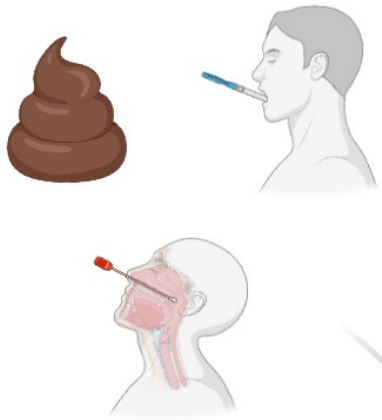
Microbiome workshop – data generation and processing

September 2024

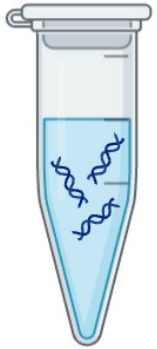
IKMB
Institute of Clinical
Molecular Biology

Microbiome science - from sample to data



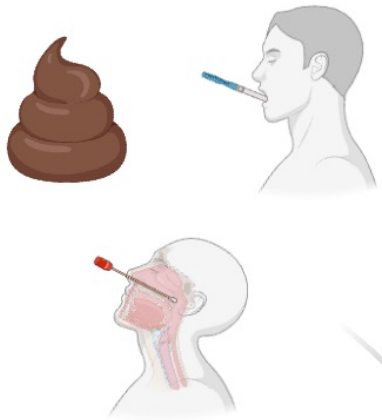
Sample collection & storage

DNA/RNA extraction



Data generation & processing

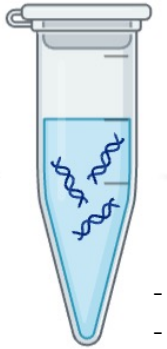
Microbiome science - from sample to data



Sample collection & storage

- Sampling method (swab, tube, …)
- Sampling buffer (RNAlater, glycerol, …)
- Storage conditions (snap freezing, -20/-80°C)

DNA/RNA extraction



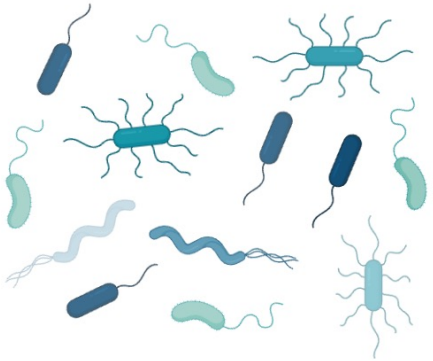
- Extraction kit used
- DNA or RNA or both?
- Cell disruption (Proteinases, Bead beating)

Data generation & processing

- 16S rRNA gene or Shotgun metagenomics
- Short reads or long reads
- Data annotation

16S rRNA gene
amplicon sequencing

shotgun
metagenomics



16S rRNA gene
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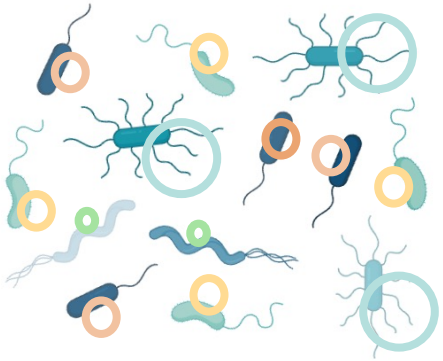

16S rRNA gene
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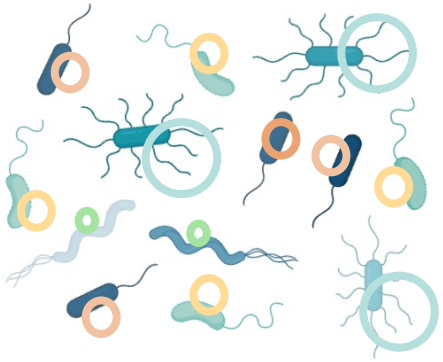
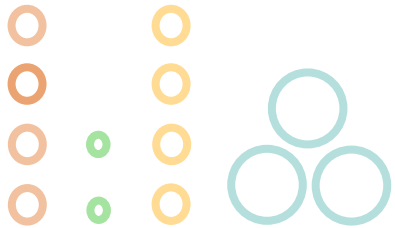
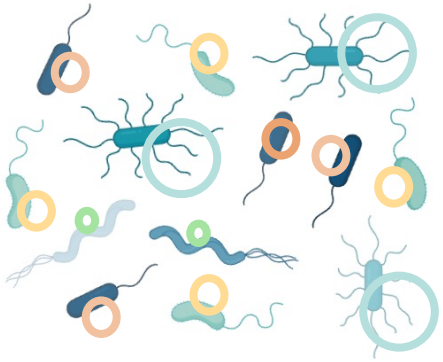




