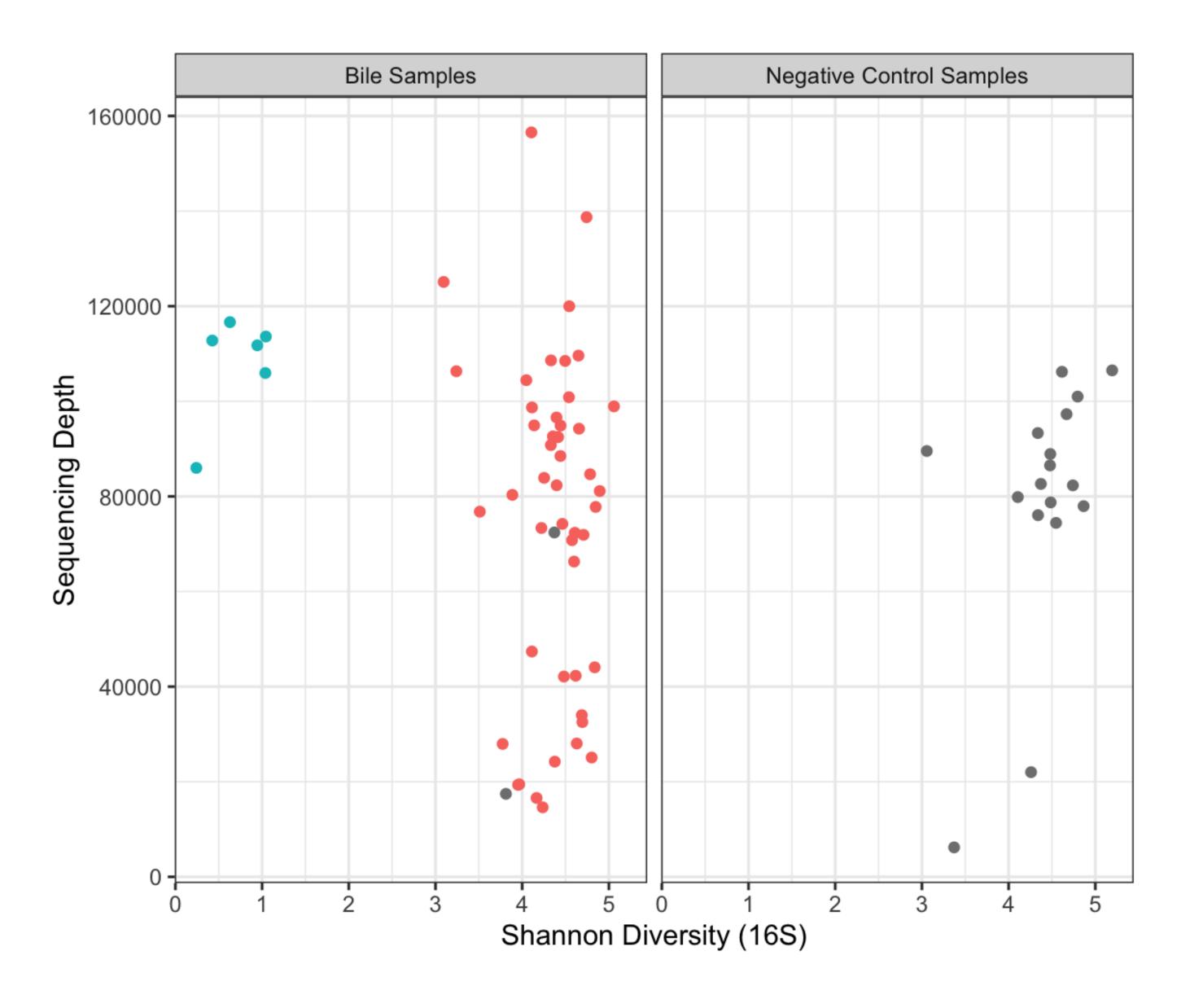
**Sample 716-518 (HBD)** 

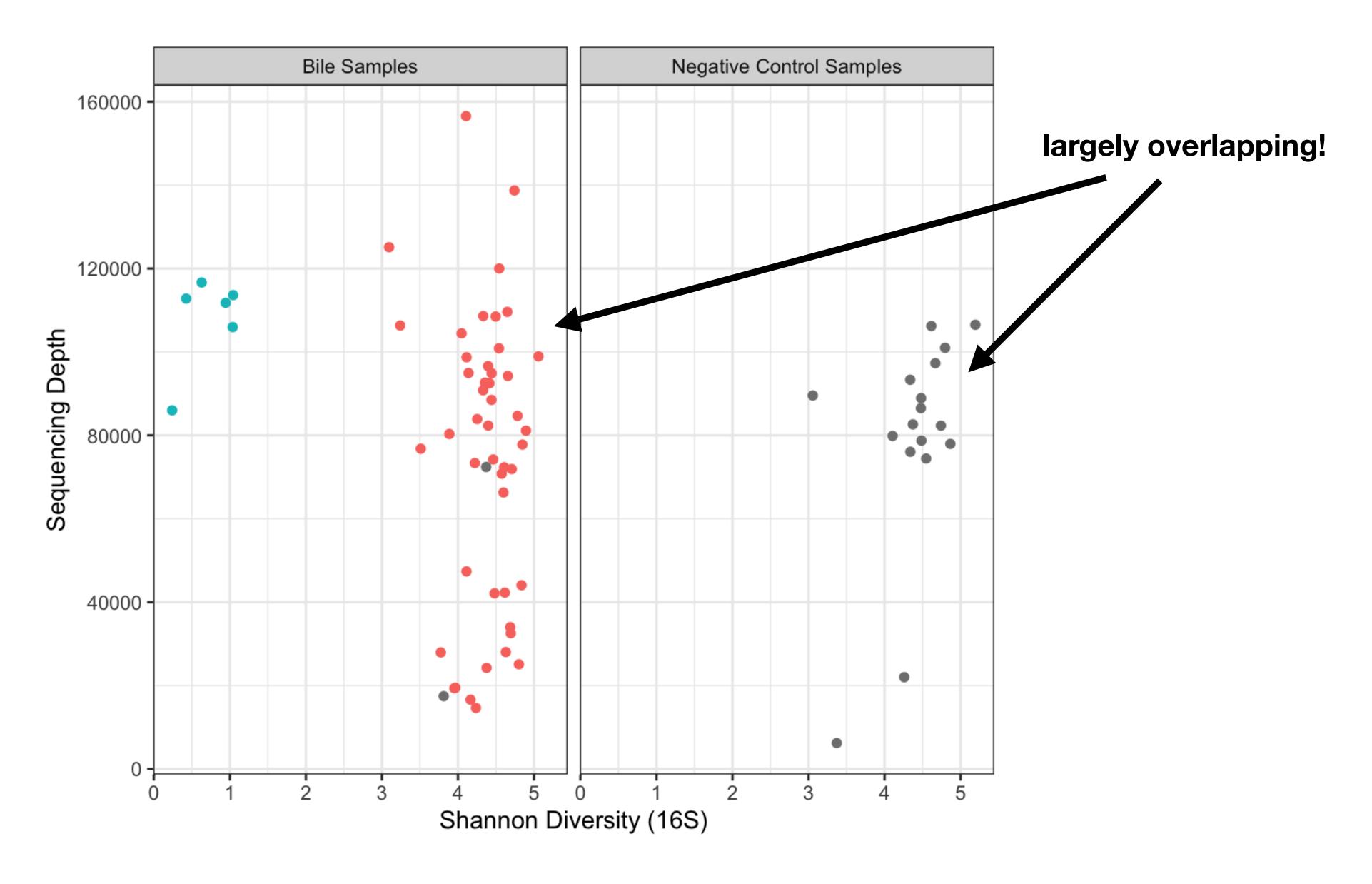
#### The 16S sequencing results for an example bile sample.

