

Finding who is out there?

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Jagiellonian University

## **Disclaimer**

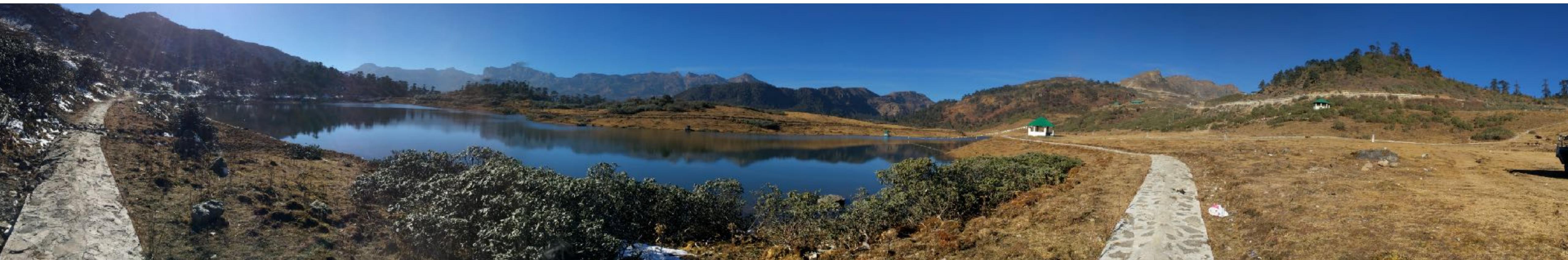
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# **Finding who is out there?**

## **Traditional and modern tools for investigating habitat**

**Arpan Kumar Basak, Krakow, March 2022**

# This is a habitat

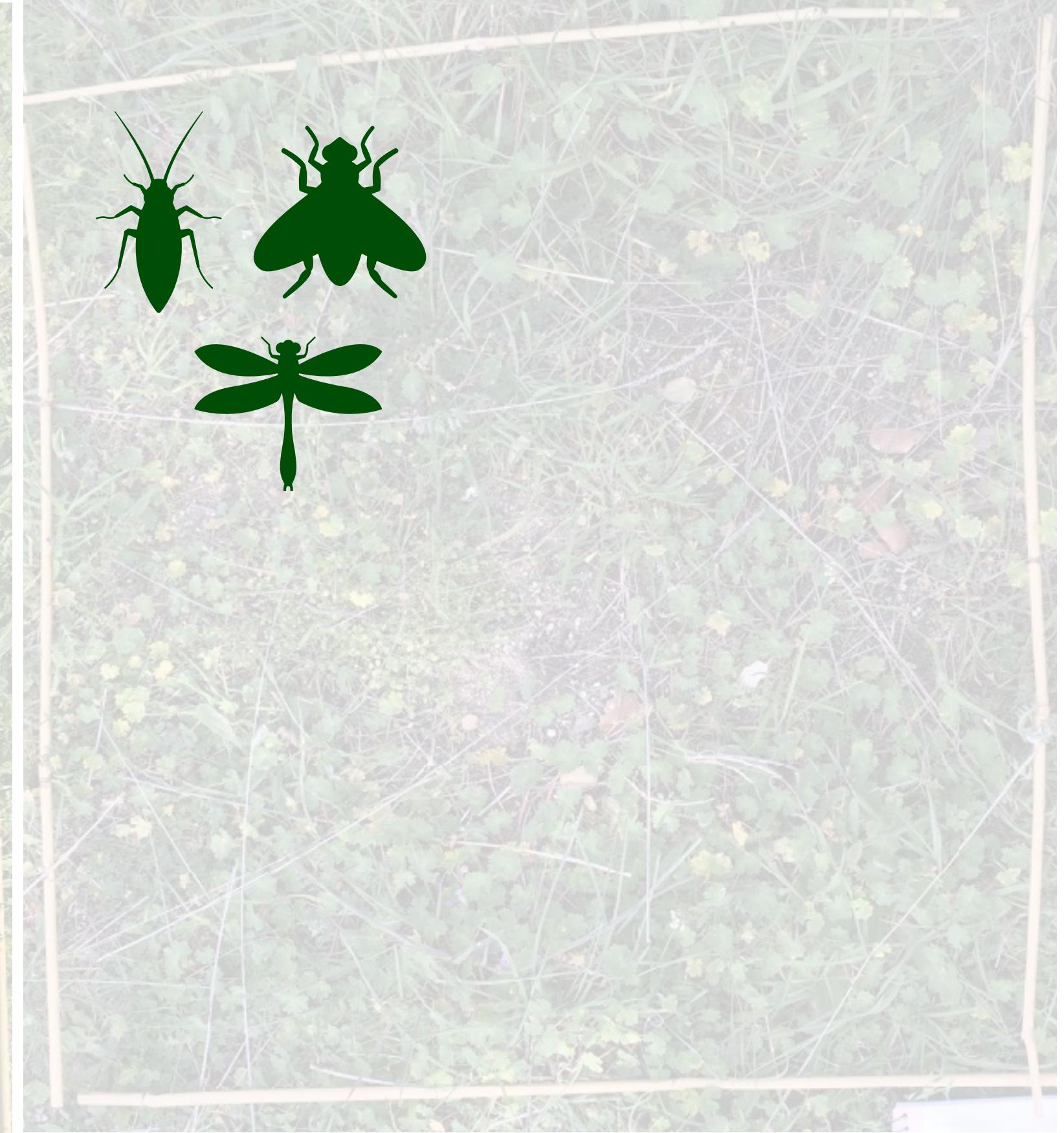
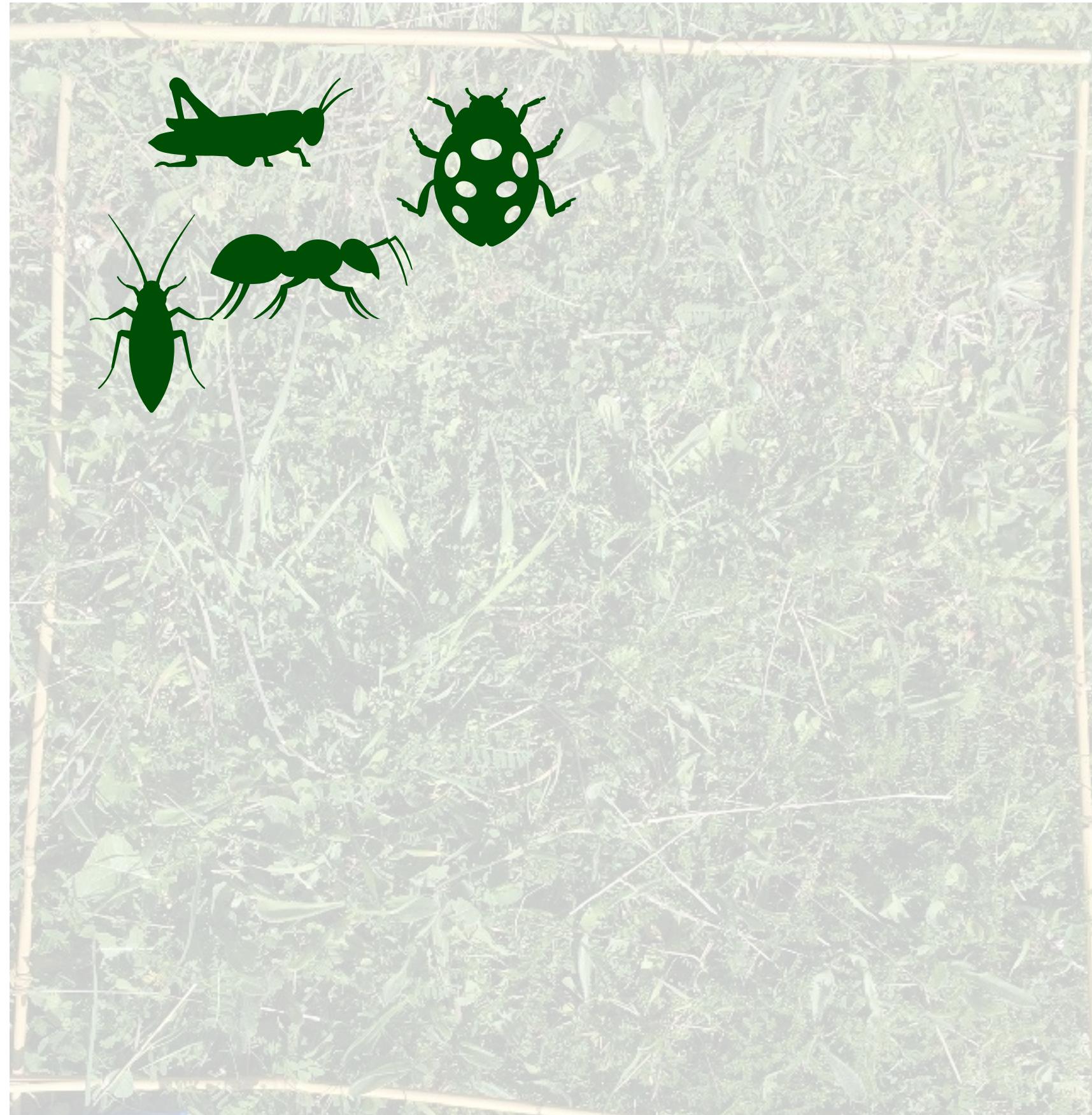


Tawang, Arunachal Pradesh, India

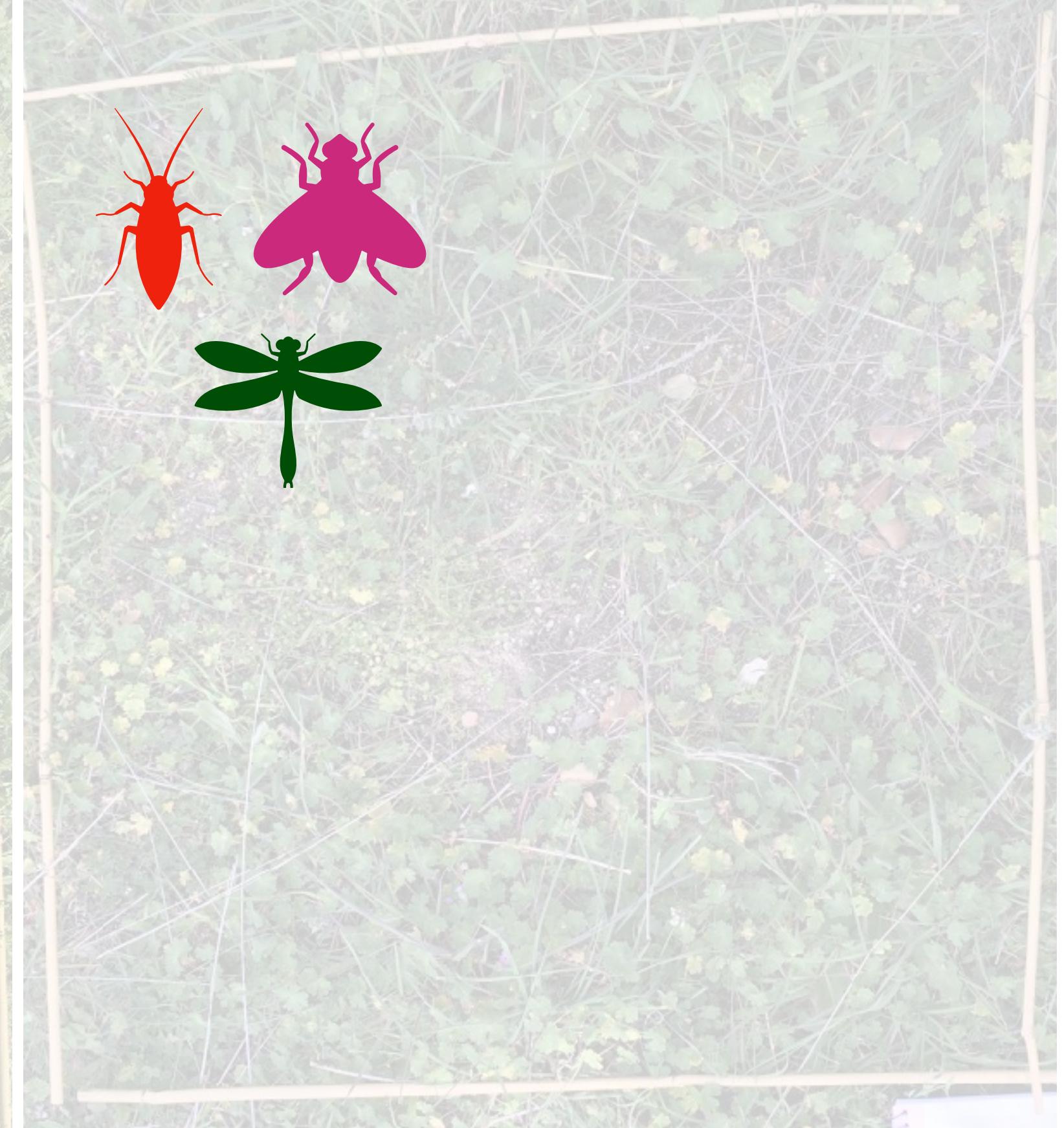
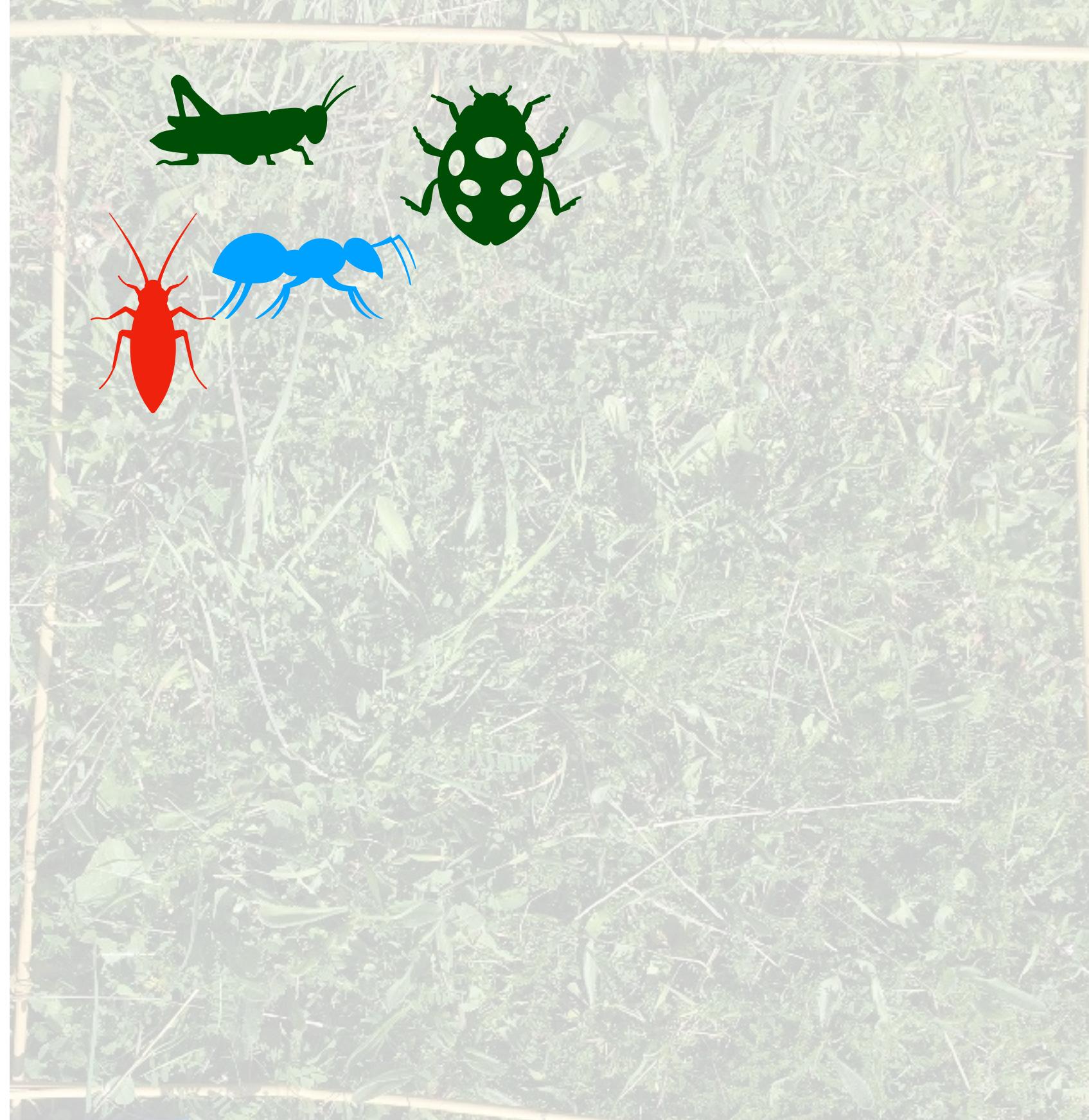
# Identification of species in the macrohabitat



