

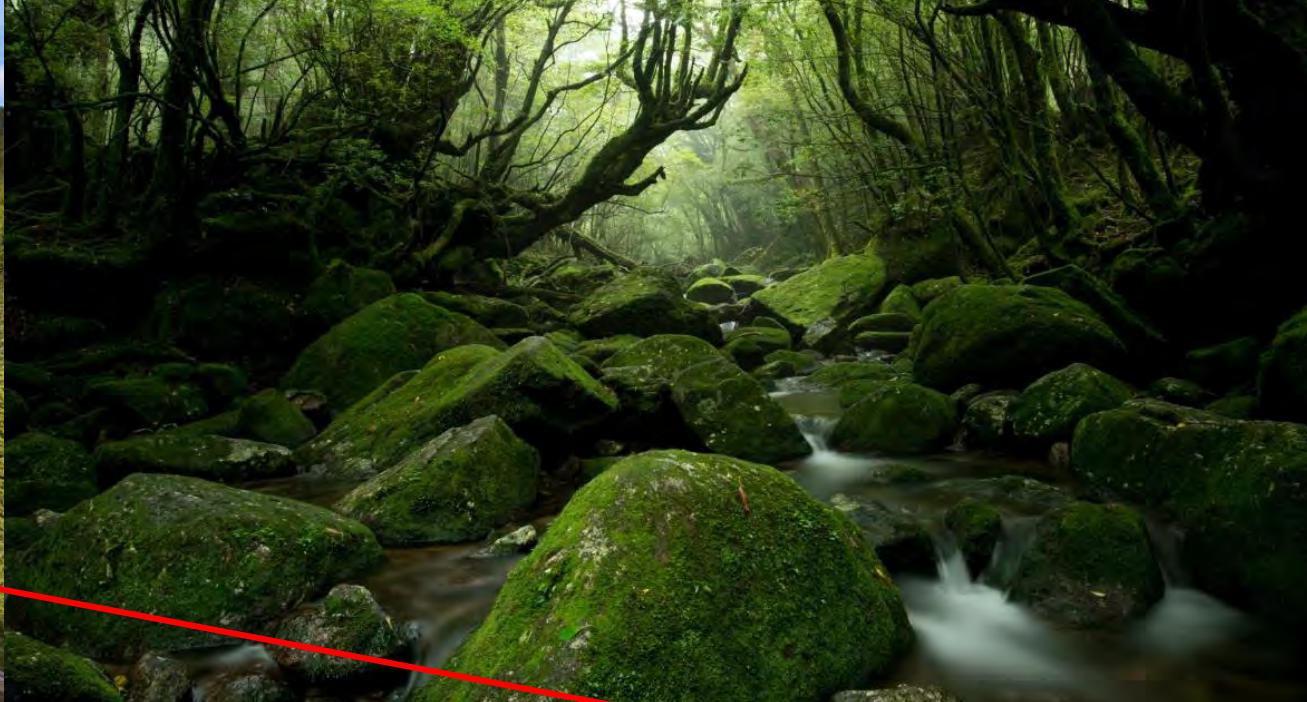
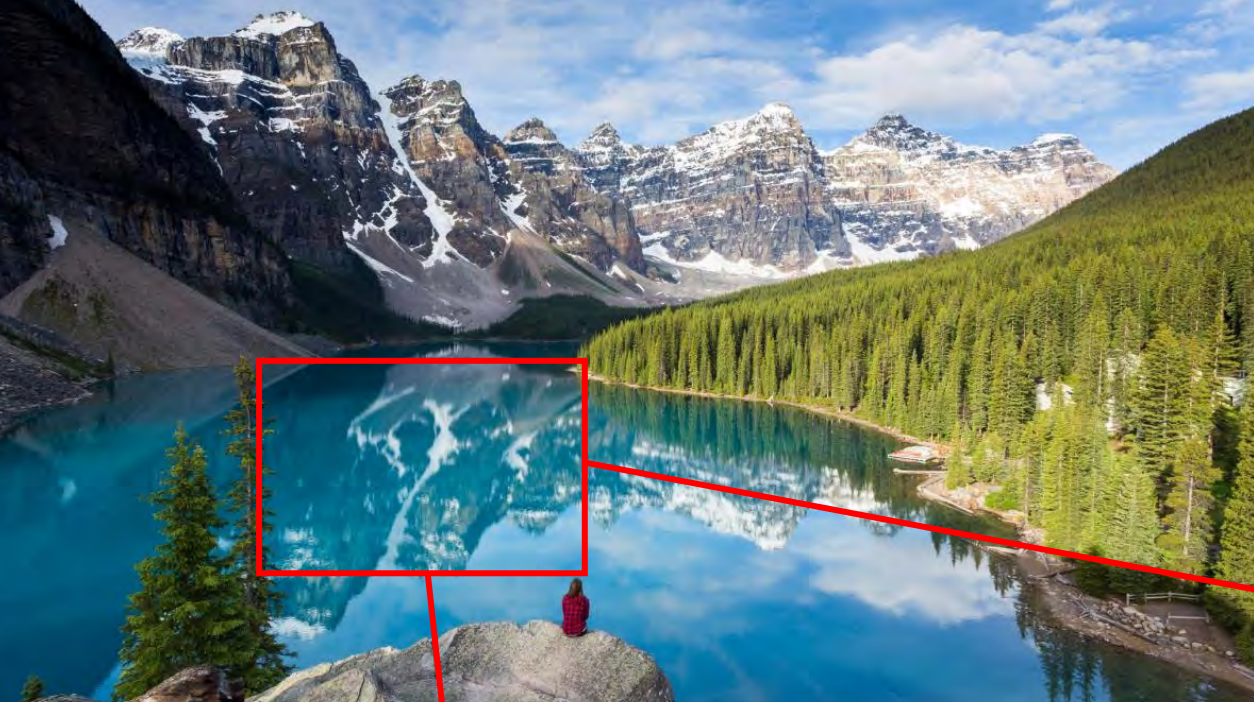
# BIOL 432