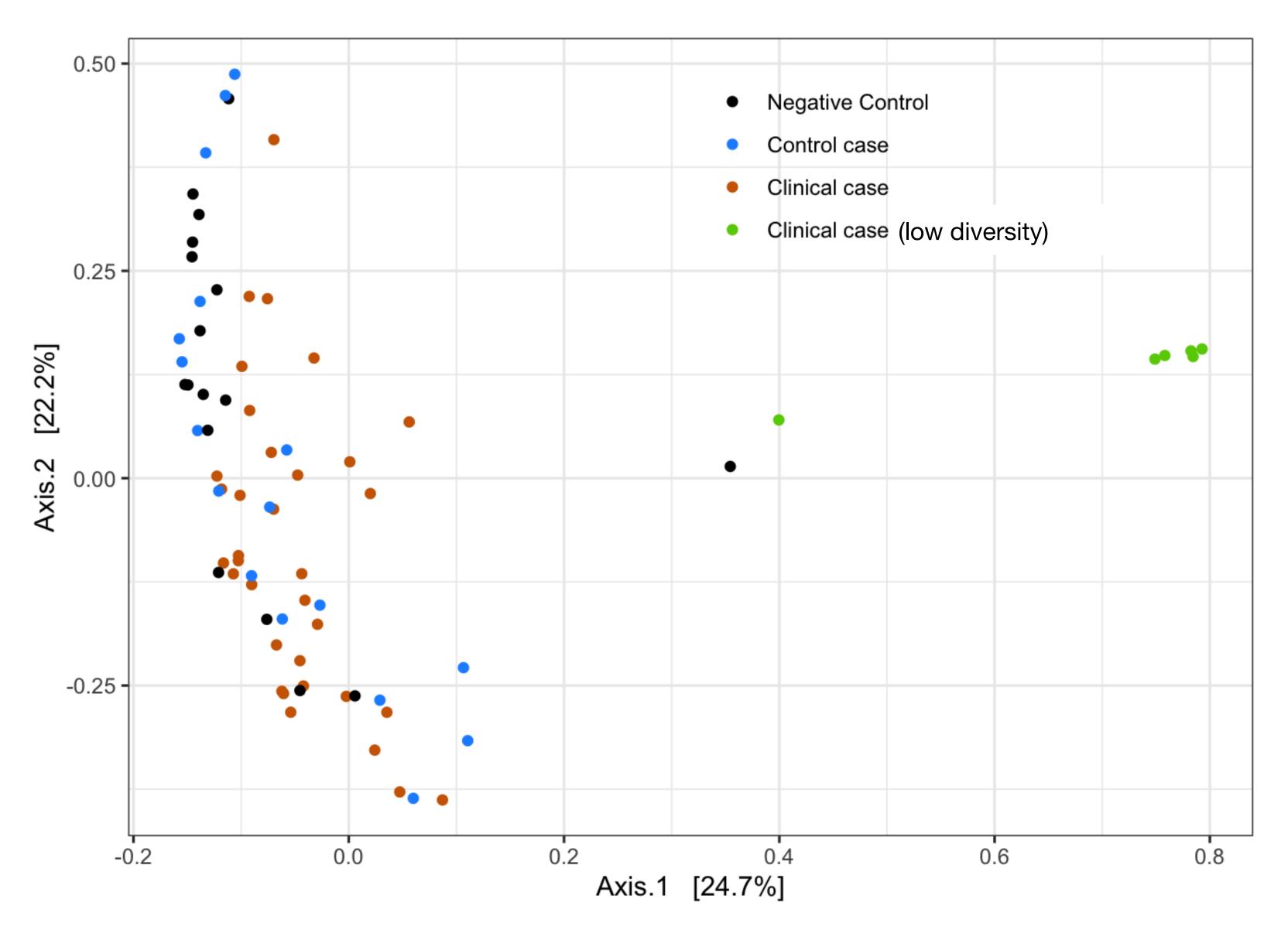
There appears to be a complex microbiome containing many genera and a substantial number of taxa that can't even be classified at the genus level!