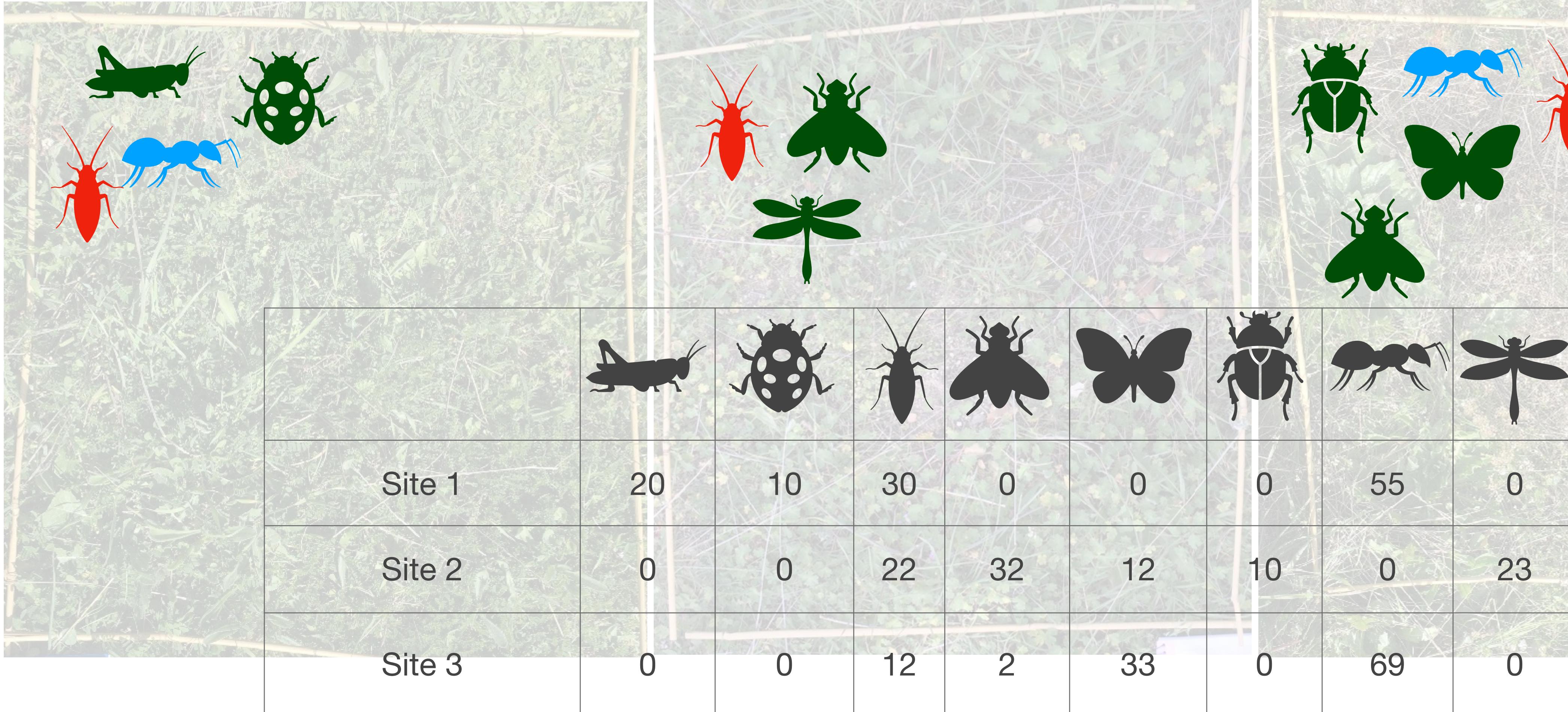
# What? - Identification of fauna in the macrohabitat



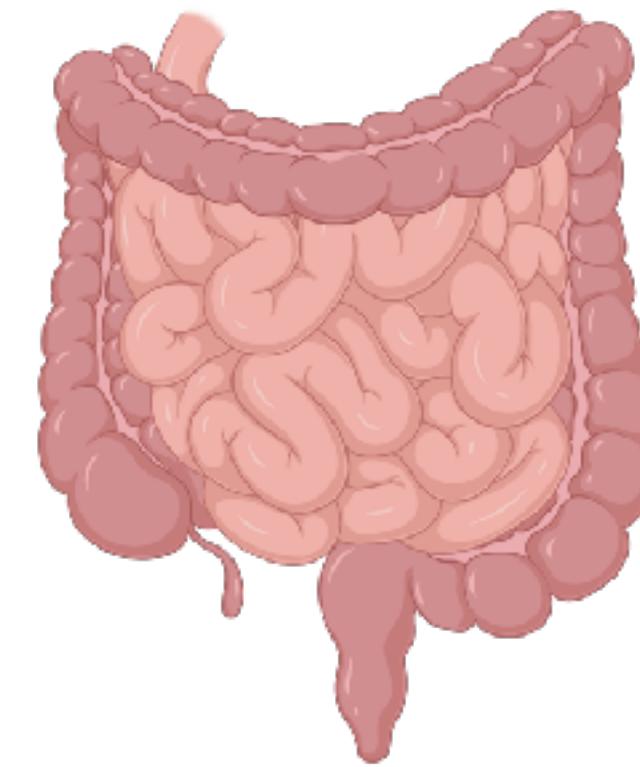
# Observe and count the type and number of species



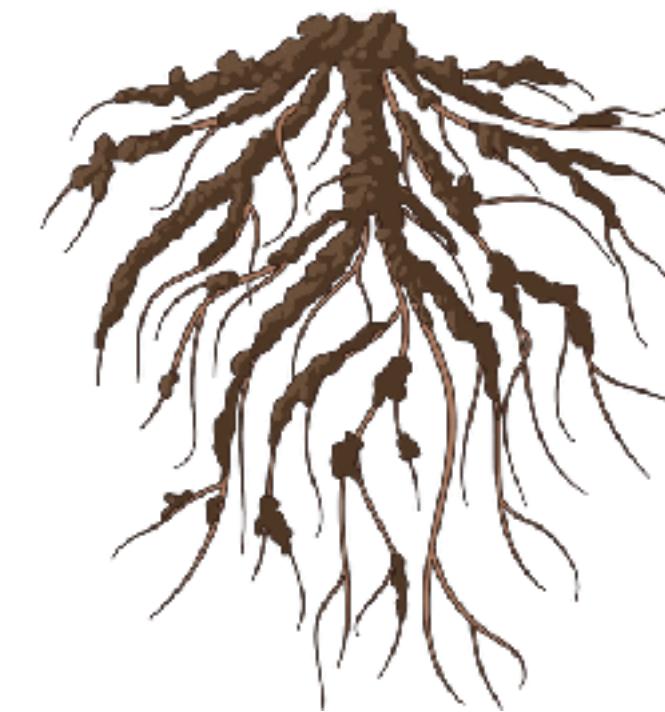
# Observe and count the type and number of species



# From an observational perspective what do you see?

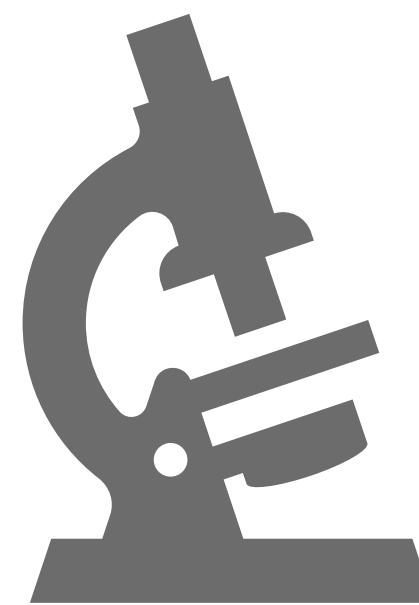


Mirco habitat

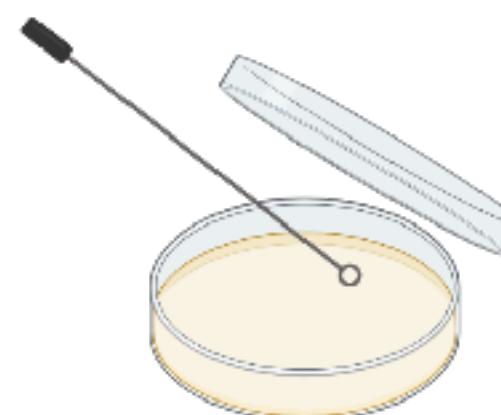


Investigation of microhabitat

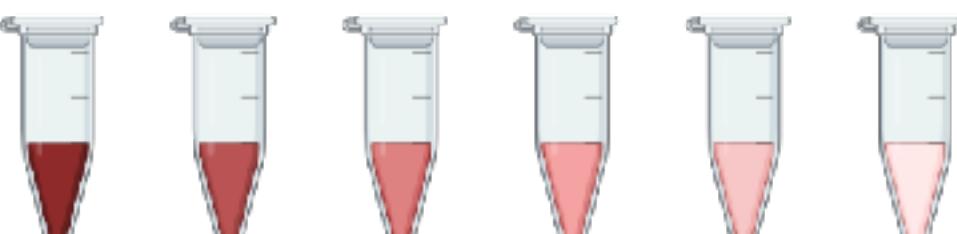
# Traditional or classical microbiology techniques



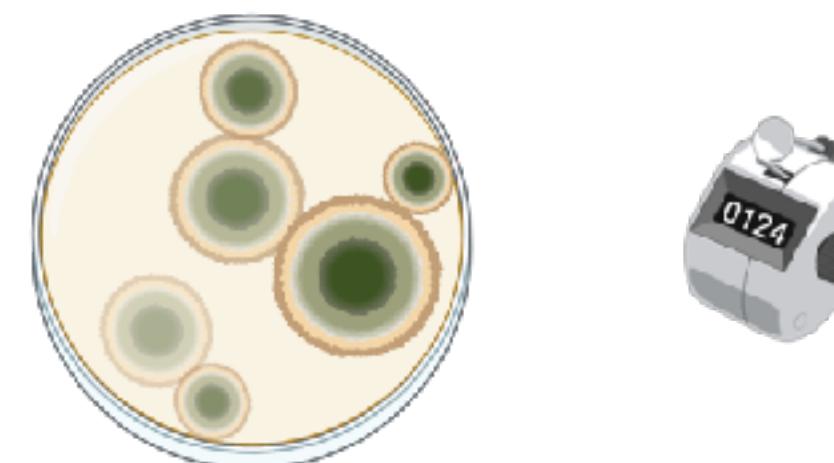
Microscopic observation



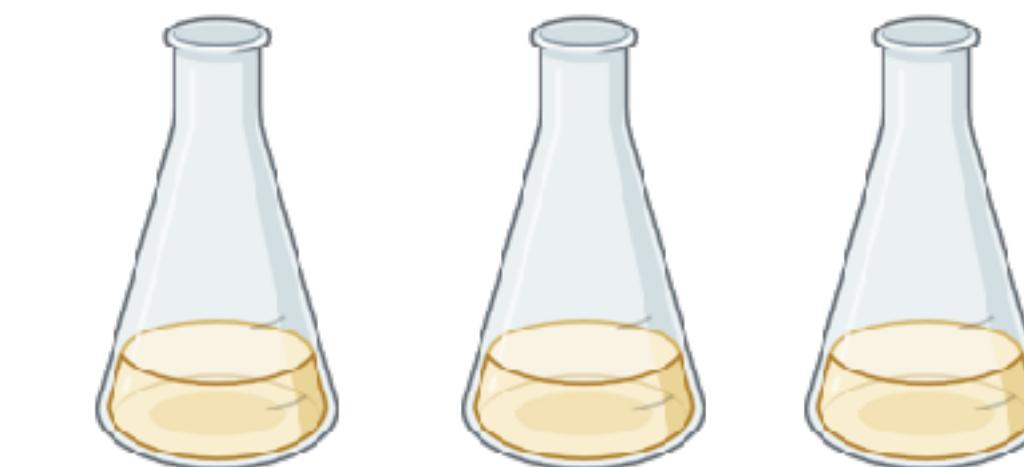
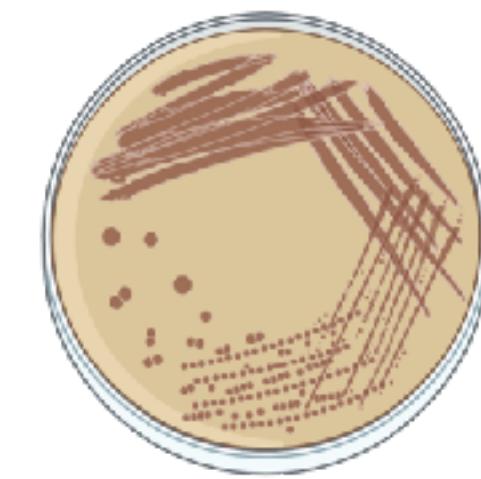
Isolate single colony    Streak in specific media