16S rRNA gene amplicon sequencing

shotgun metagenomics

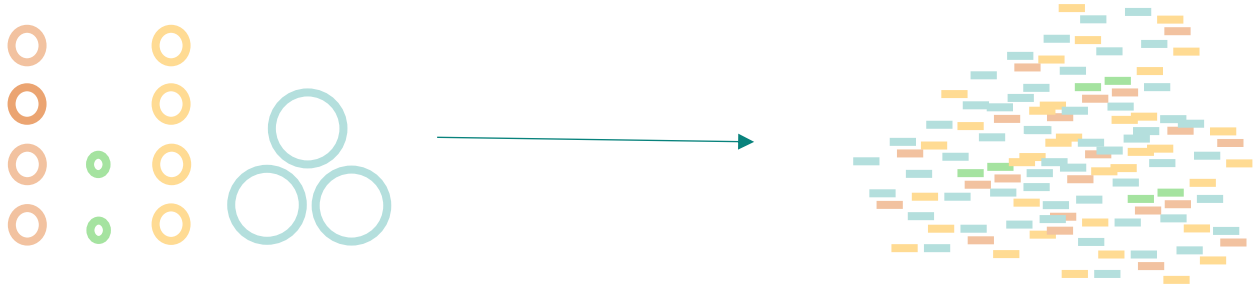


Full genome information + + +

More data + / -

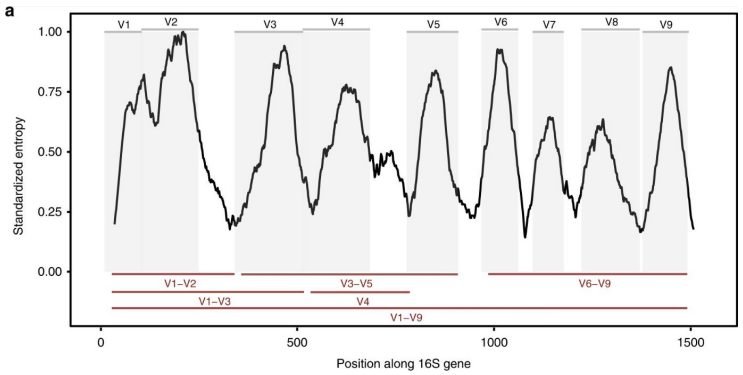
More expensive - -

Bias towards high abundant taxa -



Johnson *et al.* 2019

16S rRNA gene amplicon sequencing



16S rRNA gene: conserved and variable regions

PCR target + Molecular clock
= taxon information

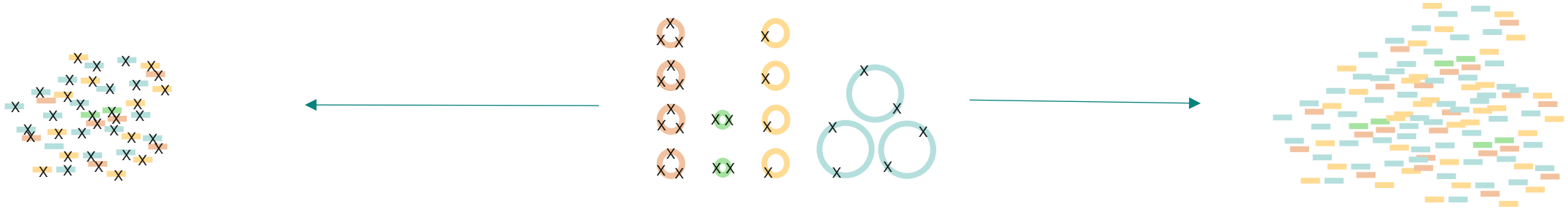
shotgun metagenomics

Full genome information + + +

More data + / -

More expensive - -

Bias towards high abundant taxa -



Johnson *et al.* 2019

What we have: The FastQ format

Identifier — @HWI-EAS209_0006_FC706VJ:5:58:5894:21141#ATCACG/1

Sequence — TTAATTGGTAAATAAATCTCCTAATAGCTTAGATNTTACCTTNNNNNNNNNNTAGTTTCTTGAGA

+ sign & identifier — +HWI-EAS209_0006_FC706VJ:5:58:5894:21141#ATCACG/1