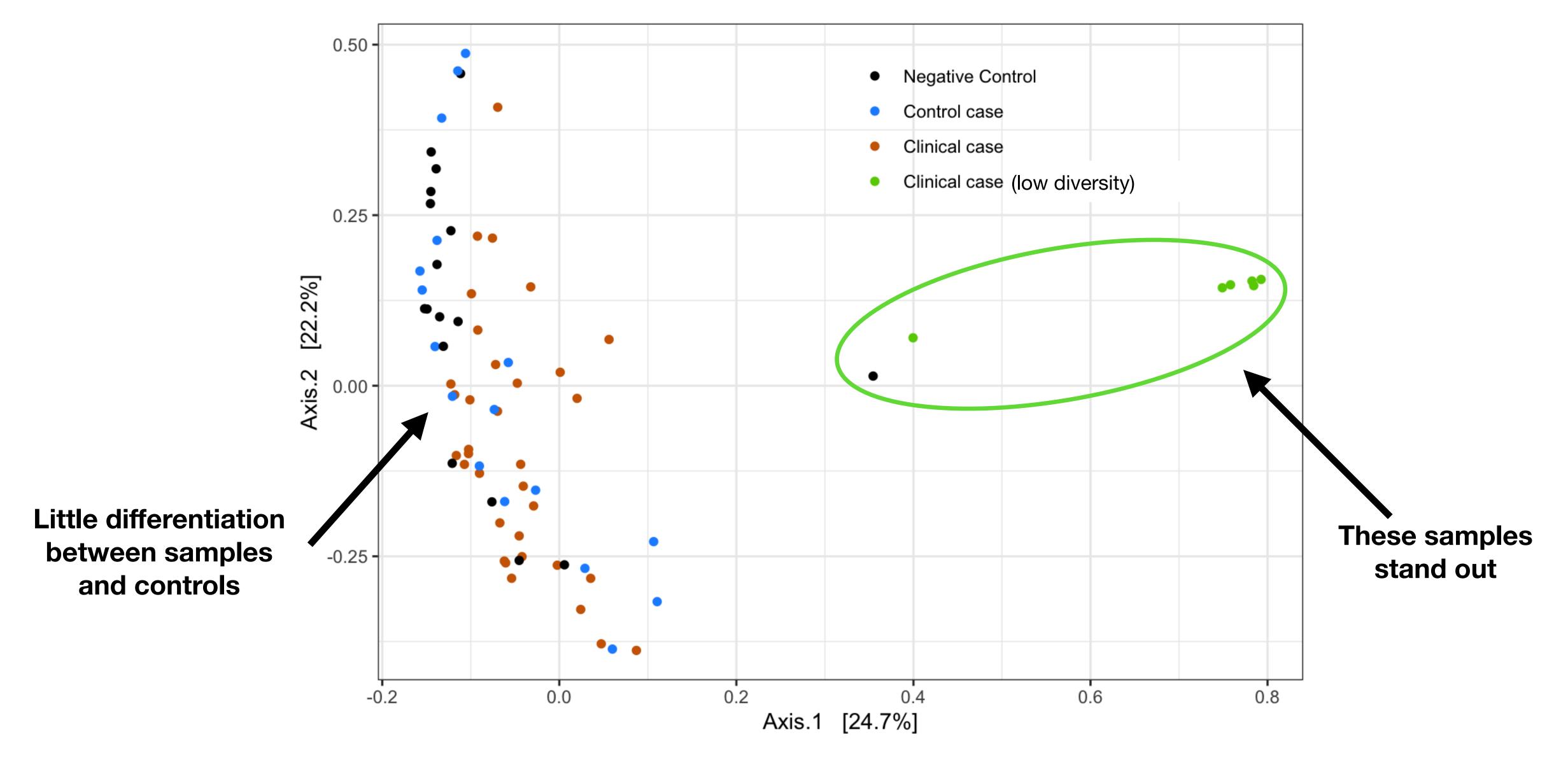
But what do our negative controls look like?