Serial dilution



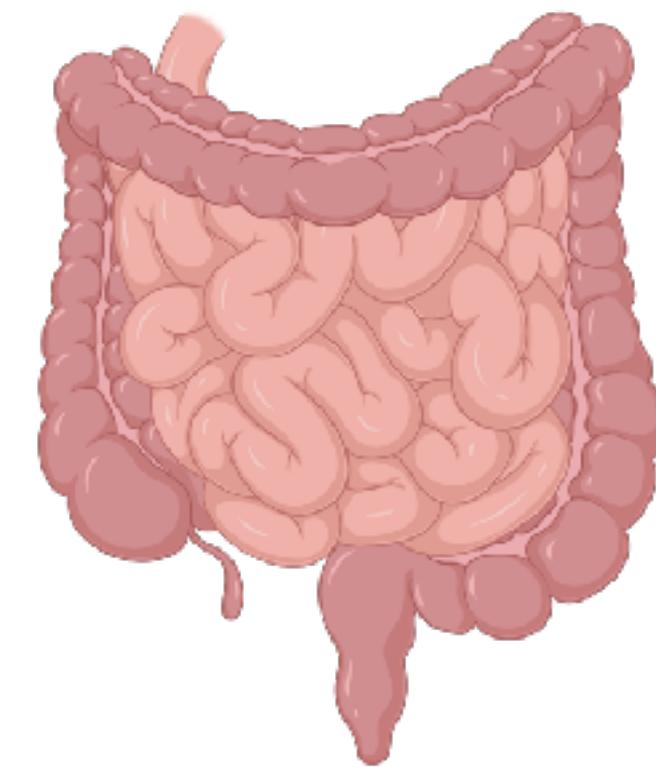
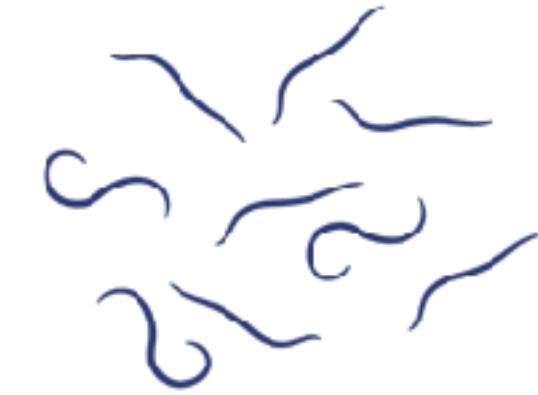
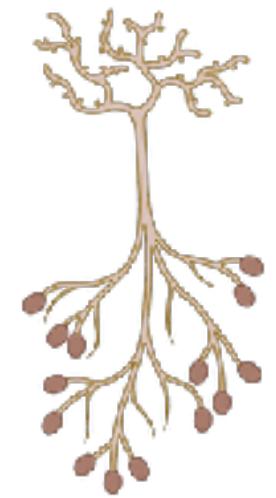
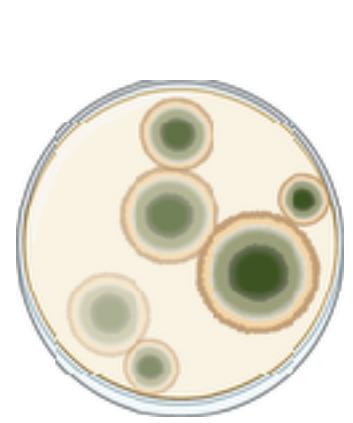
In different media



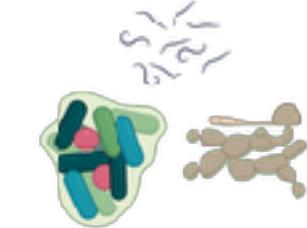
Liquid culture for studying metabolism

Limitations in observational characterisation

# From an observational perspective what do you see?



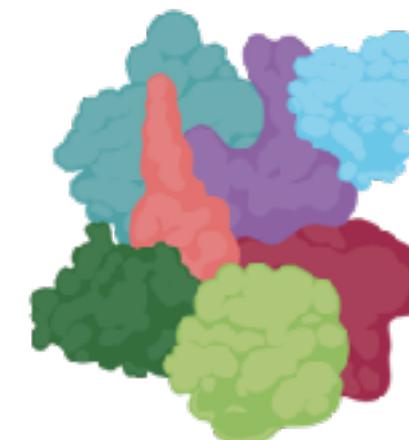
Mirco habitat



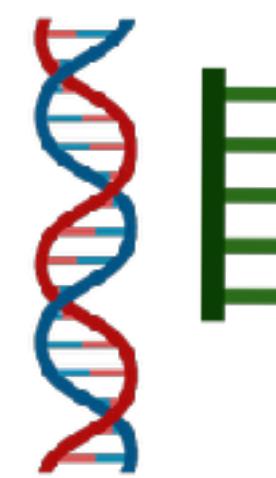
Investigation of molecular parameters

# This is not just a stool sample but it has a lot of biological entities

Abundance of proteins  
or peptides

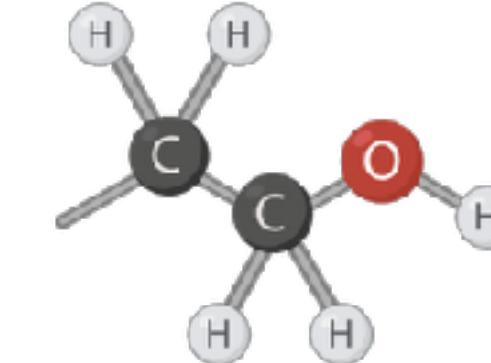
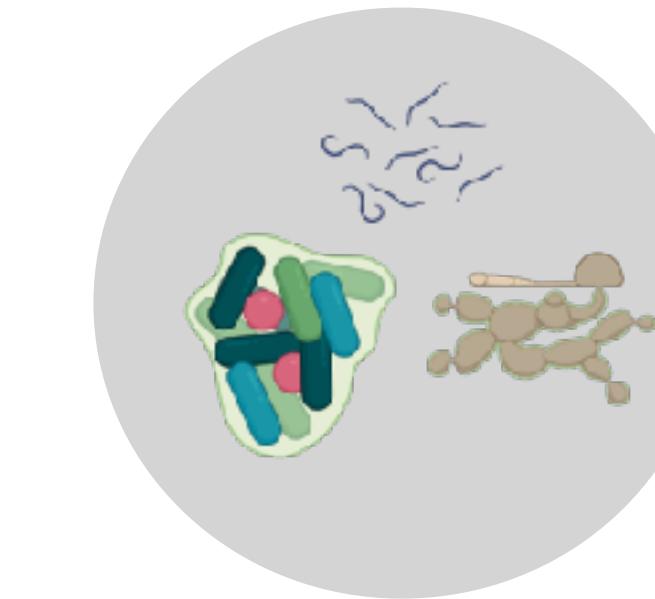


Proteins



Nucleic acids (DNA/RNA)

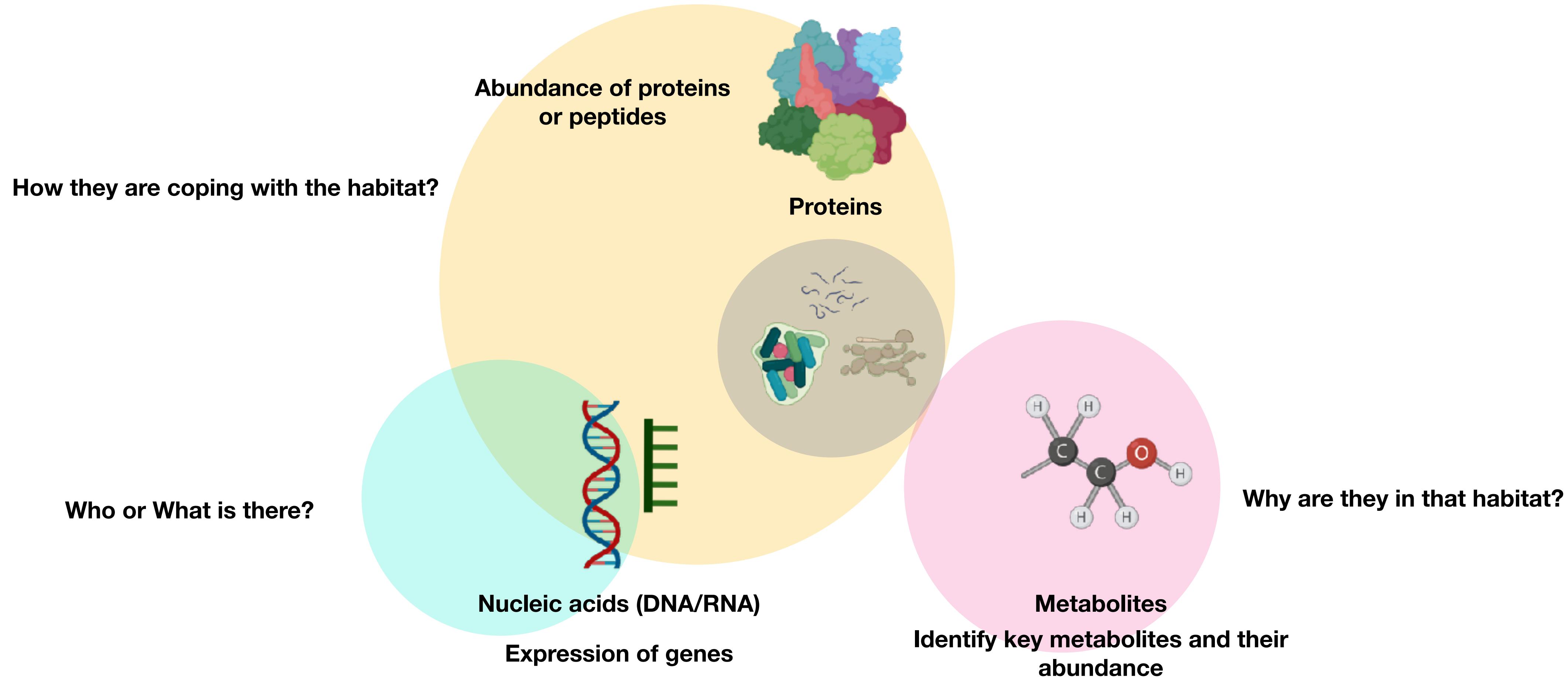
Expression of genes



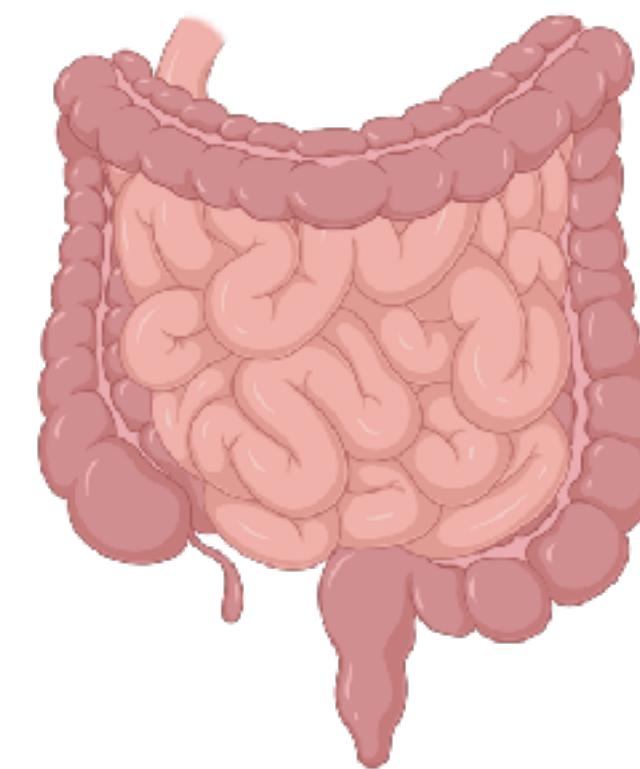
Metabolites

Identify key metabolites and their  
abundance

# The mystery behind the stool sample



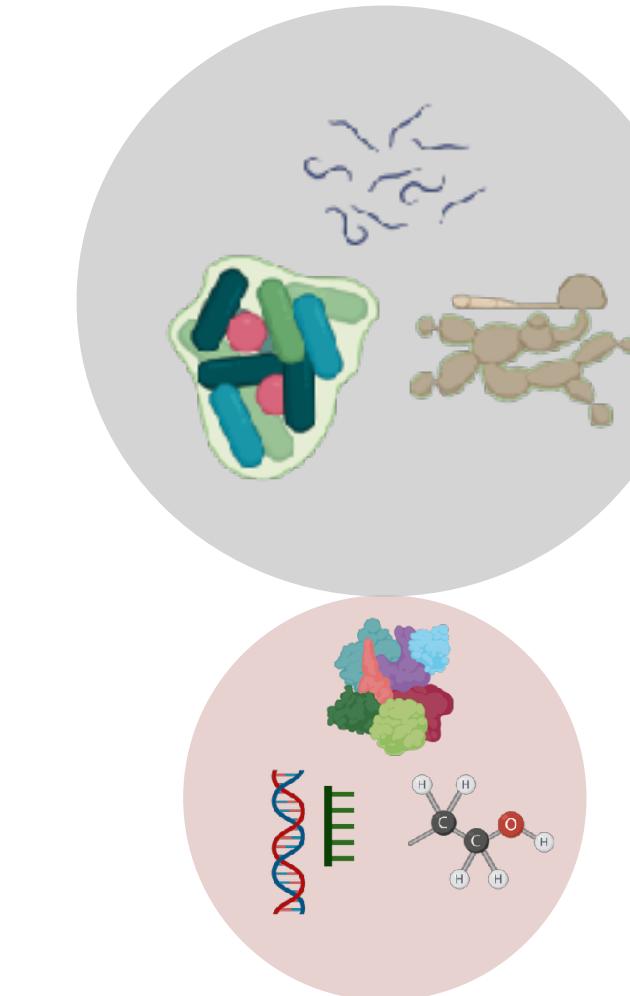
# From a molecular perspective what do you see?