Quality scores — efcfffffcfeefffcffffffddf`feed]`]_Ba_^__[YBBBBBBBBBBRTT\]][]dddd`

Base T

phred Quality] = 29

https://compgenomr.github.io/book/fasta-and-fastq-formats.html

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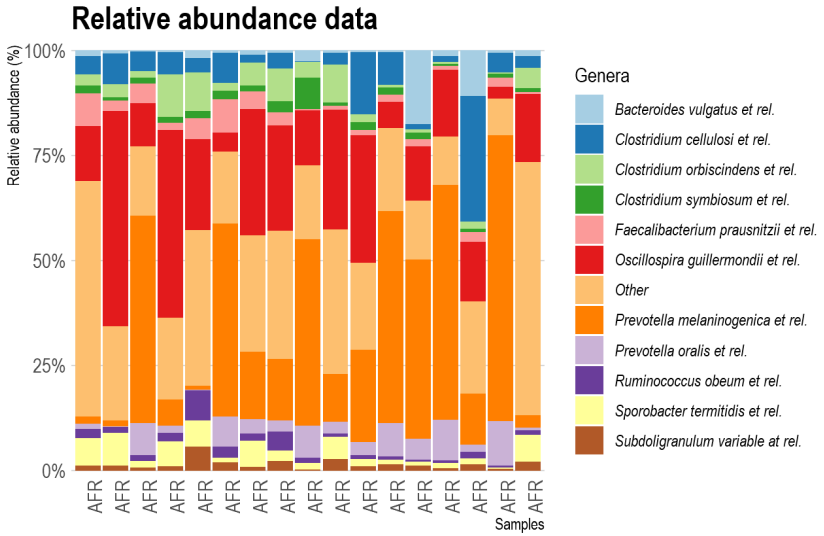

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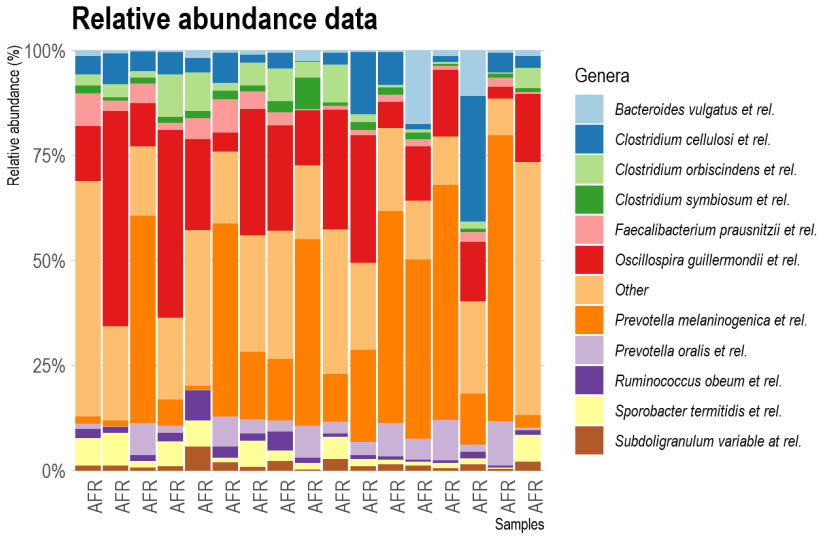
What we want:



How we get there:

qiime2

Bolyen *et al.* 2019

DADA2

Callahan *et al.* 2019

https://compgenomr.github.io/book/fasta-and-fastq-formats.html

16S data workflow with

qiime2docs

Version: 2024.5

Table of Contents

Quick search

Go

QIIME 2 user documentation

This site is the official user documentation for QIIME™ 2, including installation instructions, tutorials, and other important information. Visit http://qiime.org for information on QIIME™ 1.