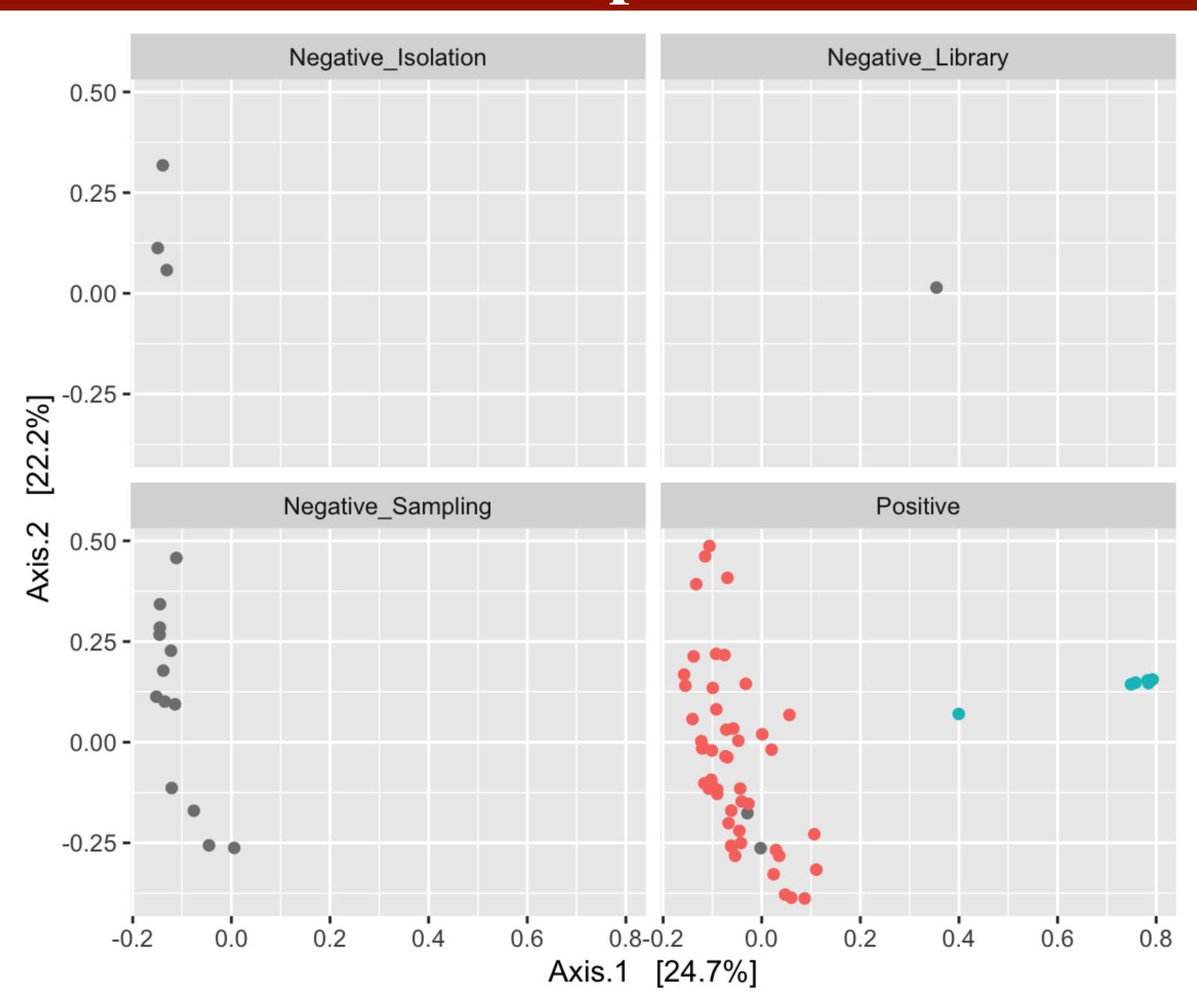








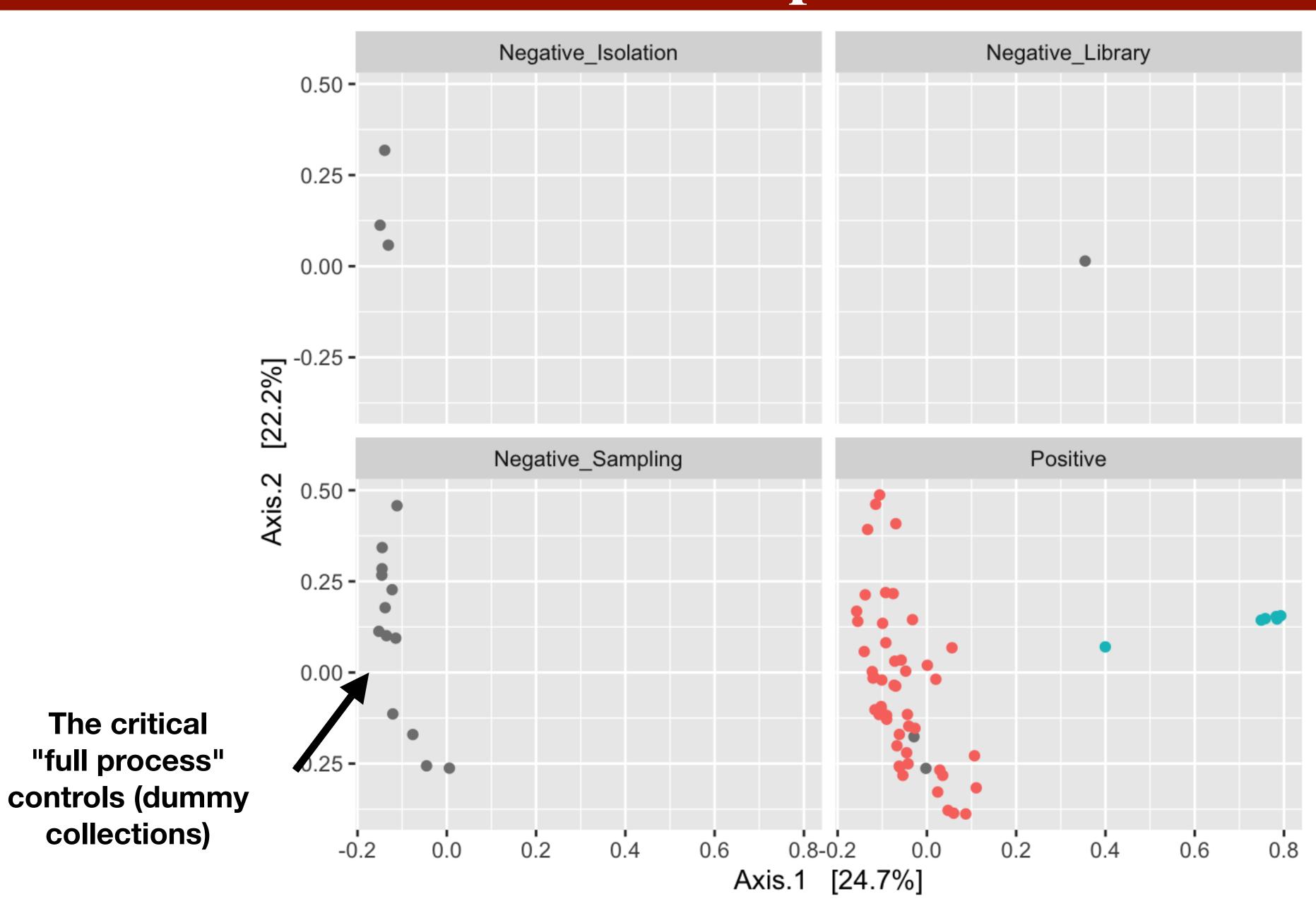




The critical

"full process"

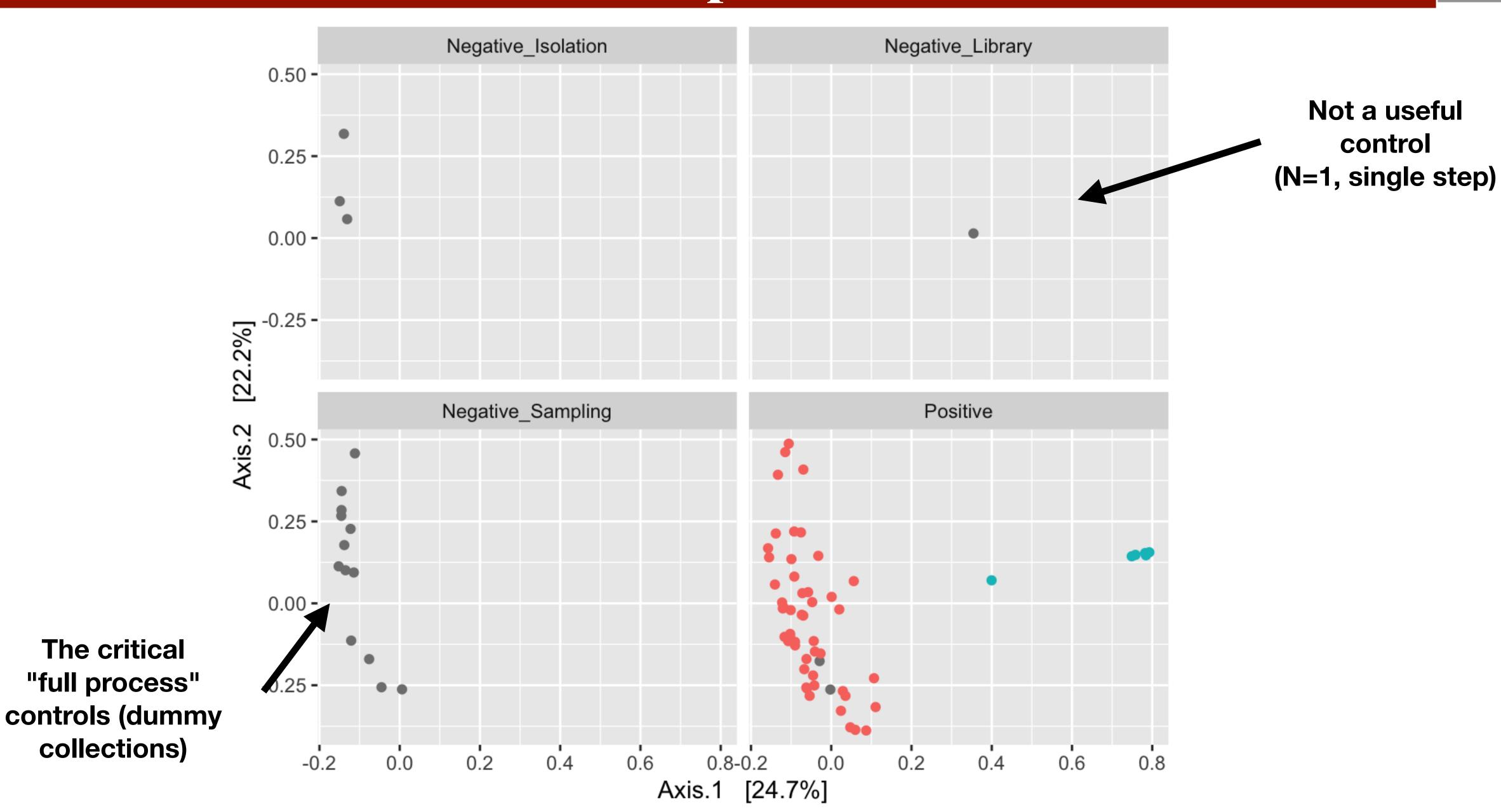
collections)