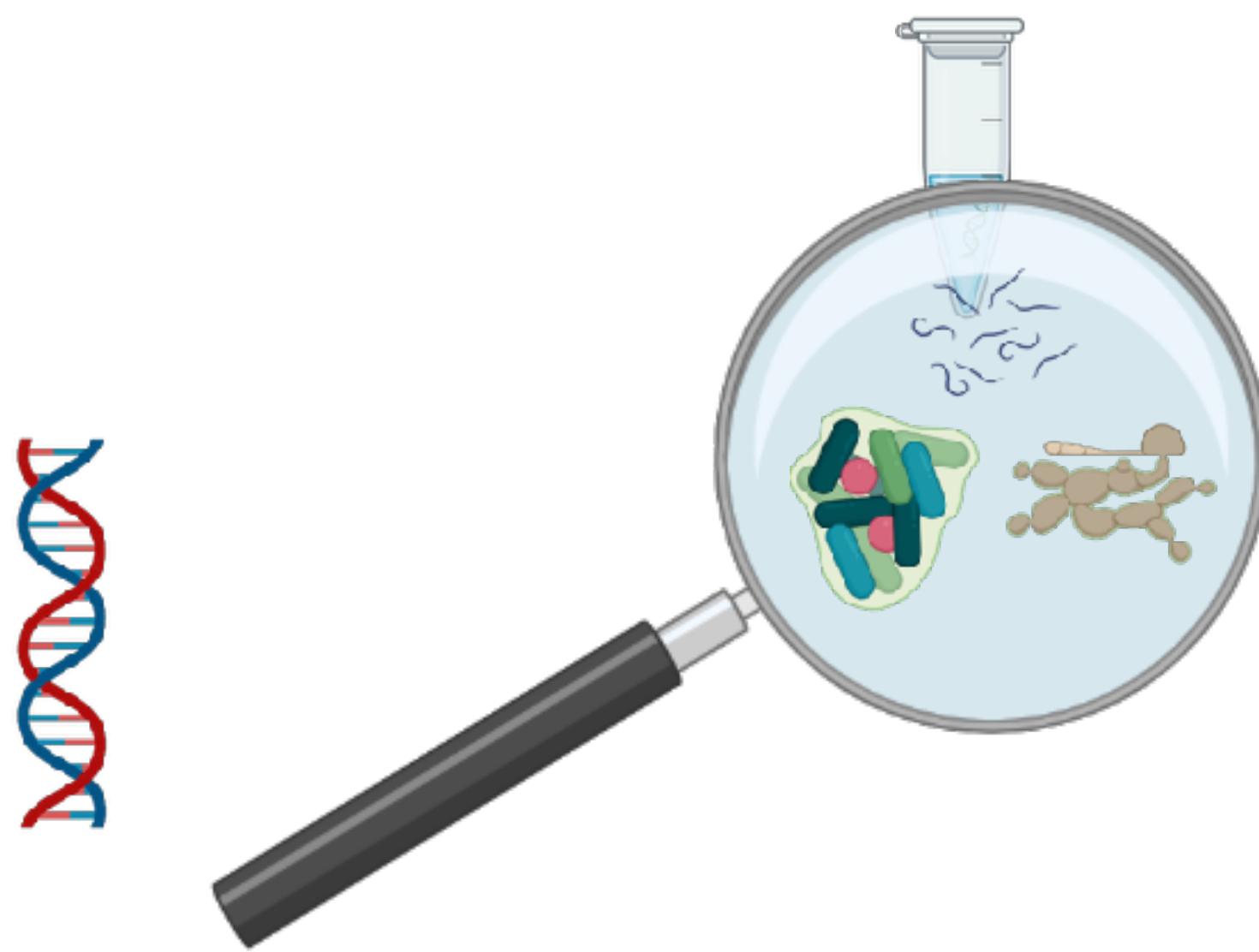
Who or What is there?



All that is in the DNA

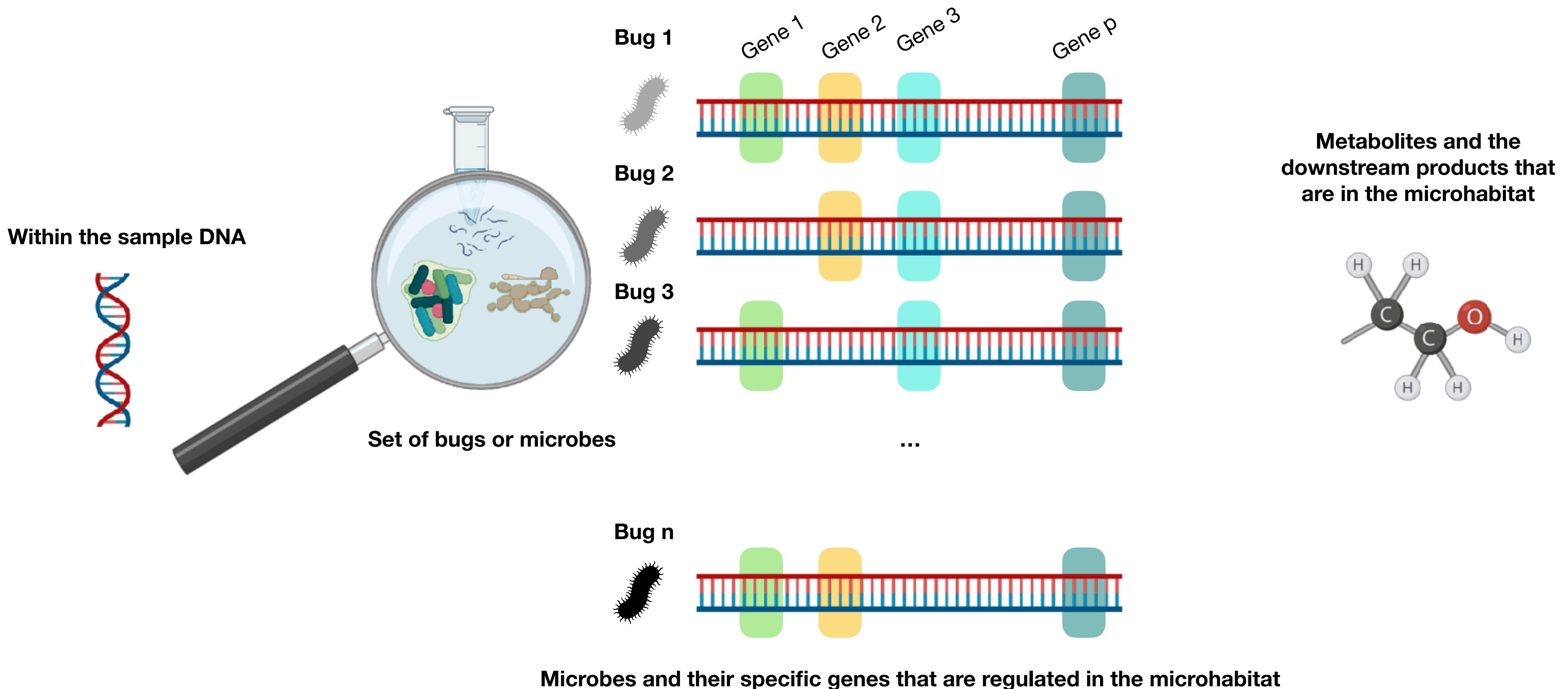


# DNA alone provides the snapshot of the habitat in molecular level

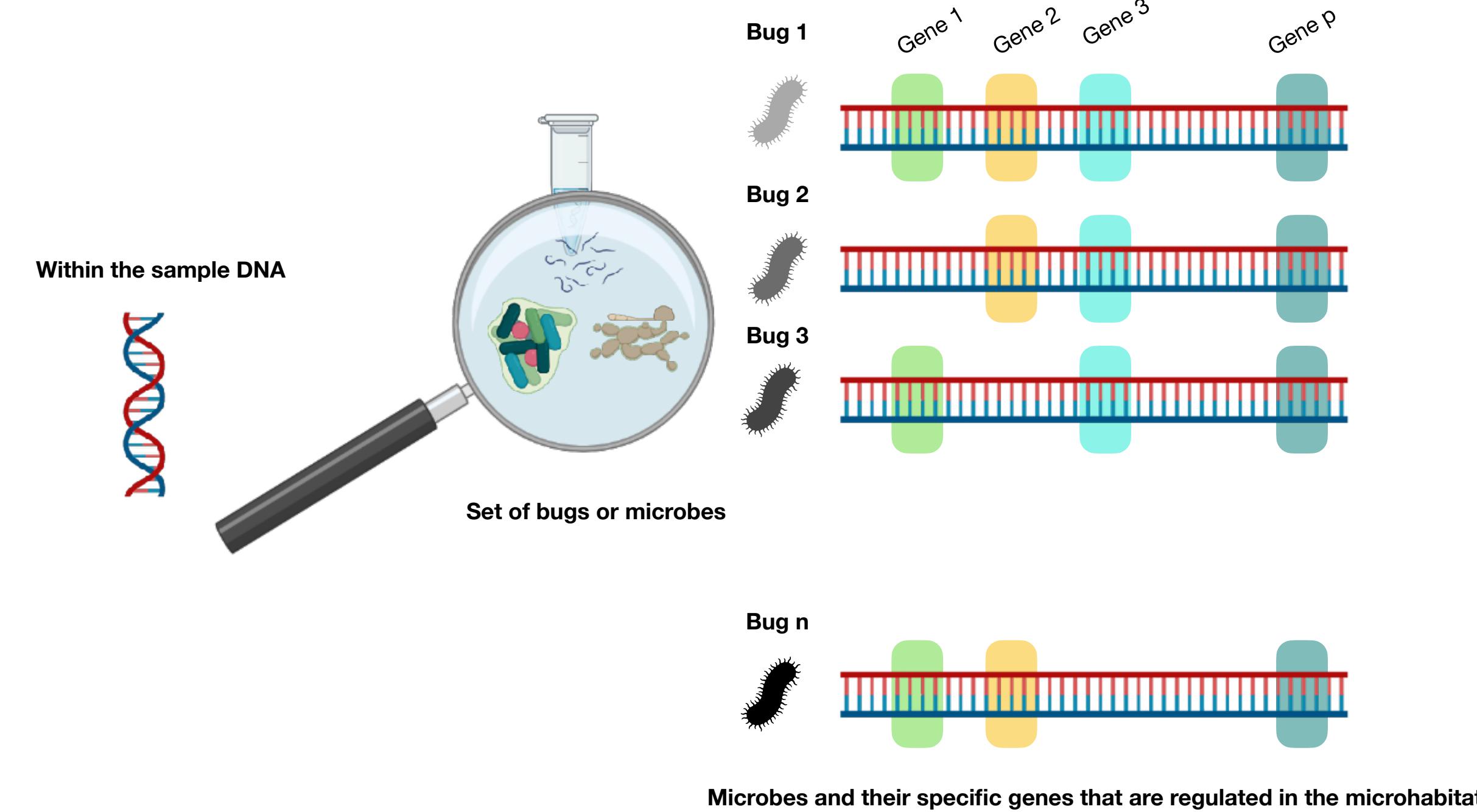


	What genes? Which species? What is their abundance?							
	20	10	30	0	0	0	55	0
Gut	20	10	30	0	0	0	55	0
Root	0	0	22	32	12	10	0	23
Poop	0	0	12	2	33	0	69	0

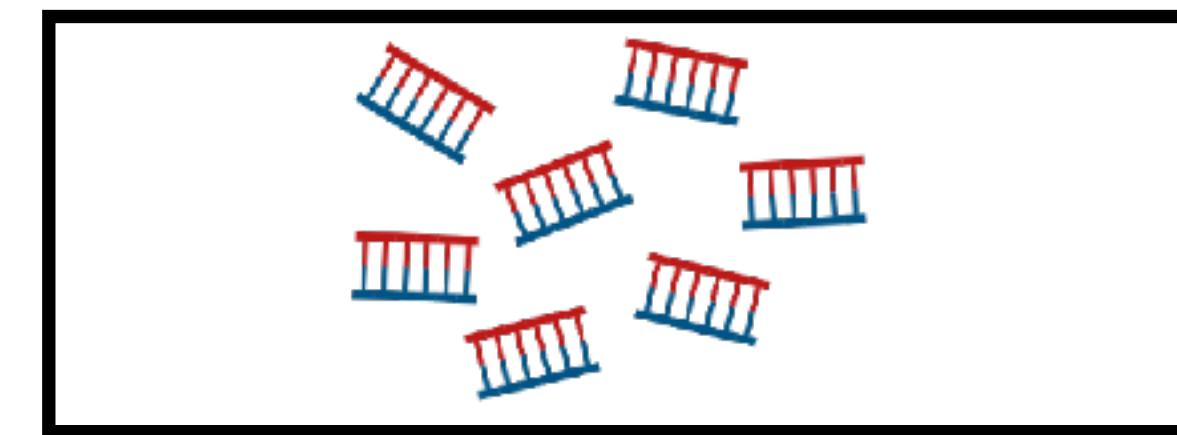
# Let's see what's in the genome of the sample?



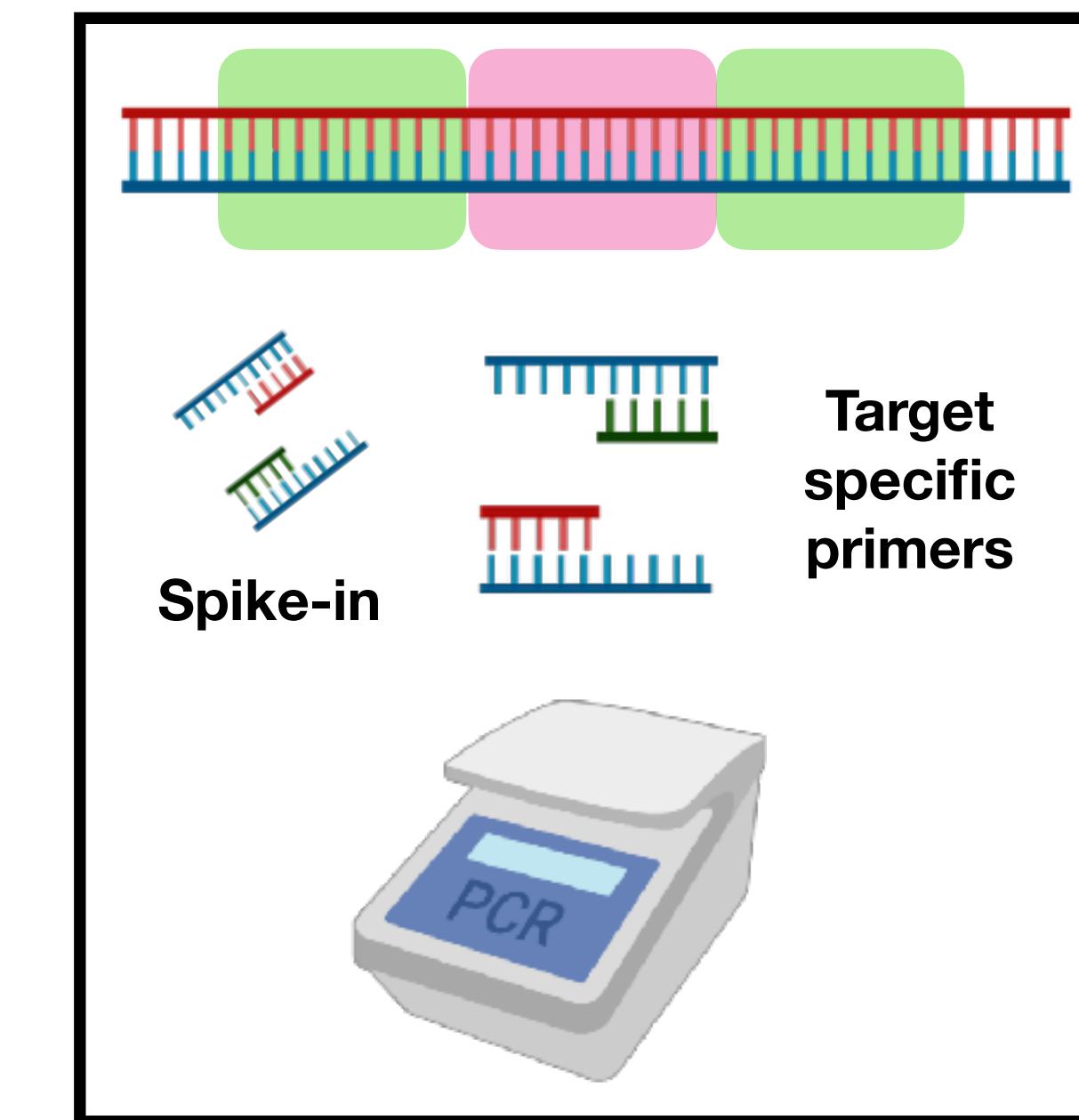
# Modern molecular techniques to look into the microhabitat