Getting started

Check out the getting started guide to begin using QIIME 2.

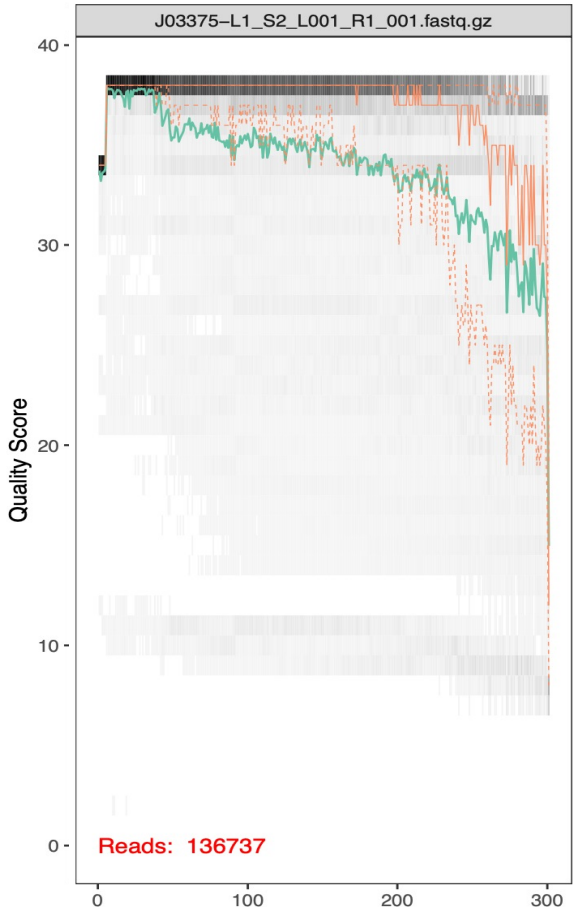
Table of contents

16S data workflow with qiime2 + DADA2

- Tutorials
  - Overview of QIIME 2 Plugin Workflows
  - QIIME 2 for Experienced Microbiome Researchers
  - “Moving Pictures” tutorial
  - “Moving Pictures” tutorial - Multiple Interface Edition
  - Fecal microbiota transplant (FMT) study: an exercise
  - “Atacama soil microbiome” tutorial
  - Parkinson’s Mouse Tutorial
  - Importing data
  - Exporting data
  - Metadata in QIIME 2
  - Filtering data
  - Training feature classifiers with q2-feature-classifier
  - Evaluating and controlling data quality with q2-quality-control
  - Predicting sample metadata values with q2-sample-classifier
  - Performing longitudinal and paired sample comparisons with q2-longitudinal
  - Identifying and filtering chimeric feature sequences with q2-vsearch
  - Alternative methods of read-joining in QIIME 2
  - Clustering sequences into OTUs using q2-vsearch
  - Utilities in QIIME 2
  - Phylogenetic inference with q2-phylogeny