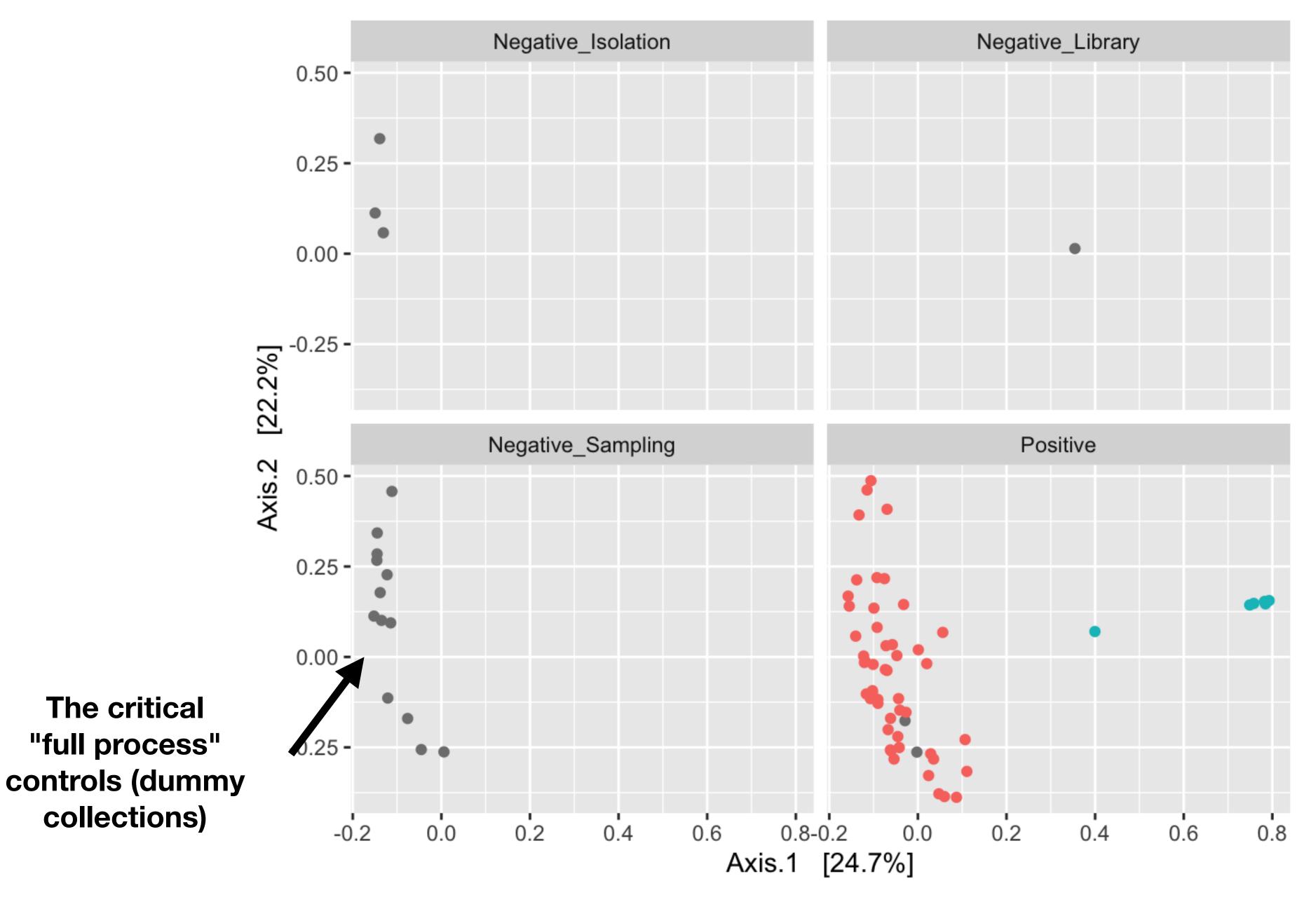


The critical

"full process"