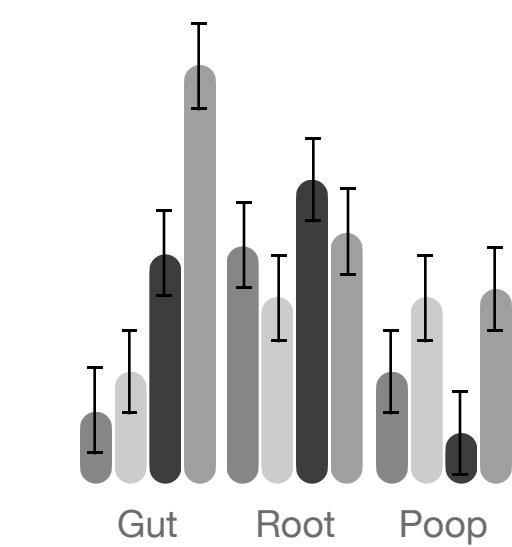
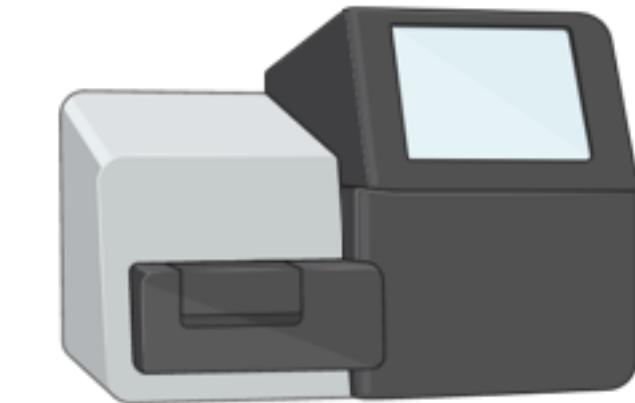
Shotgun Metagenomics



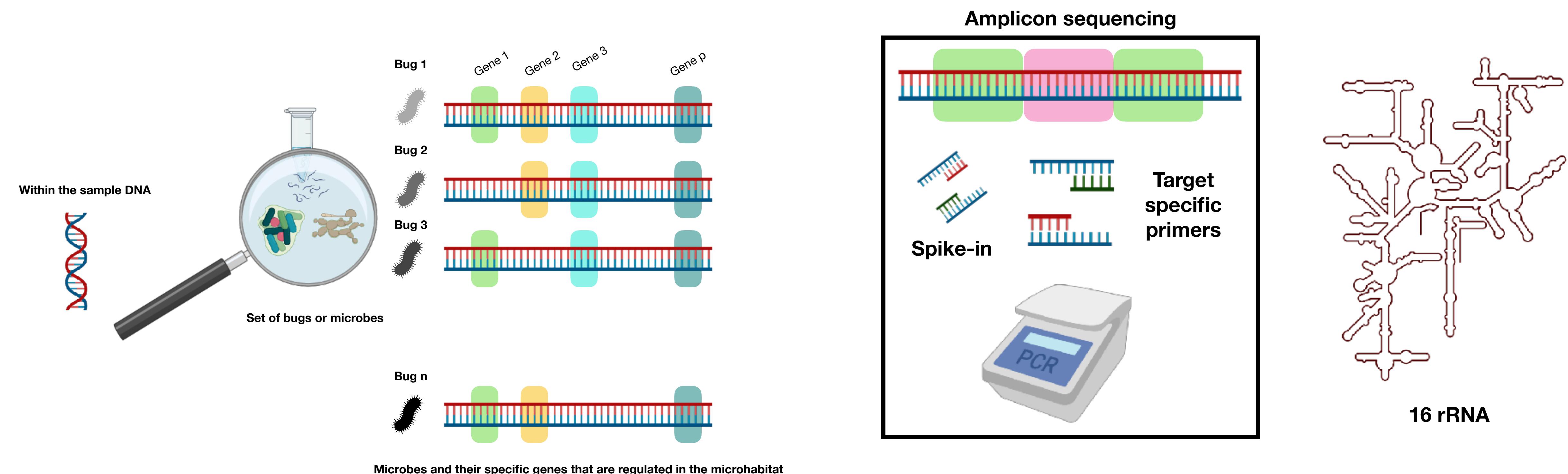
Amplicon sequencing



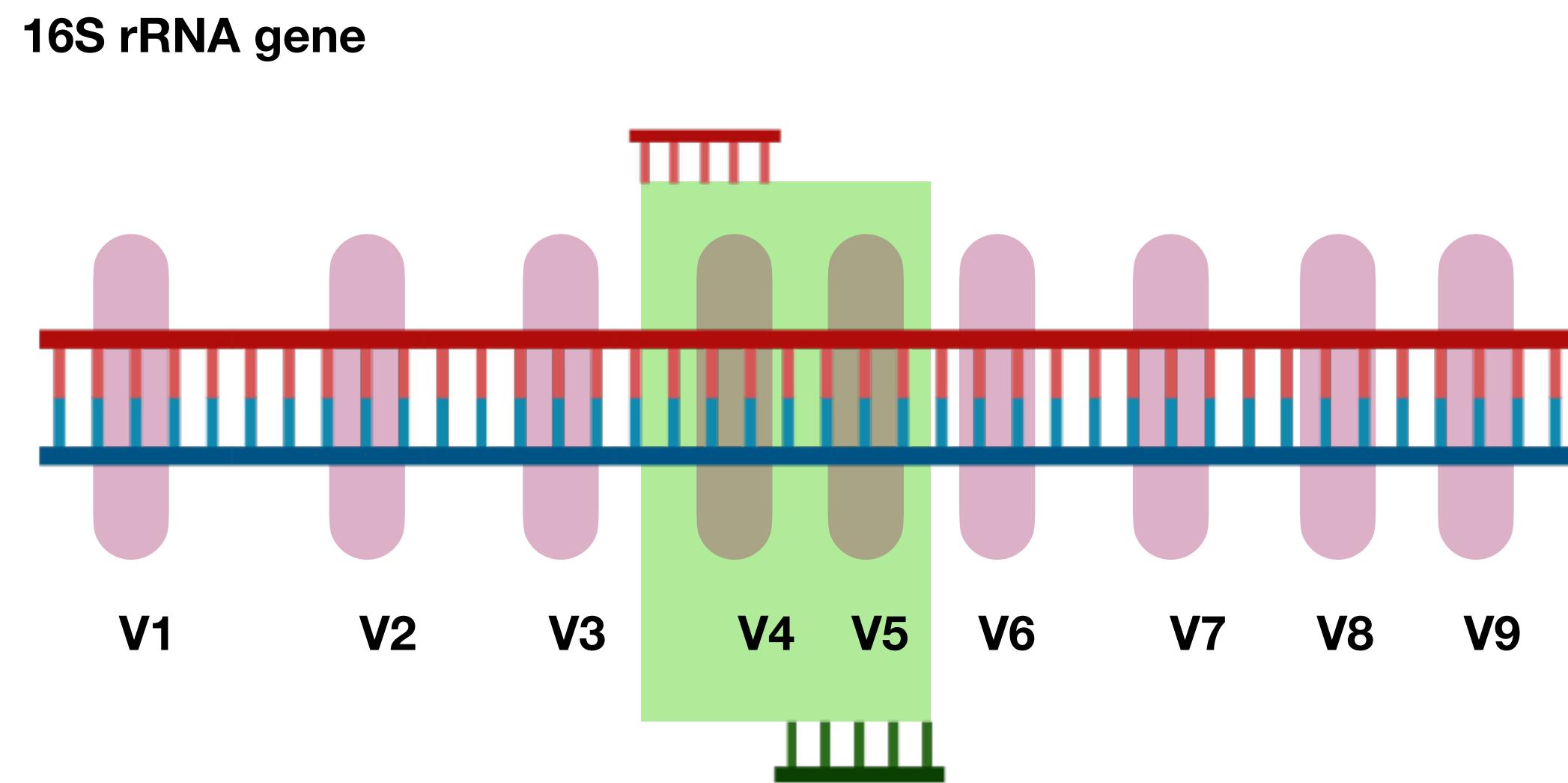
Sequencing bu Illumina  
MiSeq/NovaSeq/HiSeq



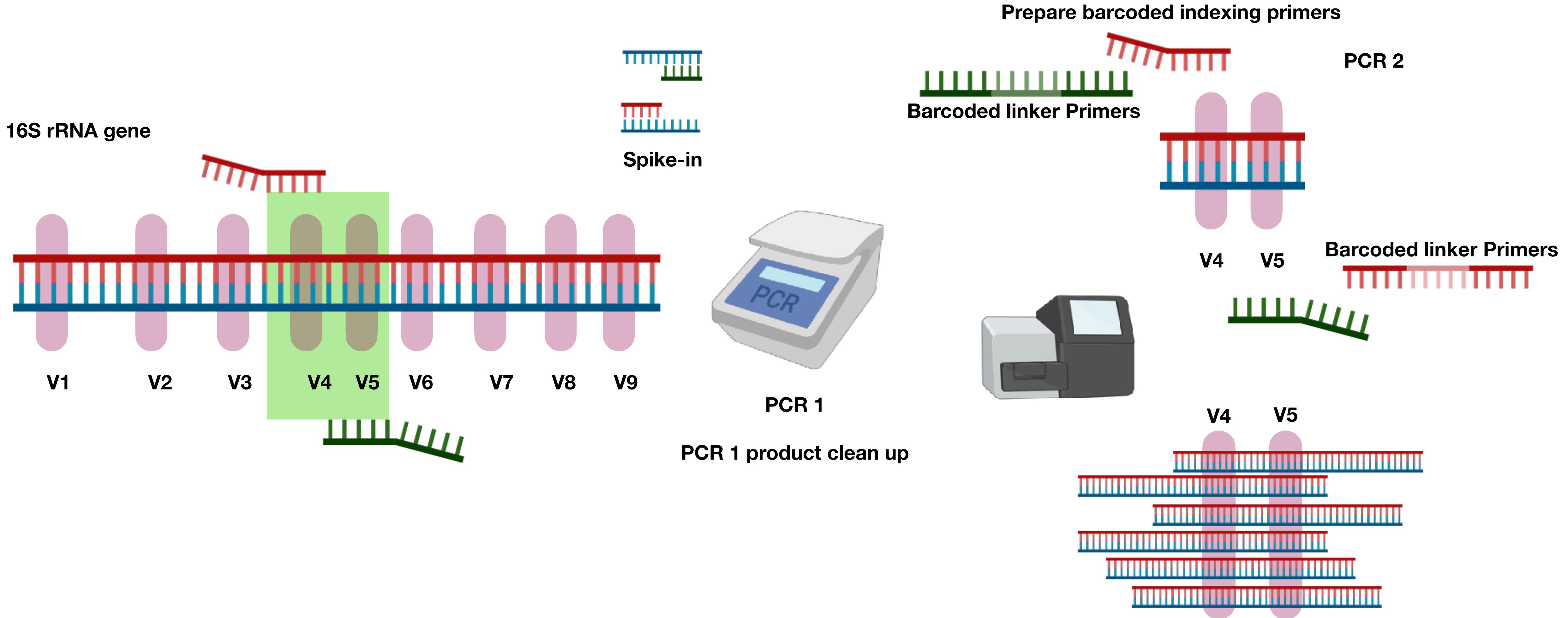
# Amplicon based meta-genomics using meta barcoding