16S data workflow with qiime2 + DADA2

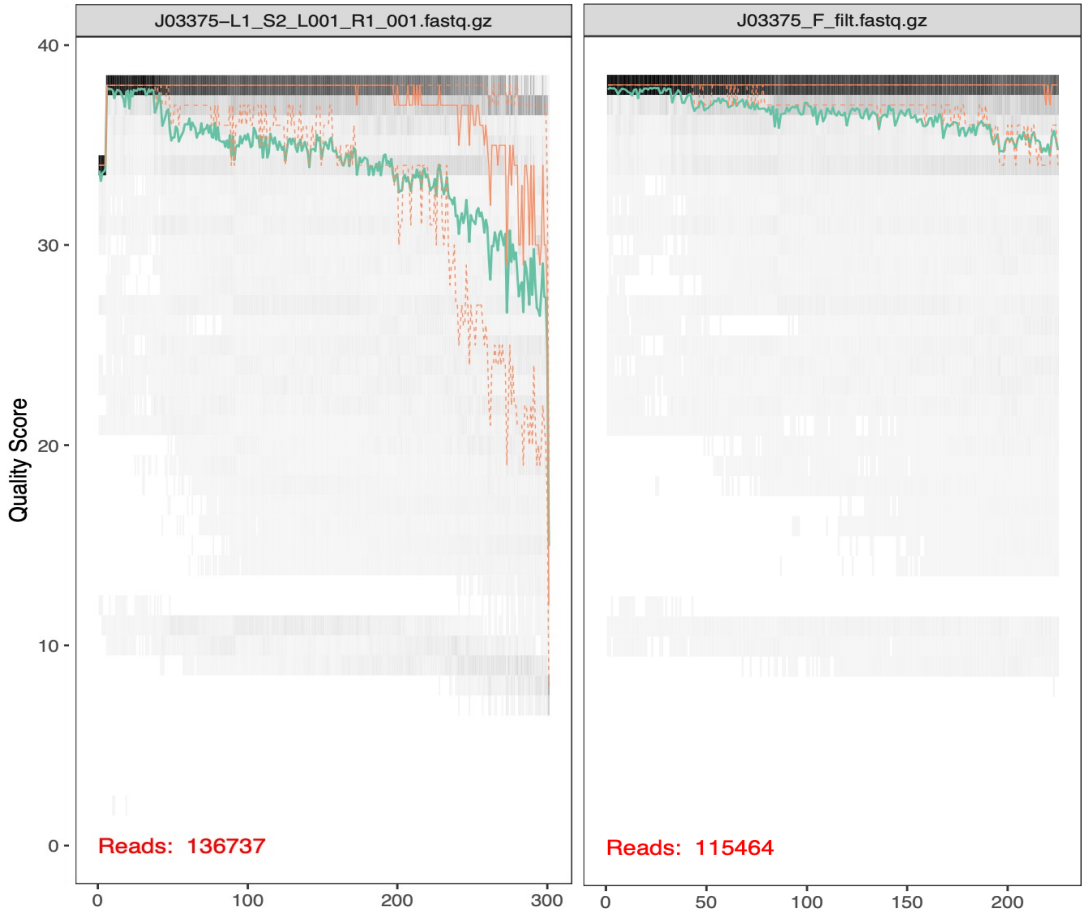
Step 1: QC and trimming



Raw

16S data workflow with qiime2 + DADA2 (simplified)

Step 1: QC and trimming



Raw

After QC

16S data workflow with qiime2 + DADA2 (simplified)