## Metabarcoding

# What is a microbiome?







 Molecular Ecology

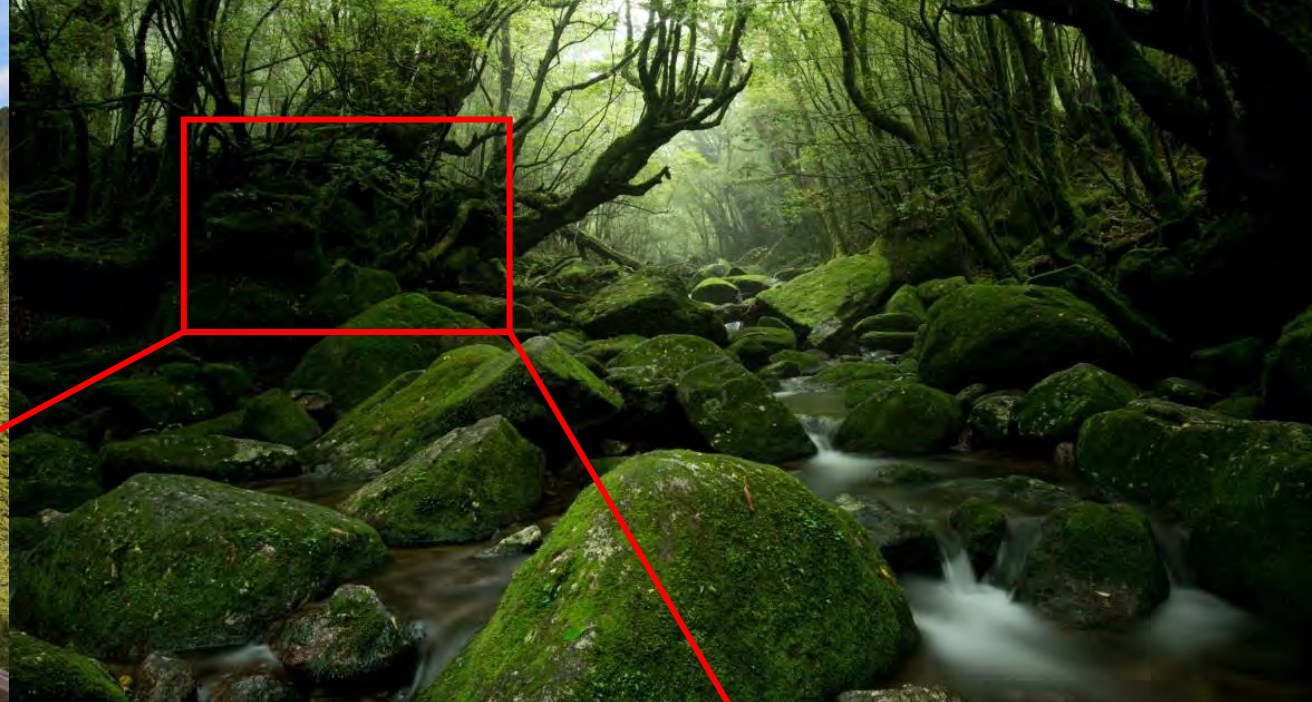
Original Article

## Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods

Bernd Hänfling , Lori Lawson Handley, Daniel S. Read, Christoph Hahn, Jianlong Li, Paul Nichols, Rosetta C. Blackman, Anna Oliver, Ian J. Winfield

First published: 20 April 2016 | <https://doi.org/10.1111/mec.13660> | Cited by:26