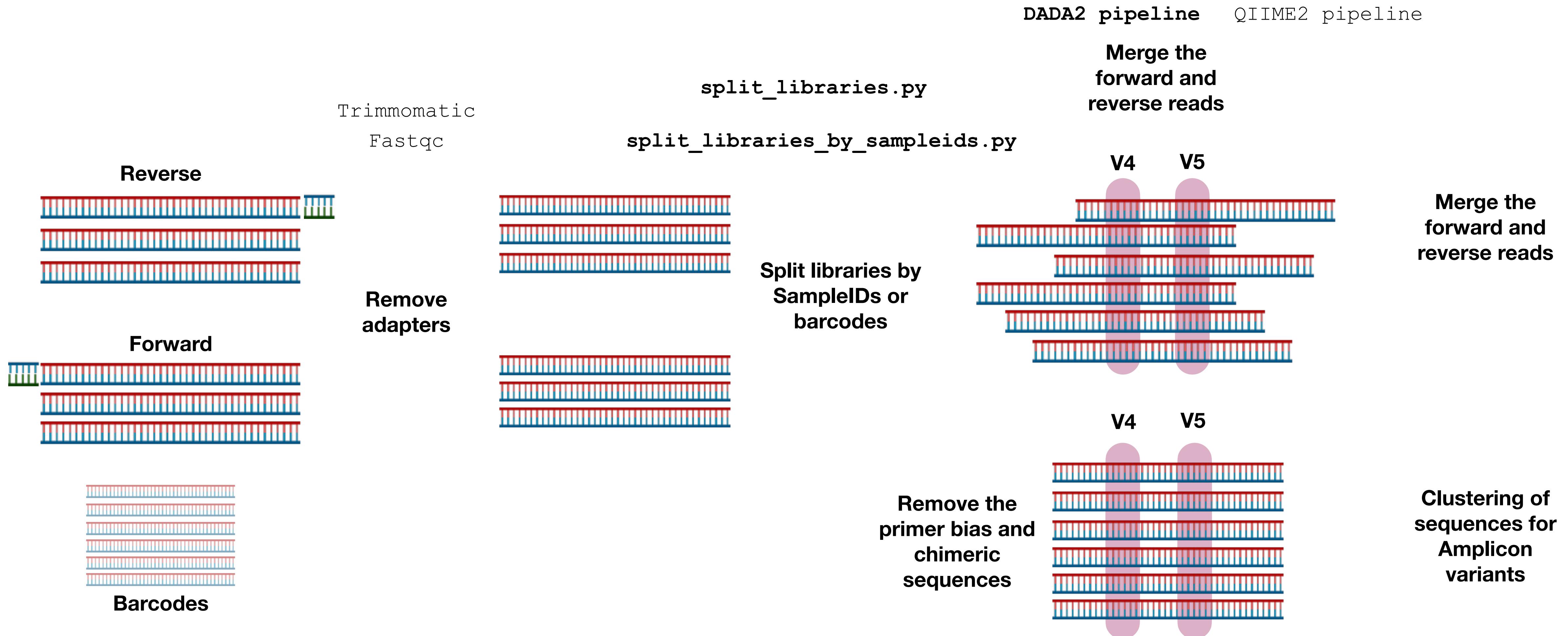
# Setup your target amplicon



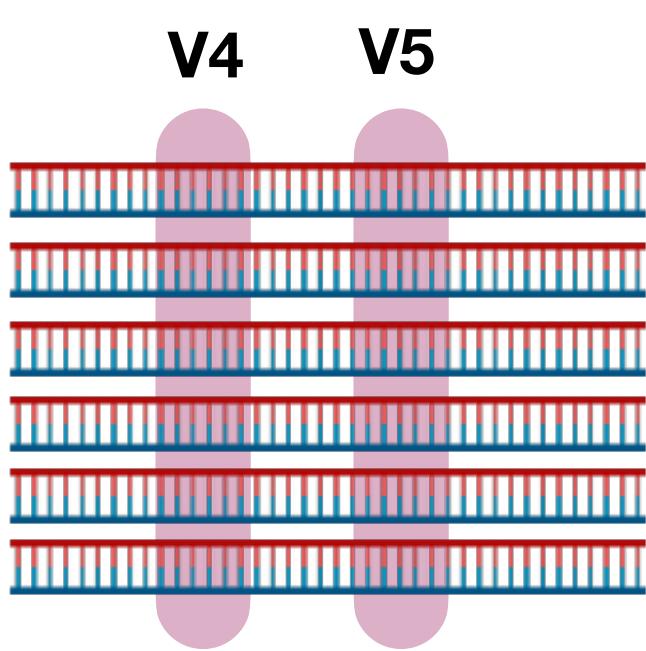
# Lab work



# Data processing



# Generating the OTU or ASV tables



**97% or X% Sequence similarity based OTU picking**

QIIME2

USEARCH

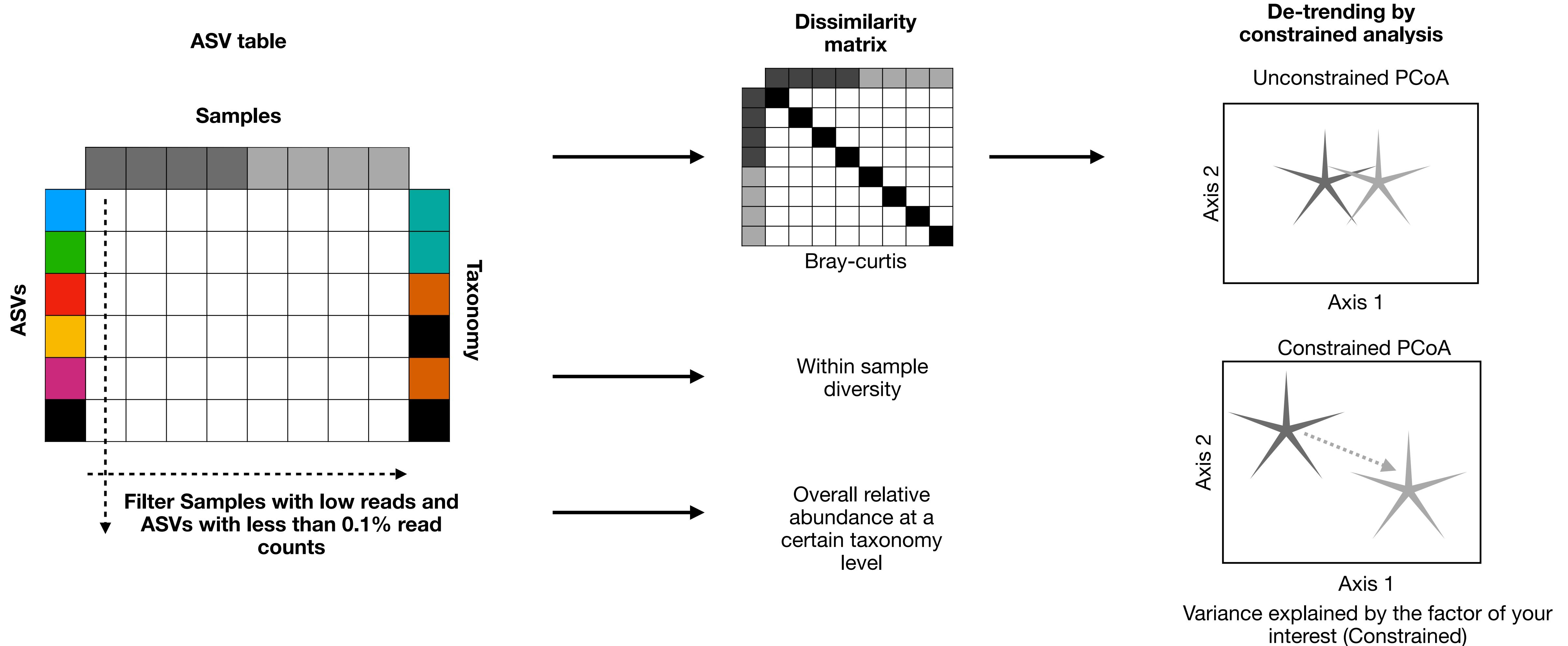
MOTHUR

**Obtain amplicon sequence variants or exact sequence variants**

**DADA2-pipeline**

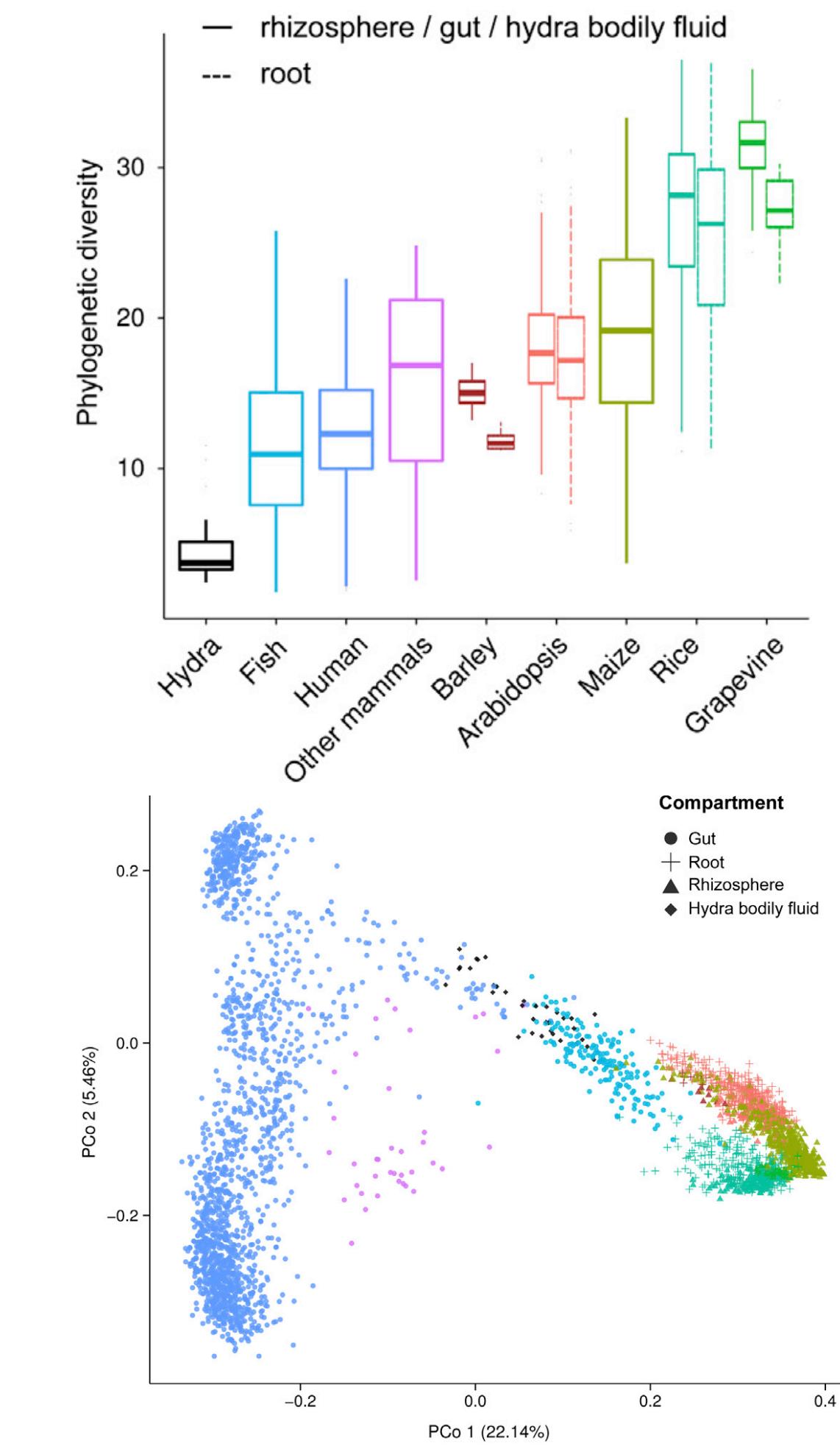
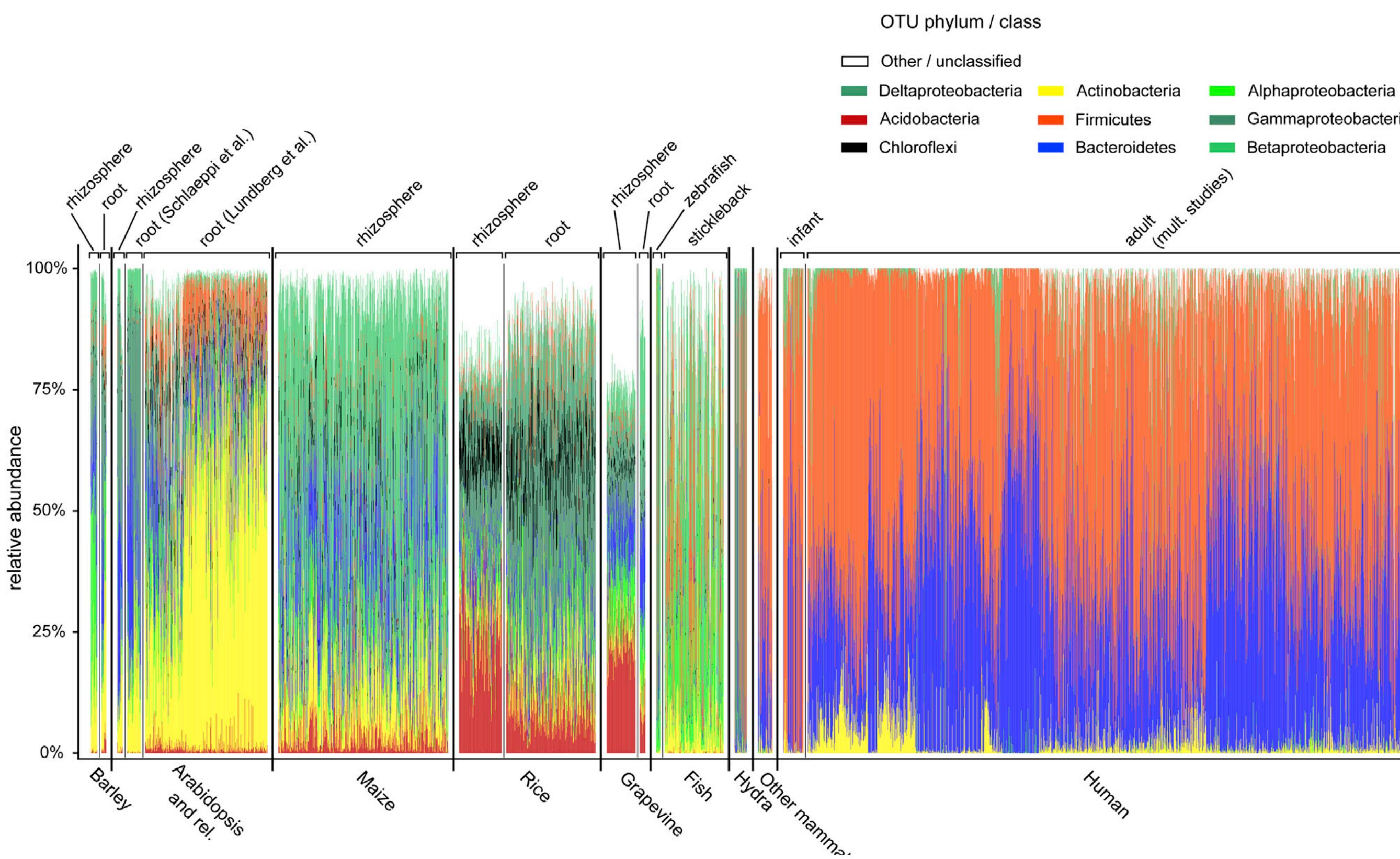
	Which amplicon?	Which taxonomy?	Which Species?						
Gut	20	10	30	0	0	0	55	0	
Root	0	0	22	32	12	10	0	23	
Poop	0	0	12	2	33	0	69	0	

# Dealing with the multivariate analysis



# Summarising the data

Hacquard et al., 2015

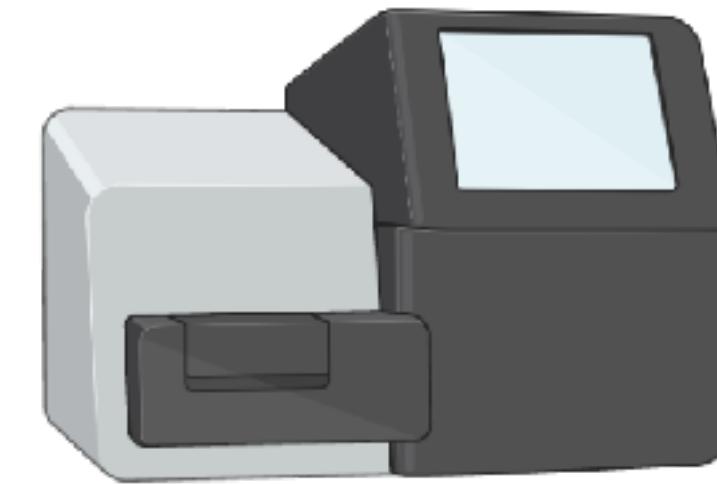
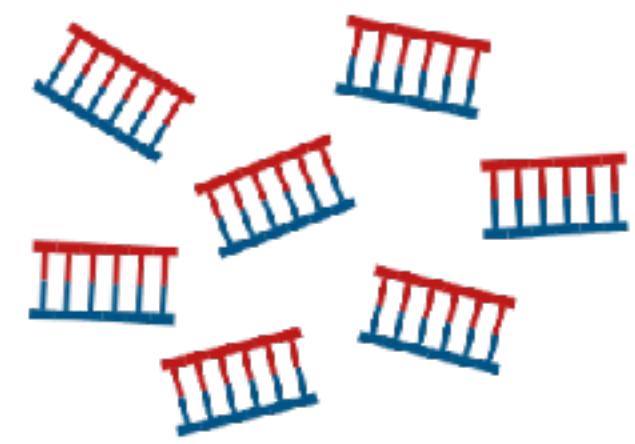