Step 1: QC and trimming

Raw

After QC

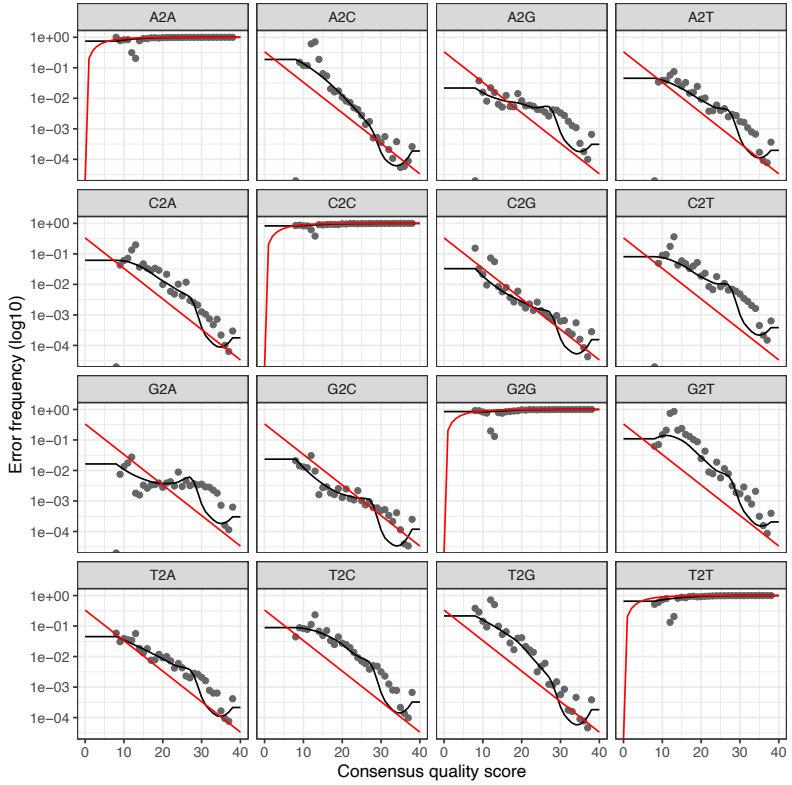
16S data workflow with qiime2 + DADA2 (simplified)

Step 1: QC and trimming

Raw

After QC

Step 2: Error correction



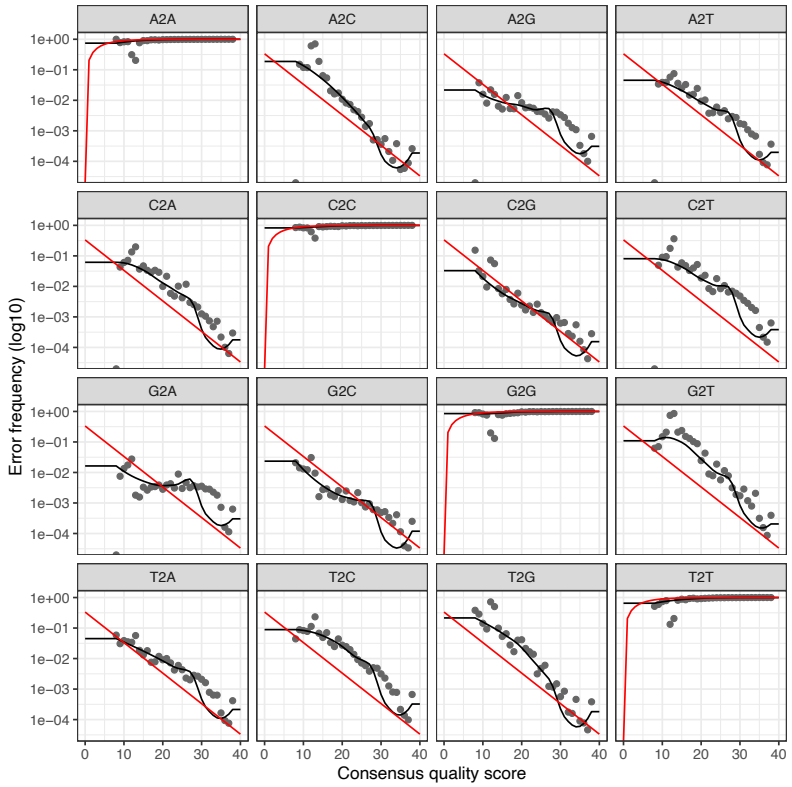
16S data workflow with qiime2 + DADA2 (simplified)

Step 1: QC and trimming

Raw

After QC

Step 2: Error correction



Step 3:
Inference of Amplicon Sequence Variants (ASVs)

Assigning taxonomic labels

Abundance Table

	ASV1	ASV2	ASV3	ASV4
S1	5	10	3	7
S2	5	0	3	0
S3	0	0	4	3

ASV sequences:

```
>ASV1
ACGCTGGCGGCATGCTTAACACATGCAAGTCGTACGAATGAAT…
>ASV2
ACGCTGGCGGTATGCCTAACACATGCAAGTCGAACGAGGTAGC…
>ASV3
ACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTAACAG…
>ASV4
ACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTAGTCA…
```

Assigning taxonomic labels

What is a taxonomy?