# ECOLOGY LETTERS

 Free Access

## The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems

Marcel G. A. Van Der Heijden , Richard D. Bardgett, Nico M. Van Straalen

First published: 29 November 2007 | <https://doi.org/10.1111/j.1461-0248.2007.01139.x>

| Cited by:1184



# Bacterial populations and metabolites in the feces of free roaming and captive grizzly bears

Clarissa Schwab, Bogdan Cristescu, Mark S. Boyce, Gordon B. Stenhouse, and Michael Gänzle



# Studying the microbiology of the indoor environment

Scott T Kelley ✉ and Jack A Gilbert

*Genome Biology* 2013 14:202

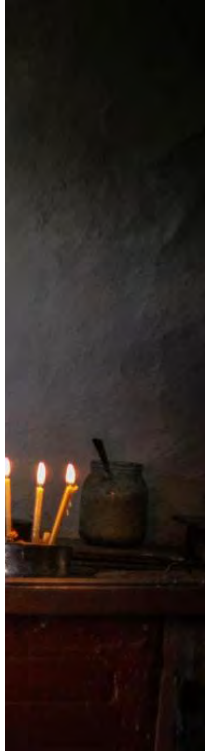
<https://doi.org/10.1186/gb-2013-14-2-202> | © BioMed Central Ltd 2013

Published: 28 February 2013









## [HTML] A human gut microbial gene catalogue established by metagenomic sequencing

J Qin, [R Li](#), [J Raes](#), [M Arumugam](#), KS Burgdorf... - nature, 2010 - nature.com

... As part of the MetaHIT (**Metagenomics** of the **Human** Intestinal Tract) project, we collected ... groups plus novel gene families (>20 proteins) recovered from the **metagenome** (top) ... Deep **metagenomic sequencing** provides the opportunity to explore the existence of a common set of ...

☆ [🔗](#) Cited by 5685 [Related articles](#) [All 23 versions](#)

## Metagenomic analysis of the human distal gut microbiome

SR Gill, [M Pop](#), RT DeBoy, PB Eckburg... - ..., 2006 - science.sciencemag.org

... size of the contigs) of genome assemblies generated from a **metagenomics** project can ... 1. Comparison of random **metagenome** reads with completed genome of Bifidobacterium longum and ... provide a benchmark for interpreting the functional predictions from **metagenomic** data ...

☆ [🔗](#) Cited by 3168 [Related articles](#) [All 32 versions](#)

## [HTML] Structure, function and diversity of the healthy human microbiome

[C Huttenhower](#), D Gevers, [R Knight](#), S Abubucker... - Nature, 2012 - nature.com

... d, e, The population size and **sequencing** depths of the HMP have well defined the ... of the **human** microbiome was provided by identifying unique marker **sequences** in **metagenomic** data 11 ... for a small subset of stool samples with 454 pyrosequencing **metagenomics** data using ...



# Marine viruses — major players in the global ecosystem

Curtis A. Suttle

*Nature Reviews Microbiology* **5**, 801–812 (2007)

doi:10.1038/nrmicro1750

[Download Citation](#)

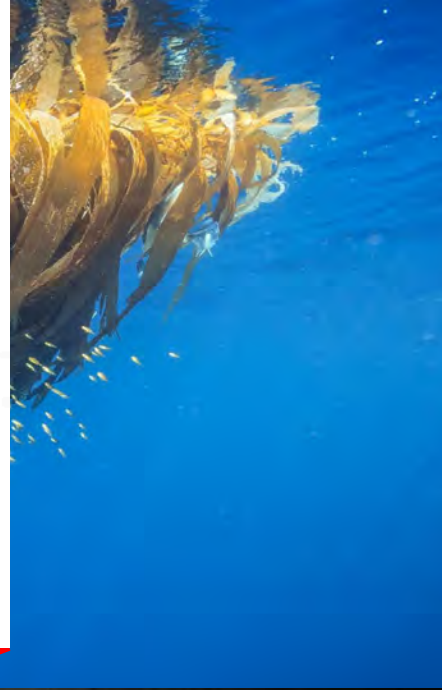
Published: 01 October 2007

# Sedimentary DNA from a submerged site reveals wheat in the British Isles 8000 years ago

Oliver Smith<sup>1</sup>, Garry Momber<sup>2</sup>, Richard Bates<sup>3</sup>, Paul Garwood<sup>4</sup>, Simon Fitch<sup>5</sup>, Mark Pallen<sup>6,\*</sup>, Vincent Gaffn...