# Shot-gun meta-genomics



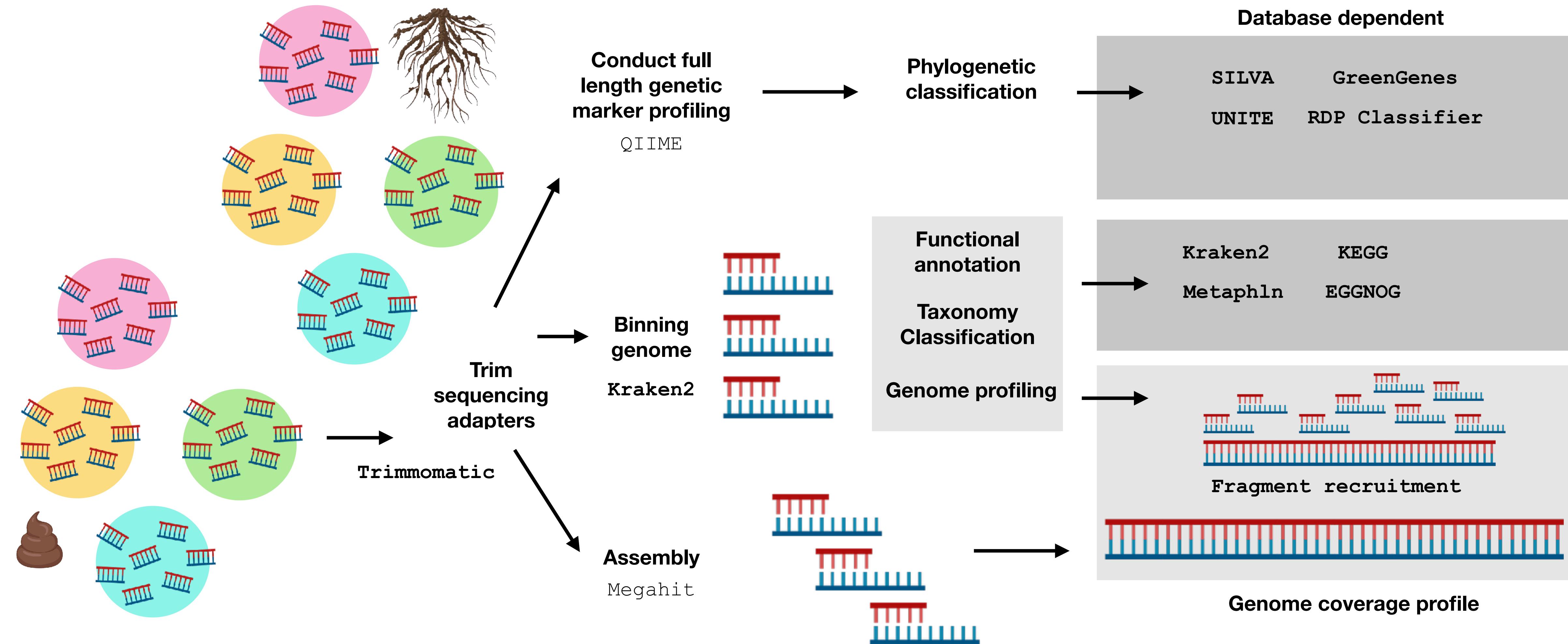
Sample genome

Random fragment generation using restriction sites

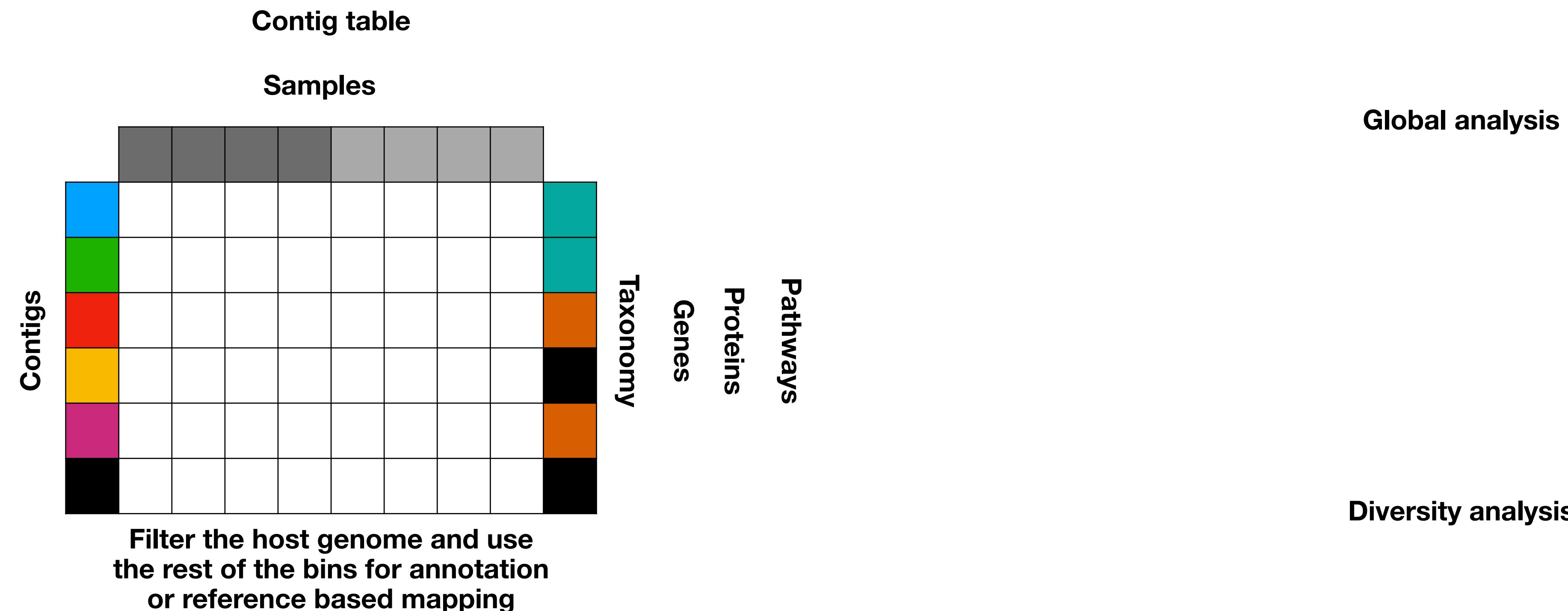


Whole genome sequencing  $\geq 300$  million reads

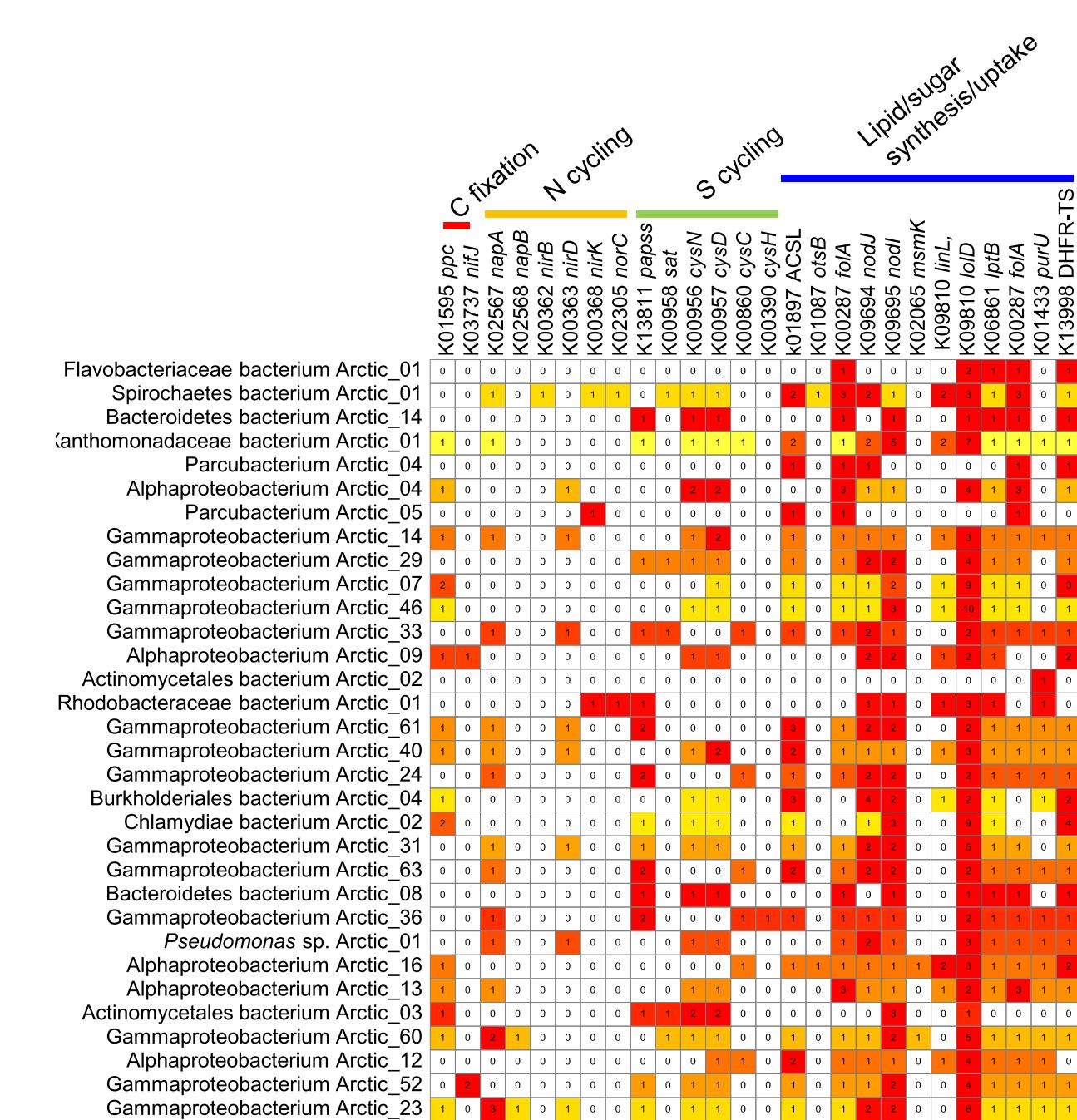
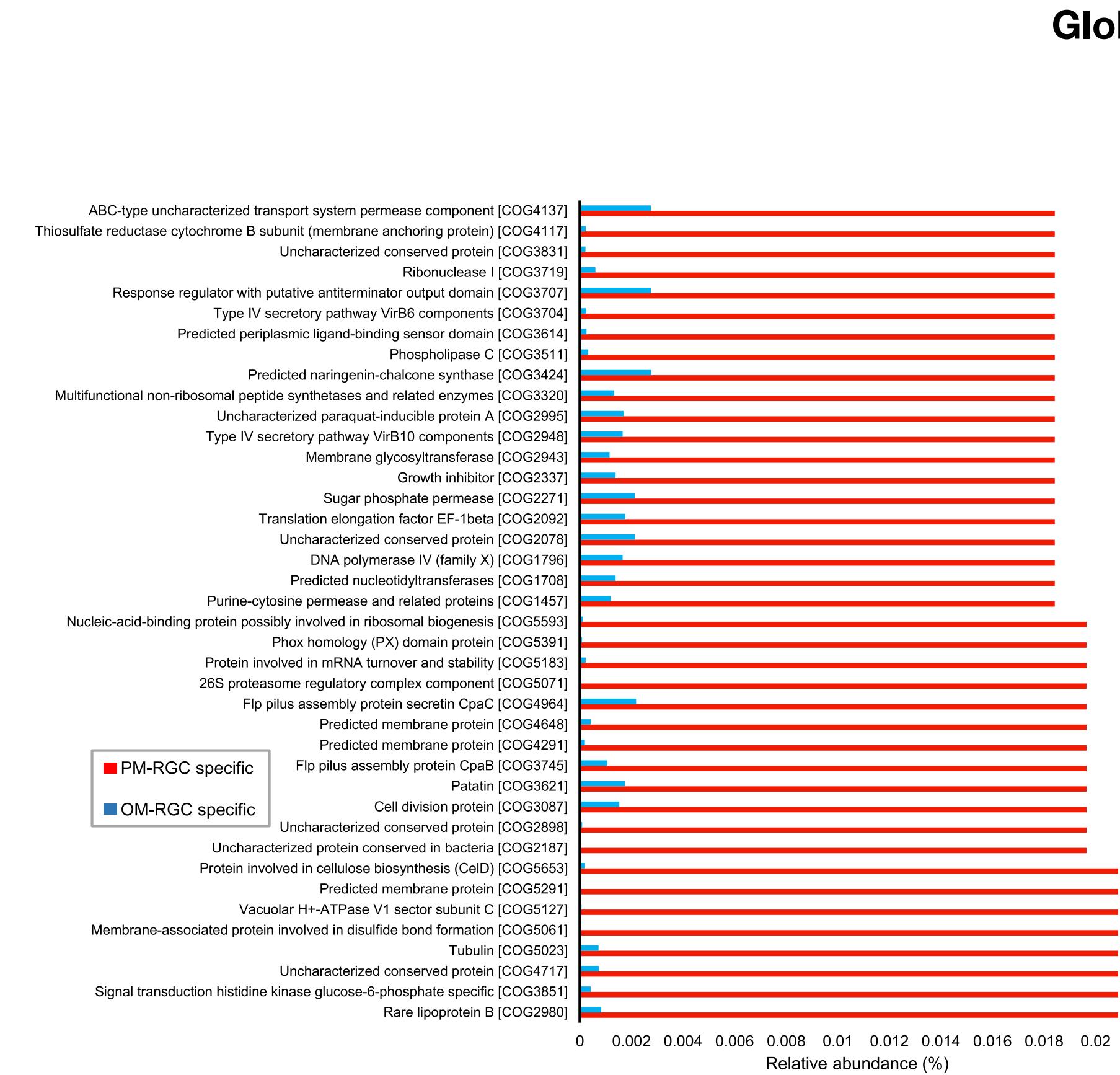
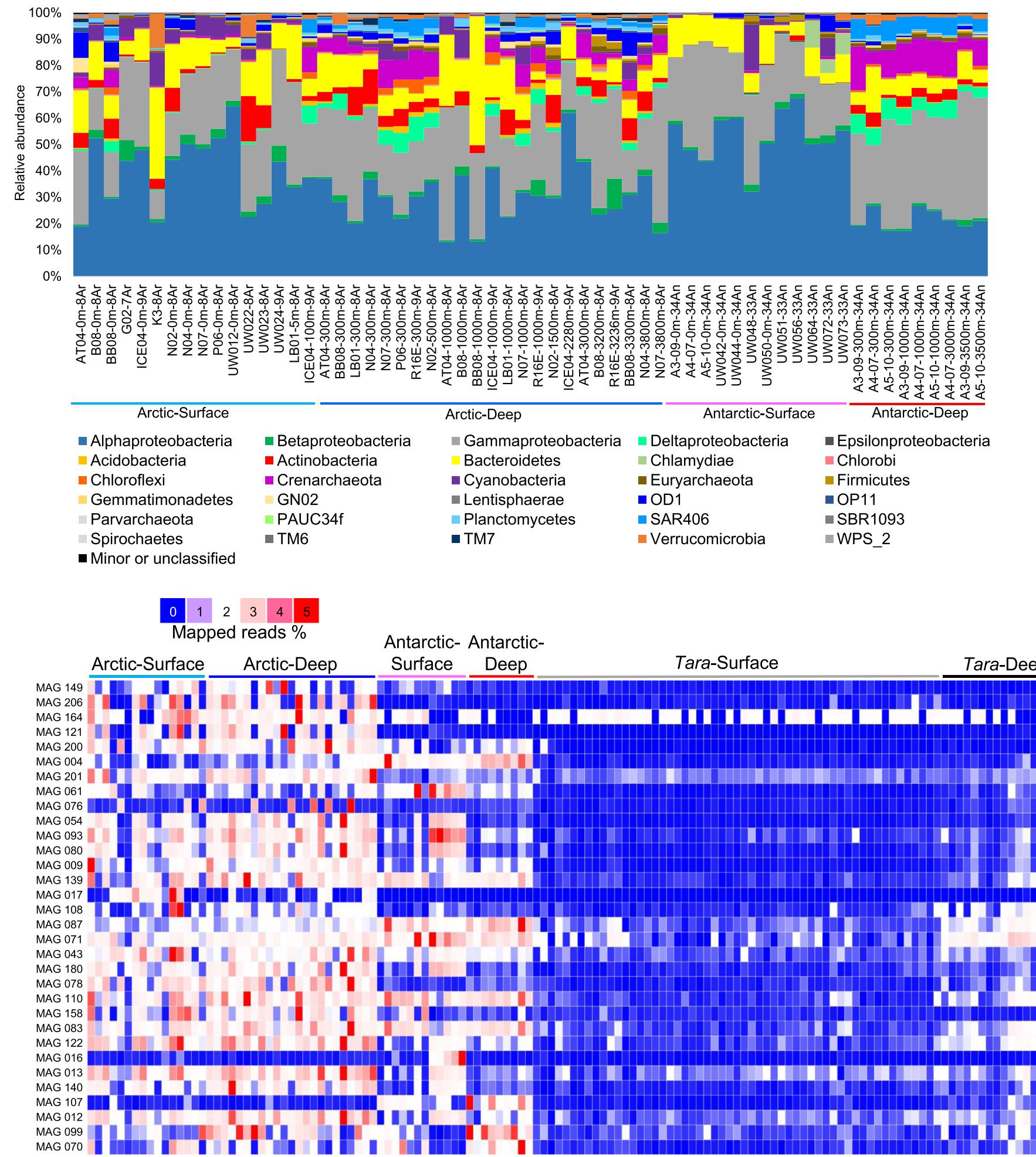
# Shot-gun meta-genomics - Data processing