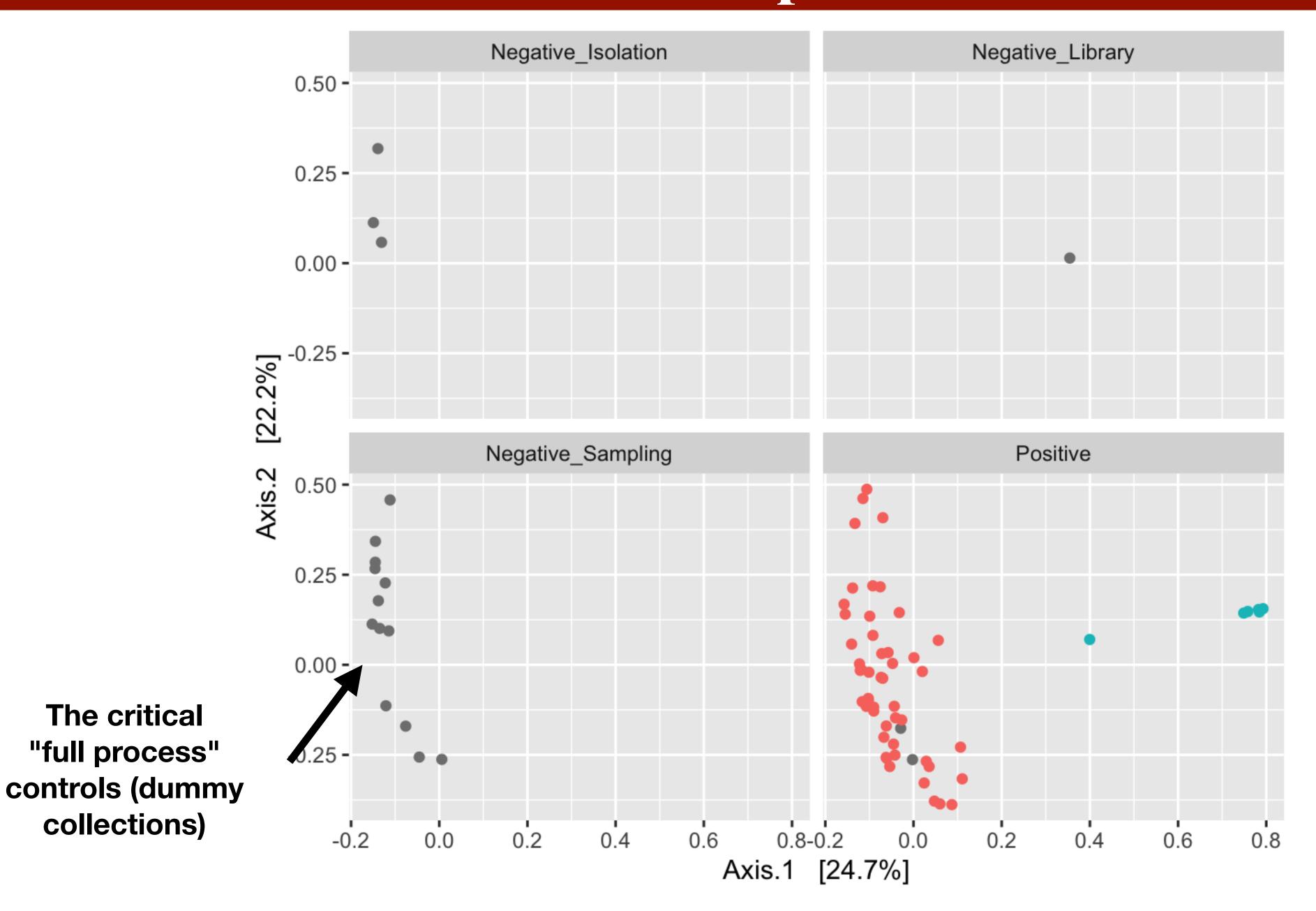
collections)





#### E. coli culture positive

- FALSE
- TRUE
- NA



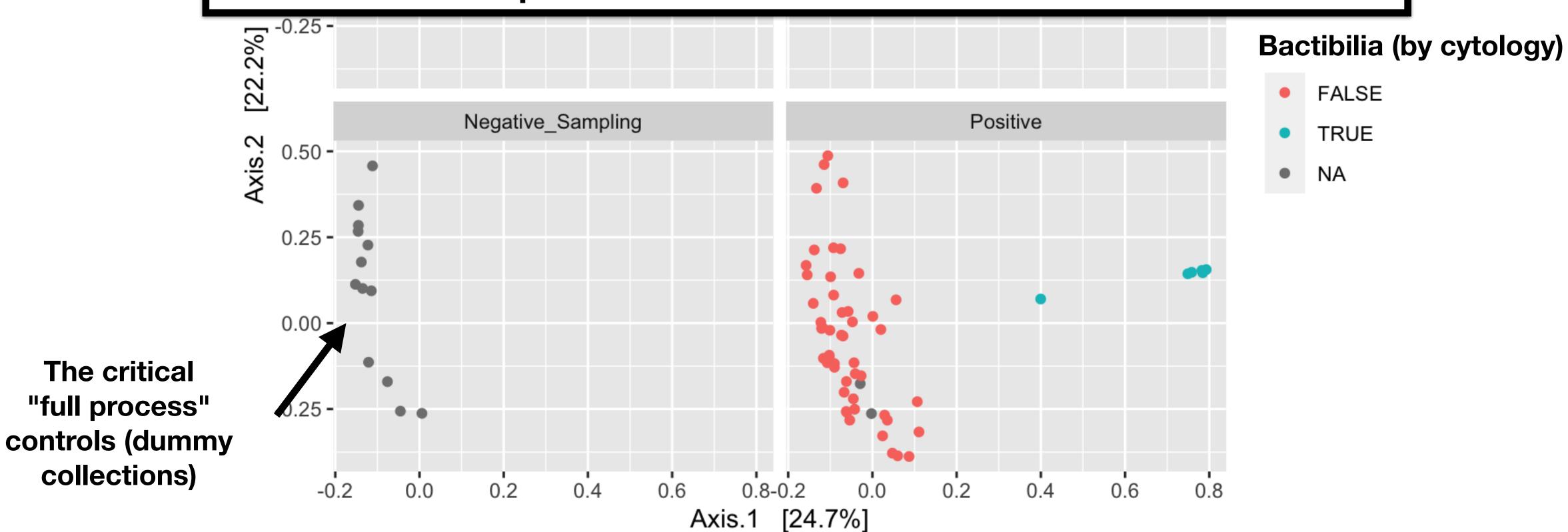
#### **Bactibilia** (by cytology)