+ See all authors and affiliations

*Science* 27 Feb 2015:  
Vol. 347, Issue 6225, pp. 998-1001  
DOI: 10.1126/science.1261278





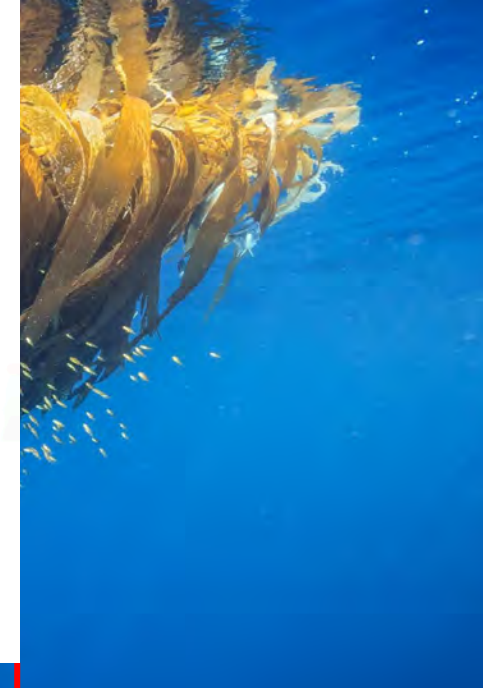
 Molecular Ecology banner

INVITED REVIEW |  Free Access

# Who is eating what: diet assessment using next generation sequencing

FRANCOIS POMPANON, BRUCE E. DEAGLE, WILLIAM O. C. SYMONDSON, DAVID S. BROWN, SIMON N. JARMAN, PIERRE TABERLET

First published: 15 December 2011 | <https://doi.org/10.1111/j.1365-294X.2011.05403.x>  
| Cited by:304



# Identifying fungi and bacteria





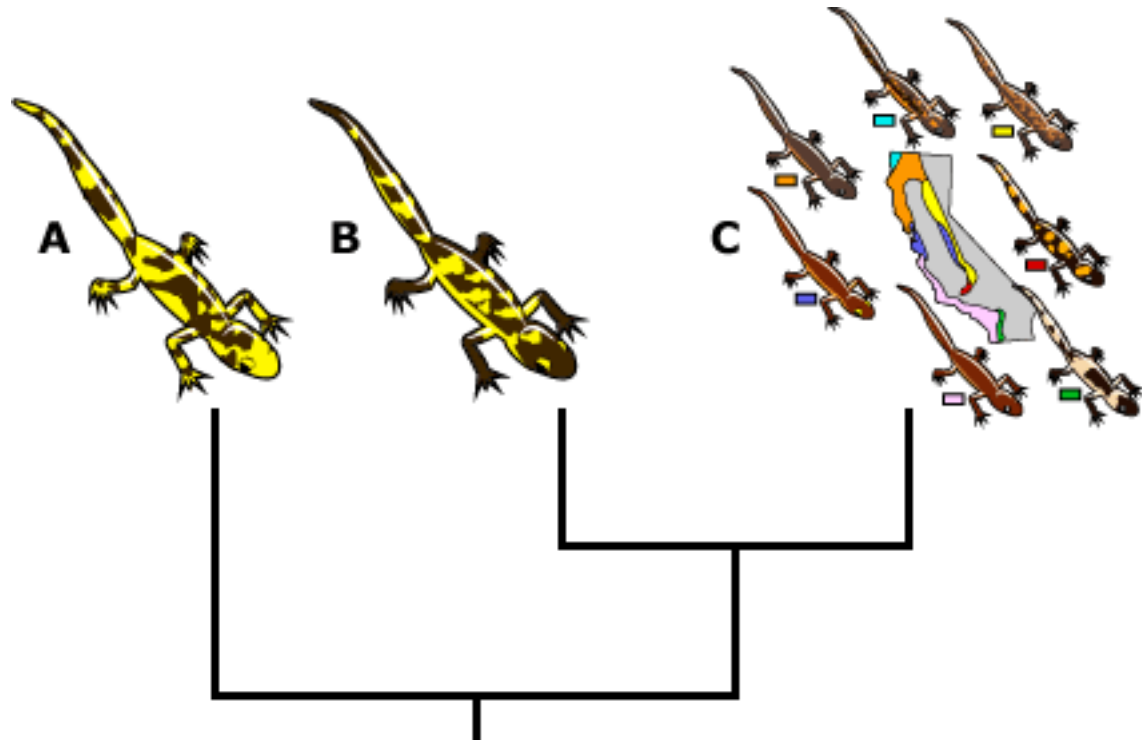
Less than 2% of bacteria  
can be cultured in the  
laboratory

# What is a species?

"Species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups."