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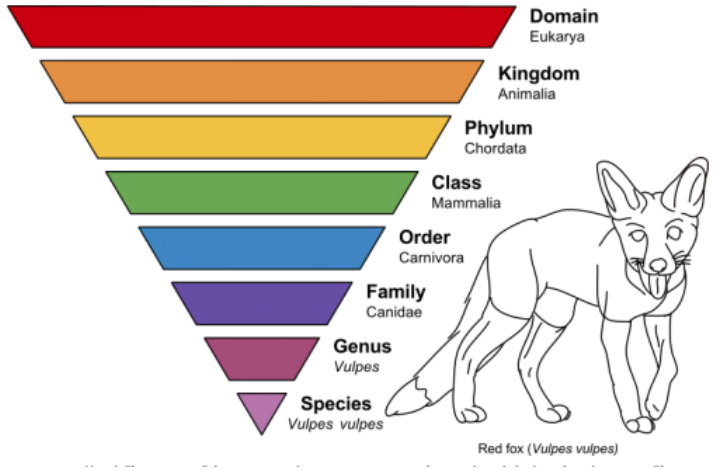

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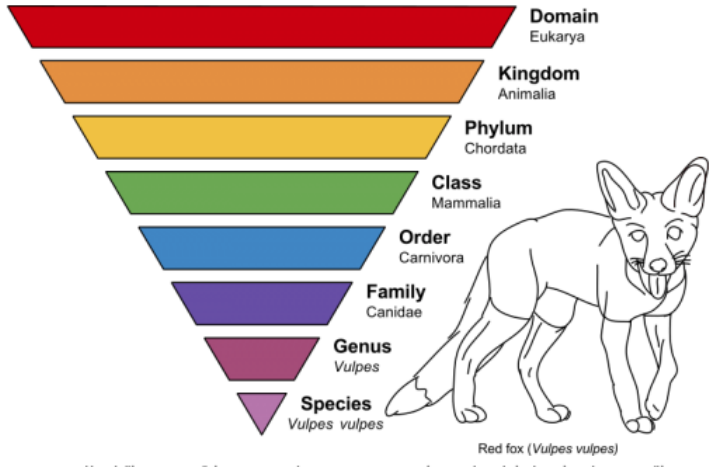

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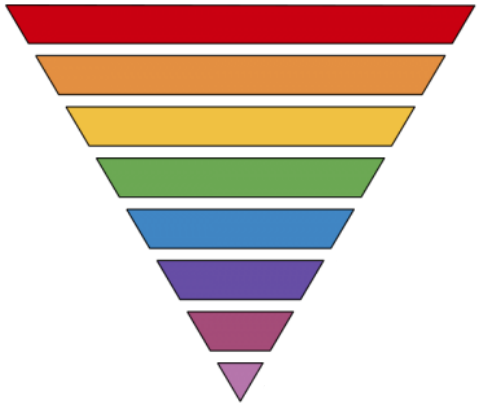
>ASV4
ACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTAGTCA…
```

Same can be done for microorganisms:



Domain	Bacteria
Kingdom	Bacteria
Phylum	Bacillota A
Class	Clostridia
Order	Oscillospirales
Family	Ruminococcaceae
Genus	Faecalibacterium
Species	Faecalibacterium prausnitzii

Assigning taxonomic labels

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Database search
(Naïve Bayesian Classifier)

Assigning taxonomic labels

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Greengenes2

silva high quality ribosomal RNA databases

GTDB TK

Ribosomal database project

Database search
(Naïve Bayesian Classifier)

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ACGCTAGCGGCAGGCTTAACACATGCAAGTCGAGGGGTAGTCA…
```
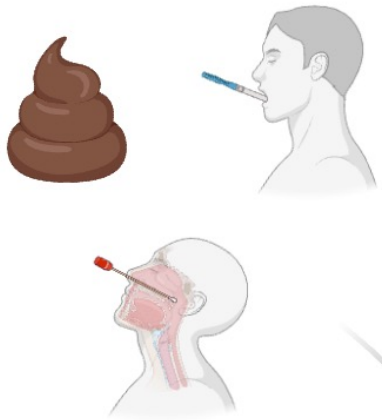
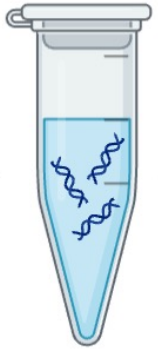