# Shot-gun meta-genomics - Data analysis

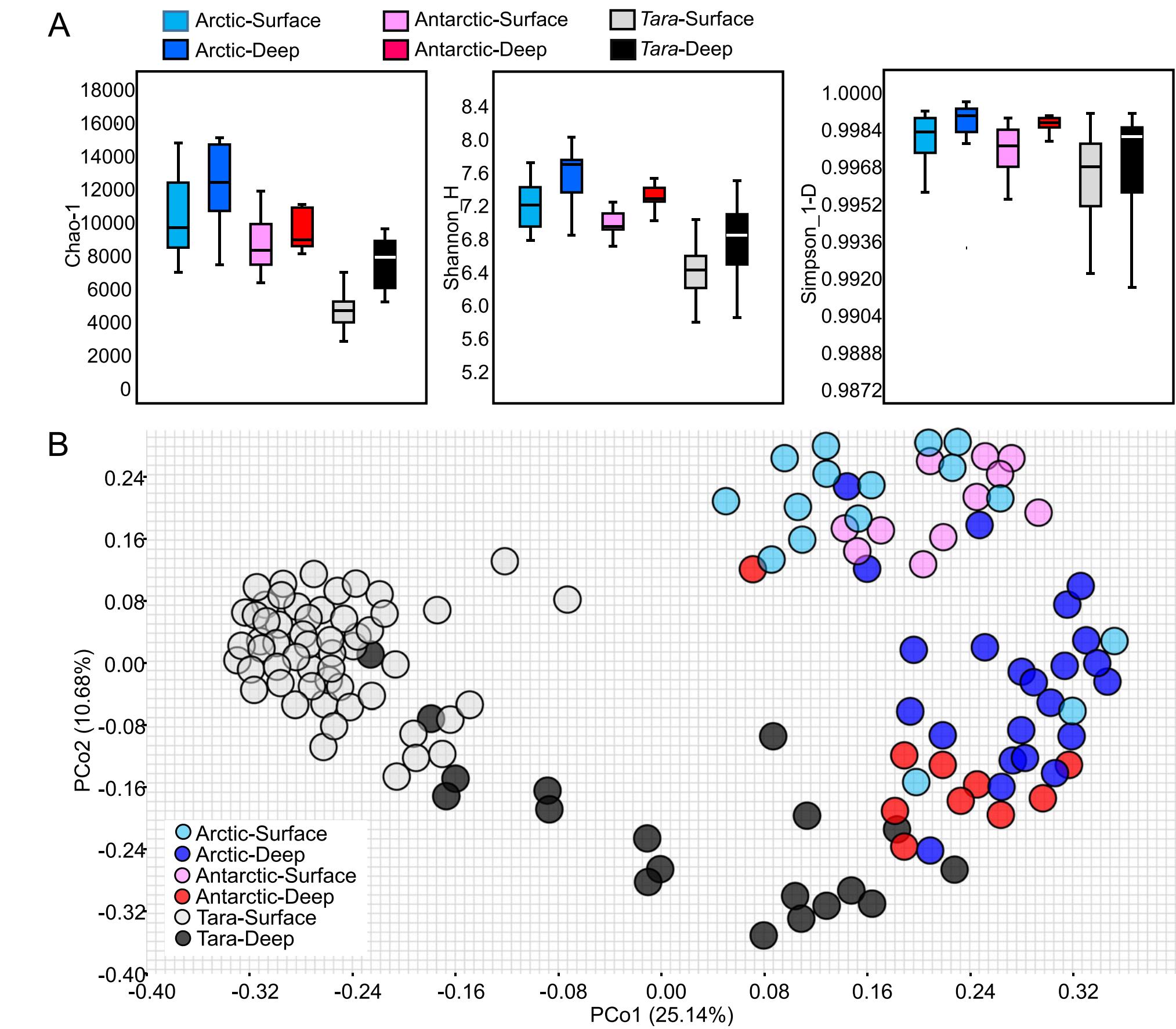


# Shot-gun meta-genomics - Data summary

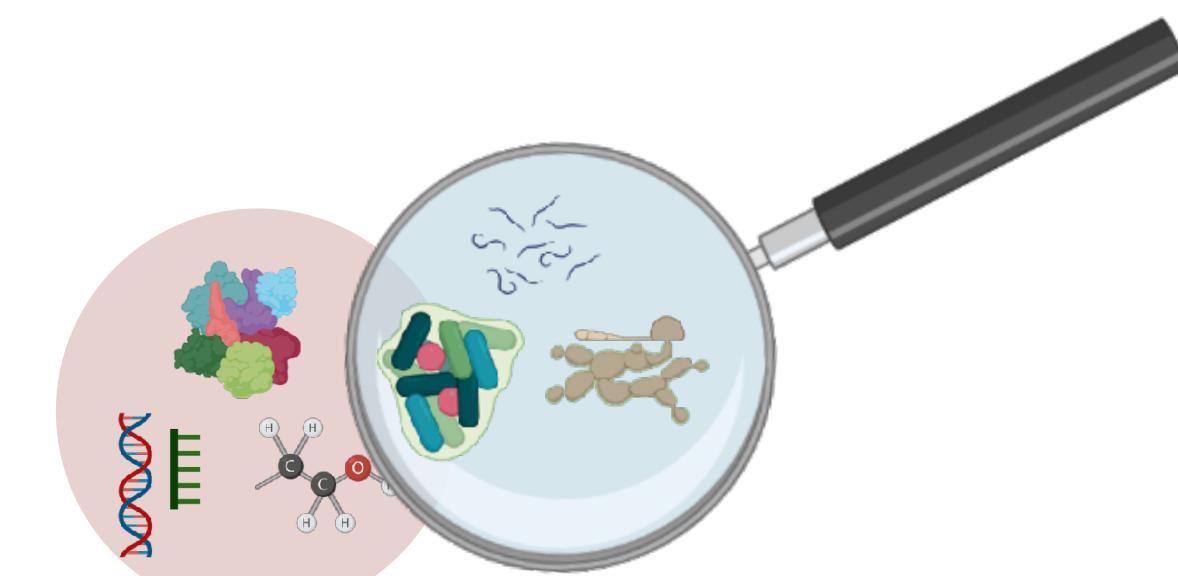
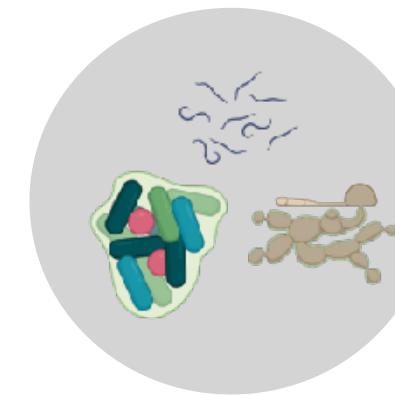
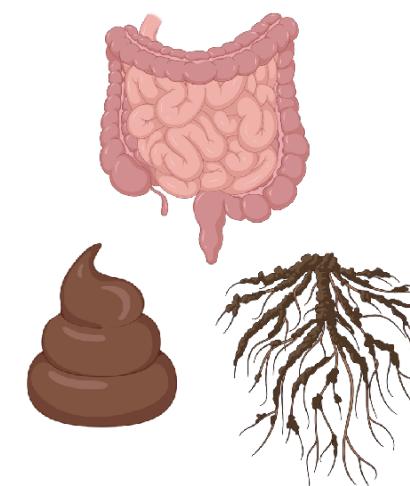


# Shot-gun meta-genomics - Data summary

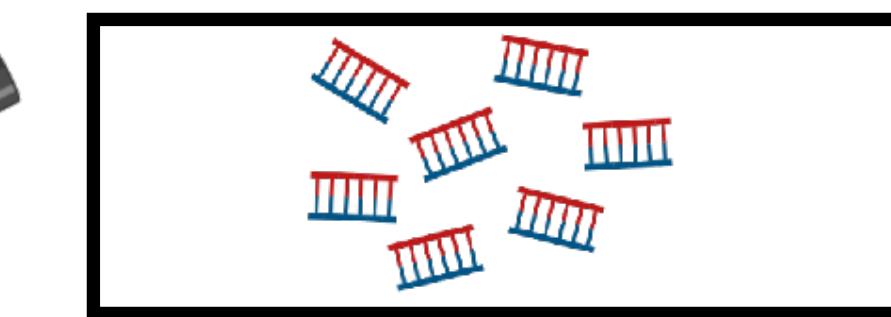
## Diversity analysis



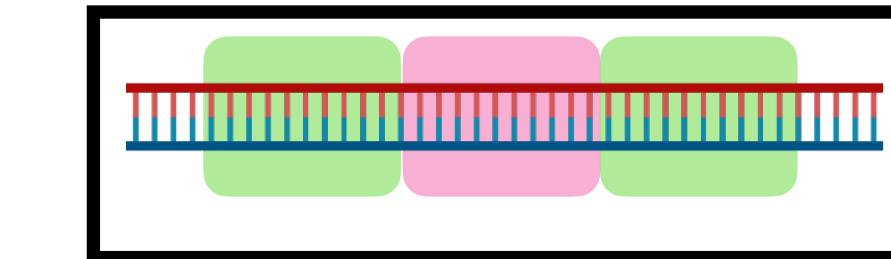
# Wrap up



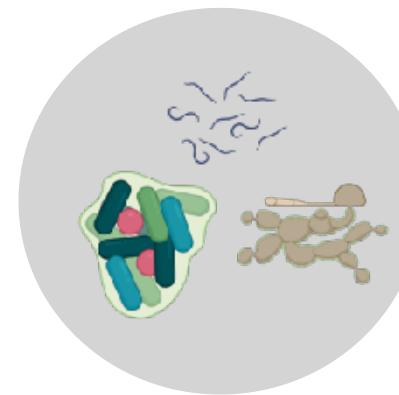
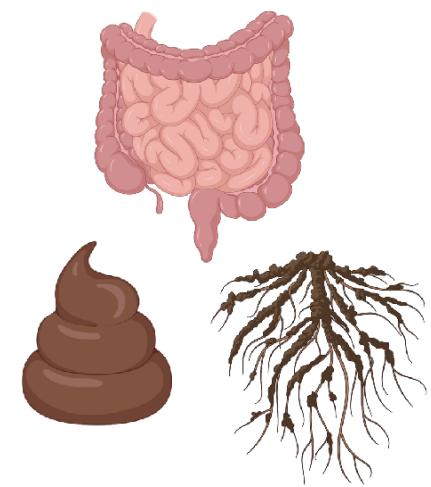
**Shotgun Metagenomics**



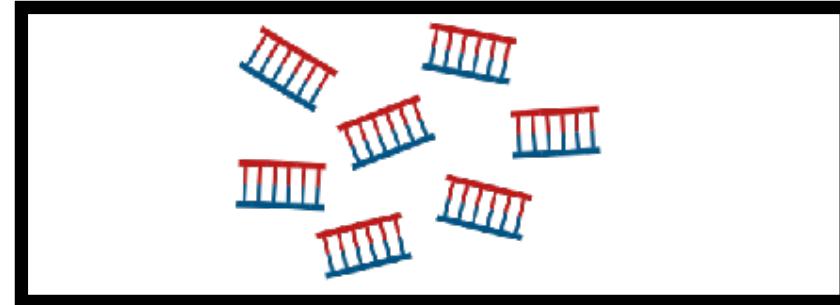
**Amplicon sequencing**



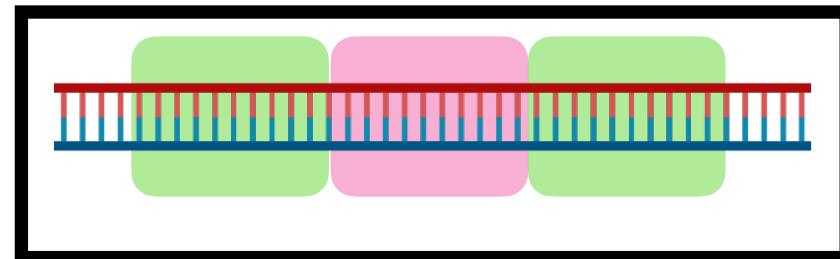
# Practical on Amplicon based sequencing



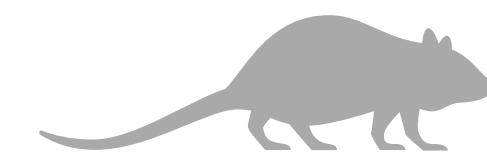
**Shotgun Metagenomics**



**Amplicon sequencing**



**Practical 1: Day 2**



Male and Female

72 h Diet treatment

Bodyweight

Microbiome

Stomach      Distal gut

**Who is out there?**

Dataset labels are shuffled

All images are illustrated using BioRender