-Ernst Mayr

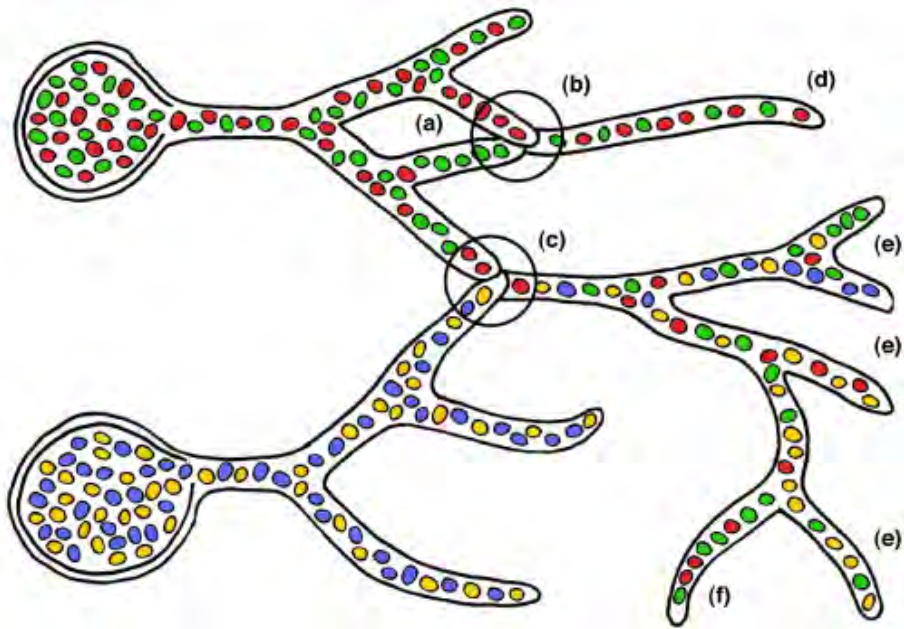
# The phylogenetic species concept



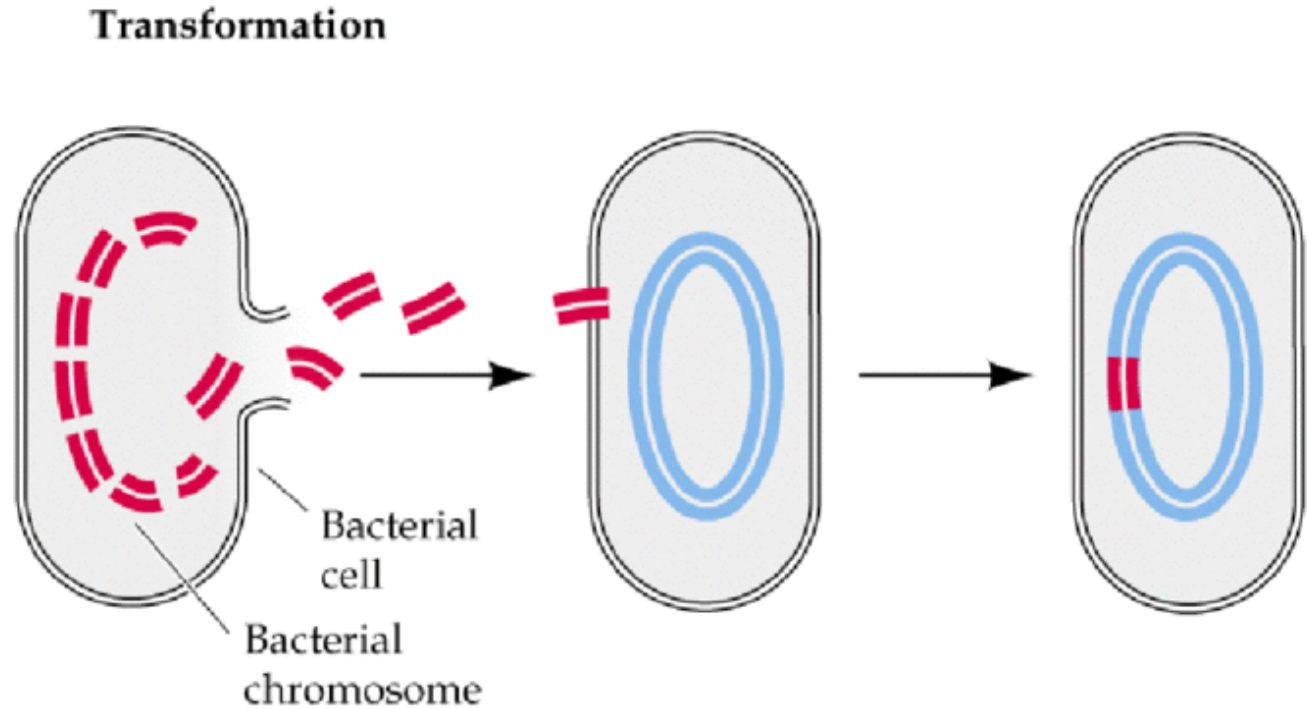


# The phylogenetic species concept

Fungal genetics:



Bacterial genetics:



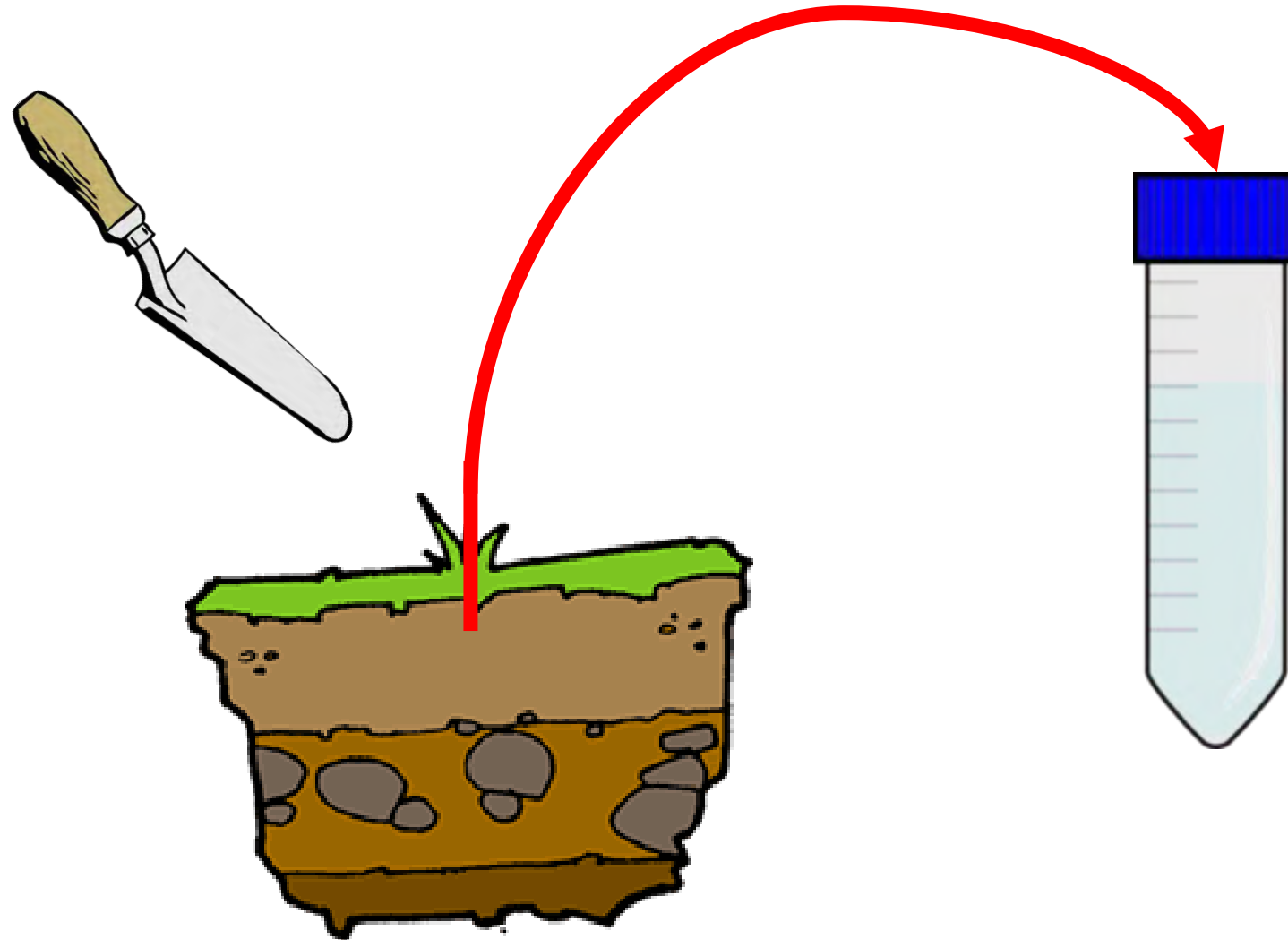
## Whole Shotgun Metagenome Sequencing

- DNA extracted and sequenced directly
  - Attempt to reconstruct (partial) individual genomes
  - Computational demanding
  - Higher taxonomic resolution
  - More expensive
- Metabarcoding
    - PCR DNA to sequence one or a few genes
    - Computationally simple
    - Lower taxonomic resolution
    - Less expensive