- FALSE
- TRUE
- NA

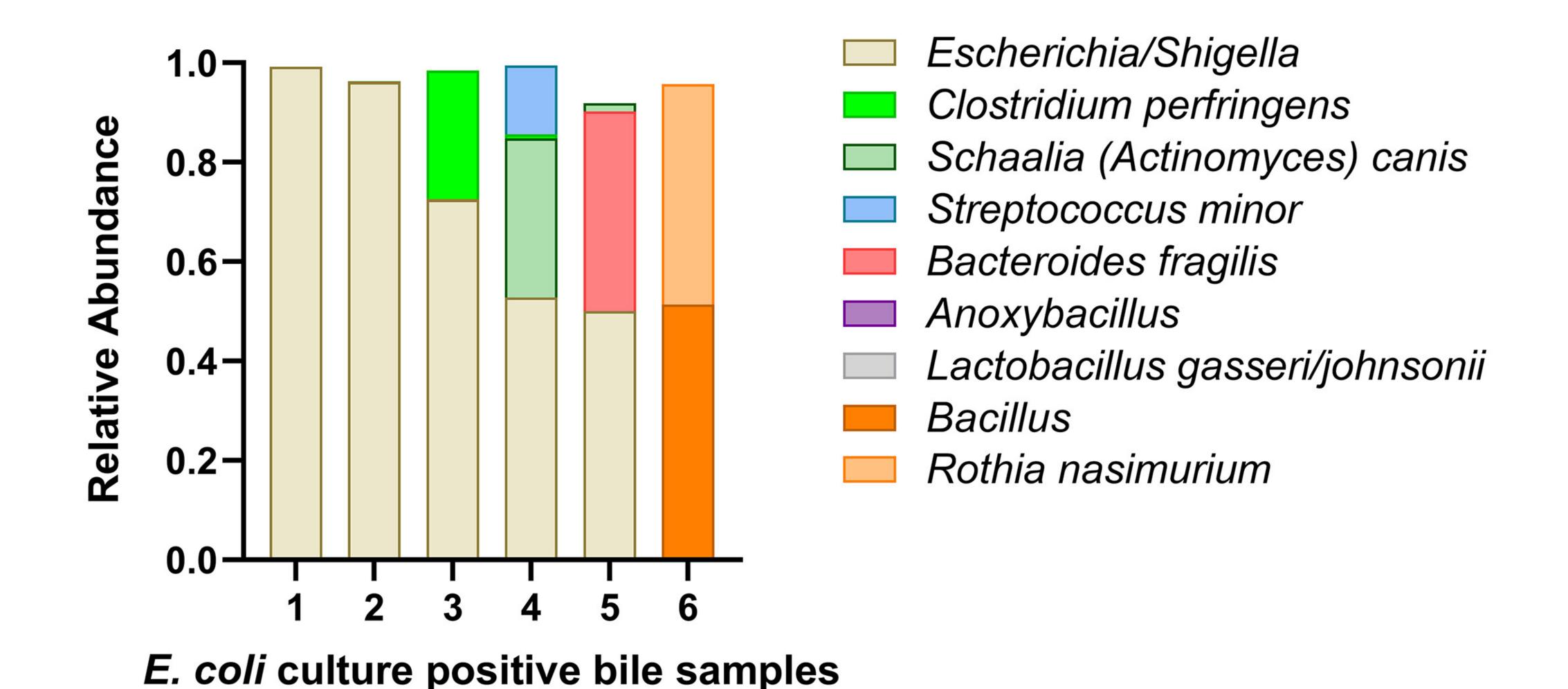
The critical

collections)

Negative\_Isolation **Negative Library** 0.50 -Perfect concordance between methods! Six samples separated from the others (and negative controls) by 16S. Six samples were E. coli culture positive. Six samples had bactibilia (bacteria present in bile by microscopy) The same six samples in each case!



#### Polymicrobial infections



#### Case Study

# The feline bile microbiome in health and hepatobiliary disease (HBD)

#### Answer 1:

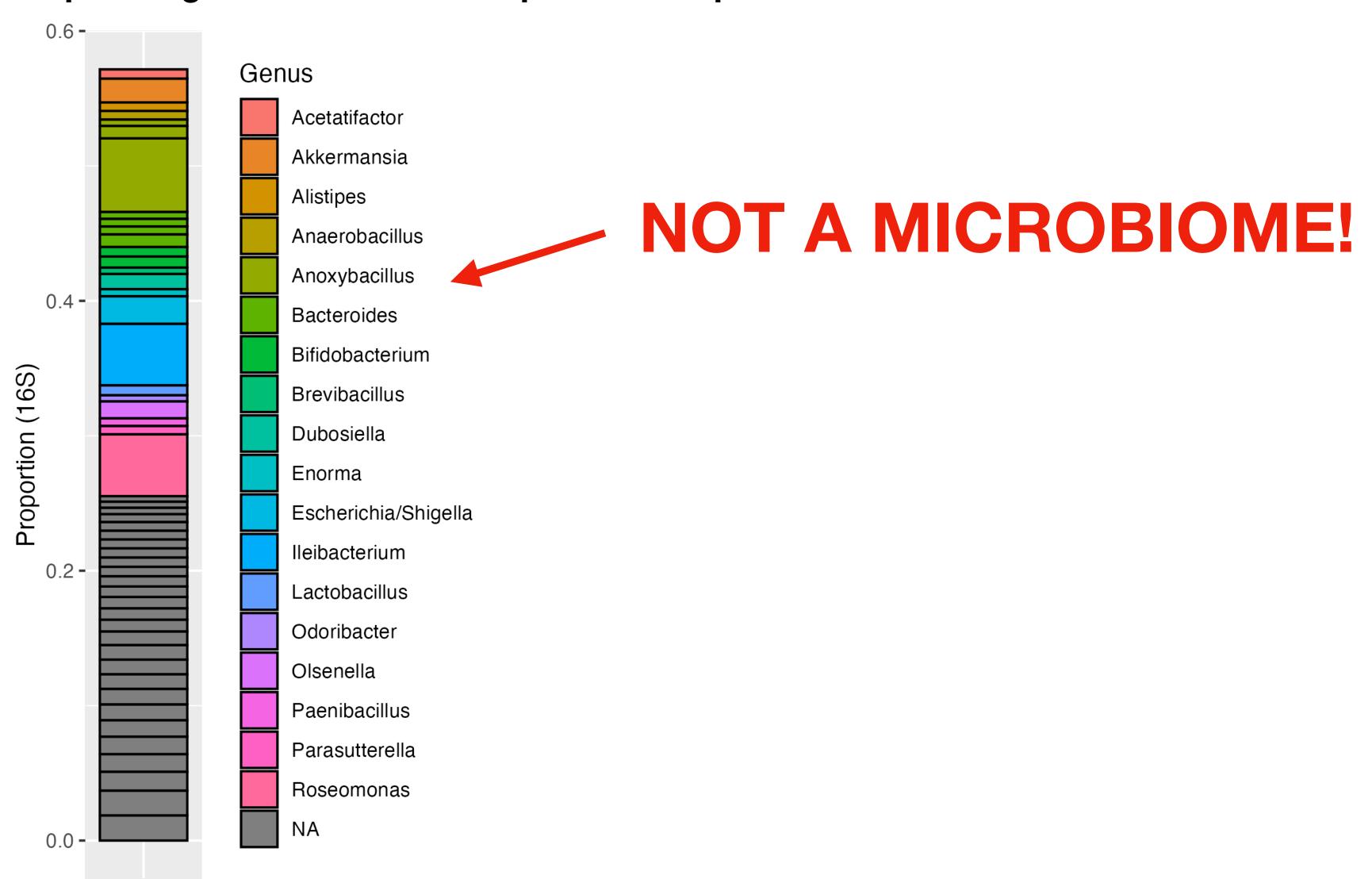
Infectious HBD is often polymicrobial, but *E. coli* culture is an effective diagnostic.