	Domain	Phylum	Class	Order	Family	Genus
ASV1	Bacteria	Bacillota_C	Negativicutes	Veillonellales	Dialisteraceae	Dialister
ASV2	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
ASV3	Bacteria	Pseudomonadota	Gammaproteo bacteria	Enterobacterales	Enterbacteriaceae	
ASV4	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Prevotella

Microbiome science - from sample to data



Sample collection & storage

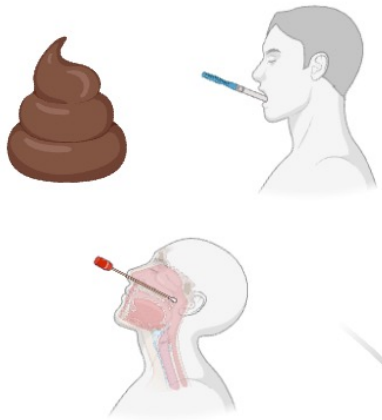
DNA/RNA extraction



Data generation & processing

Data analysis

Microbiome science - from sample to data to analysis



Sample collection & storage

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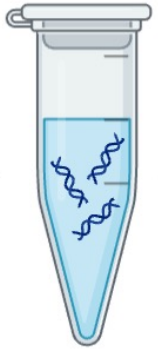

Data generation & processing

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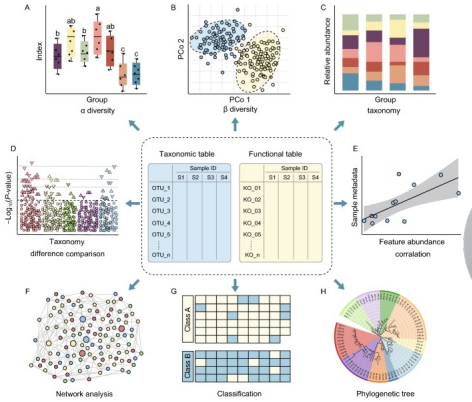

RStudio

AlphaDiversity.R | Untitled3* | Intro_R.R | 1_IgA_CoatingIndexCalculation_OT

```r
## My microbiome analyses

library(phyloseq)

setwd("/Users/apschaan/Documents/Kiel/UKSH-CAU/Classes/KMC_workshop")

taxTable <- read.csv("taxonomy_gmbc1_form.csv", header=TRUE, row.names=1)

head(taxTable)
```

11:1 (Top Level) R Script

Console | Terminal | Background Jobs

R 4.3.1 · ~/Documents/Kiel/UKSH-CAU/Classes/KMC_workshop/

```
> library(phyloseq)
> setwd("/Users/apschaan/Documents/Kiel/UKSH-CAU/Classes/KMC_workshop")
> taxTable <- read.csv("taxonomy_gmbc1_form.csv", header=TRUE, row.names=1)
> head(taxTable)
                                  Kingdom            Phylum
675c847bccbc53942ebb7b8cbb4efc4d d__Bacteria    p__Bacteroidota
da6a7cba87e0895b9cbe6037b9bd8b3b d__Bacteria    p__Bacteroidota
```

Environment | History | Connections

Import Dataset · 994 MiB · List

R · Global Environment · Taxa

Data

TaxaForHeatm…	40 obs. of 12 variables
TaxaForHeatm…	20 obs. of 2 variables

Values

taxa	"a361d6b053ce9d38182128a316635e3e"
taxa_names	chr [1:197] "2e63e8e3f9f0f102f570e7…
taxaKeep	chr [1:2174] "9492bc05eee3b253c1557…

Values

taxa	"a361d6b053ce9d38182128a316635e3e"
taxa_names	chr [1:197] "2e63e8e3f9f0f102f570e7…
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