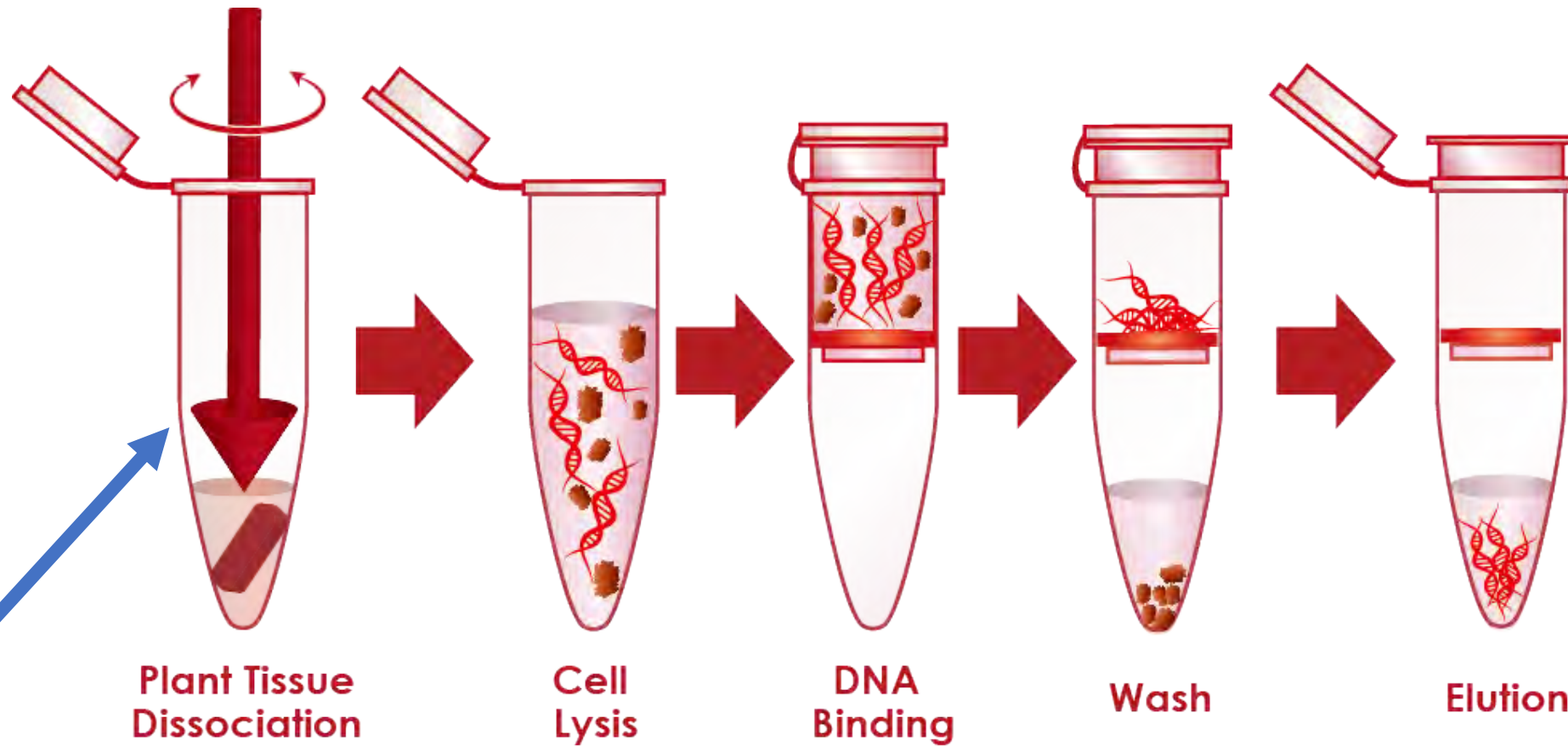
# Metabarcoding

Microbiome analysis of high-throughput sequencing data

# Step 1: collect the sample



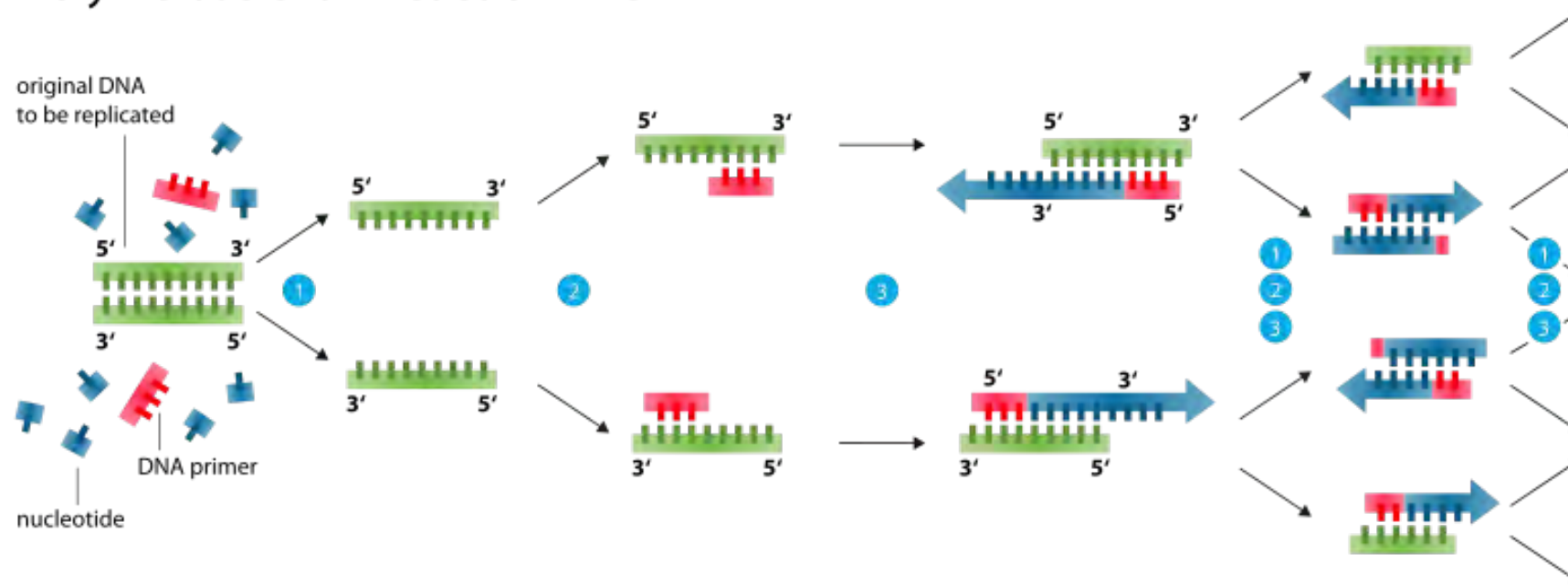
# Step 2: Extract DNA



NOTE: Bacterial