#### Answer 2:

There is no "core microbiome" of feline bile, but infections do occur.

## The Bigger Picture

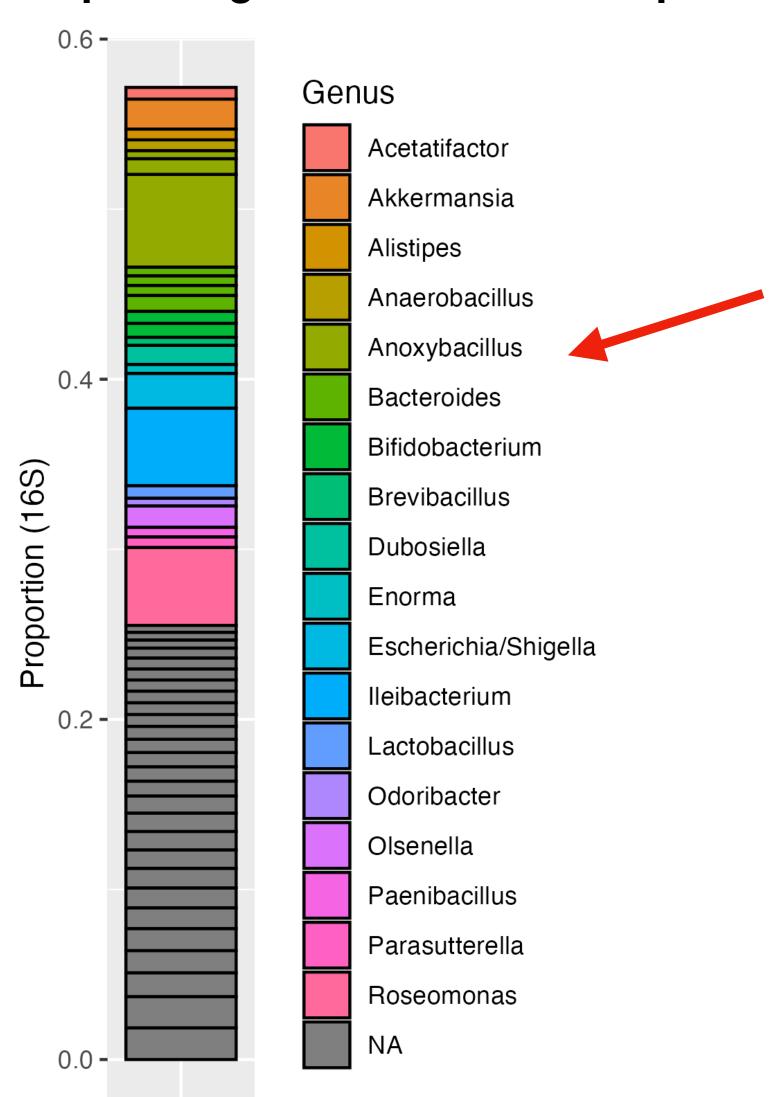
The 16S sequencing results for an example bile sample.



**Sample 716-518 (HBD)** 

#### The Bigger Picture

The 16S sequencing results for an example bile sample.



#### NOT A MICROBIOME!

What could have been a confusing and difficult to interpret low biomass study, and perhaps a mistaken report of a complex microbiome of the bile, was instead a clean, simple and interpretable story due to our study design and analytic strategy.

**Sample 716-518 (HBD)** 

#### Effective Low Biomass Microbiome Studies

- Multiple measurement modalities
- Multiple controls (min:5/96, consider a ratio e.g. 1:3)
- The right controls (full-process controls)
- Clean lab practices
- Contaminant-aware analyses
- Appropriate skepticism

In press: "Guidelines for preventing and reporting contamination in low biomass microbiome studies", Nature Microbiology

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- Appropriate skepticism

Even carefully done, sequencing only studies of low biomass samples can mislead!

Think about contamination when designing your study, not afterwards!

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#### Acknowledgements

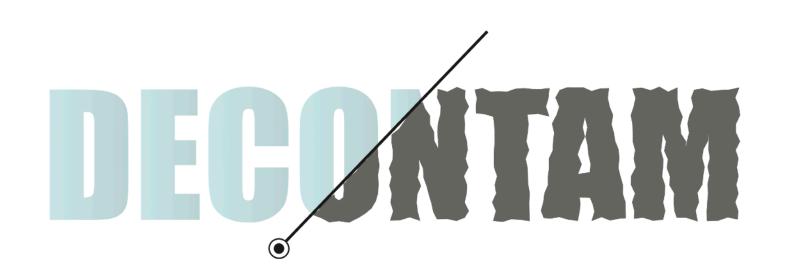


**Tanner Slead** 



**Jody Gookin** 











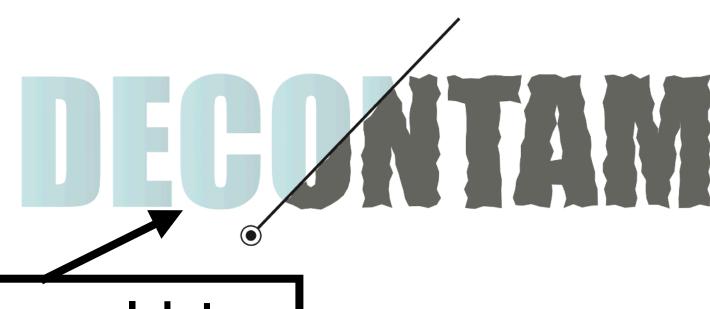
#### Acknowledgements



**Tanner Slead** 



**Jody Gookin** 



Software from my lab to identify and remove contaminants in microbiome sequencing data!



Thanks to the study cats, the pet owners, and the North Carolina State Veterinary Hospital!