Cell-wall dissociation (microbeads or chemical disruption)

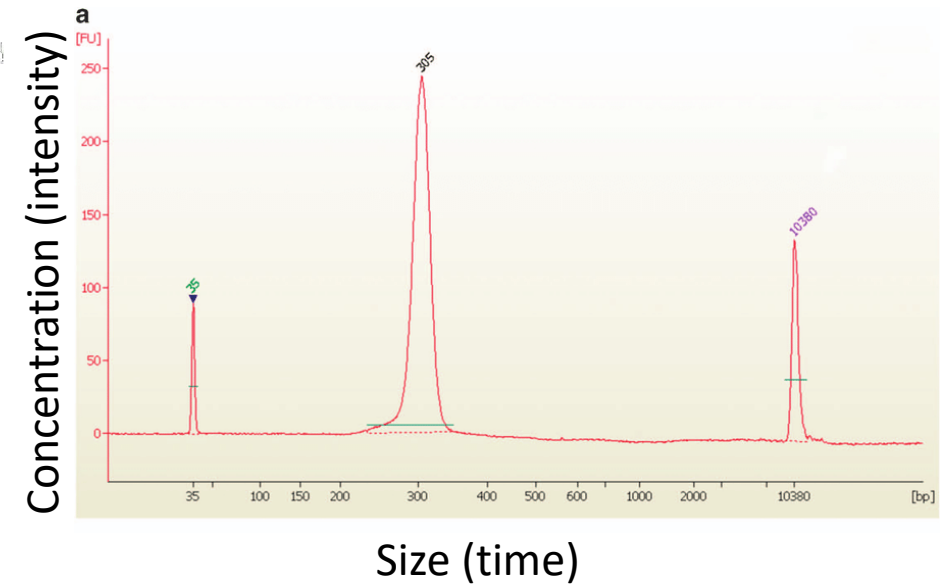
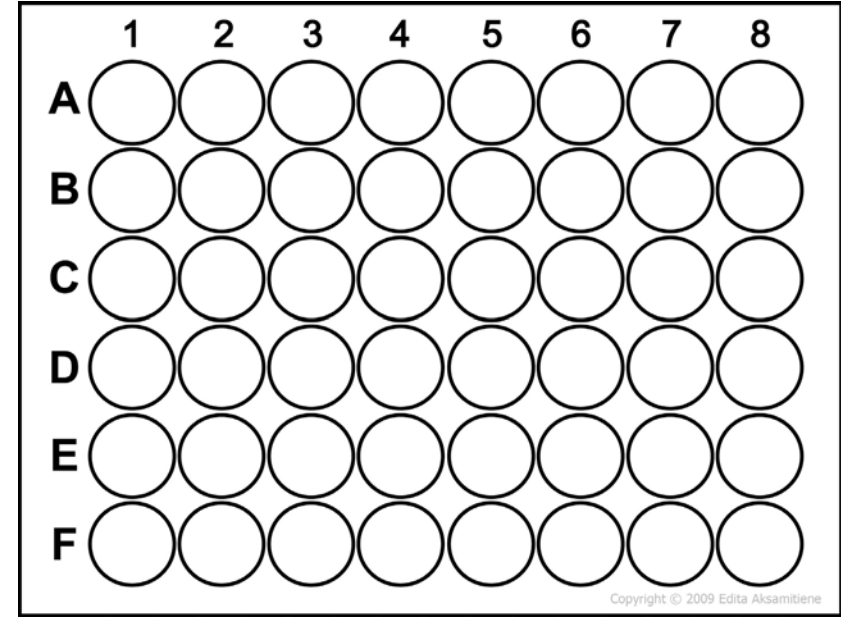
# Step 3: PCR (add a barcode? An Illumina attachment?)

## Polymerase chain reaction - PCR



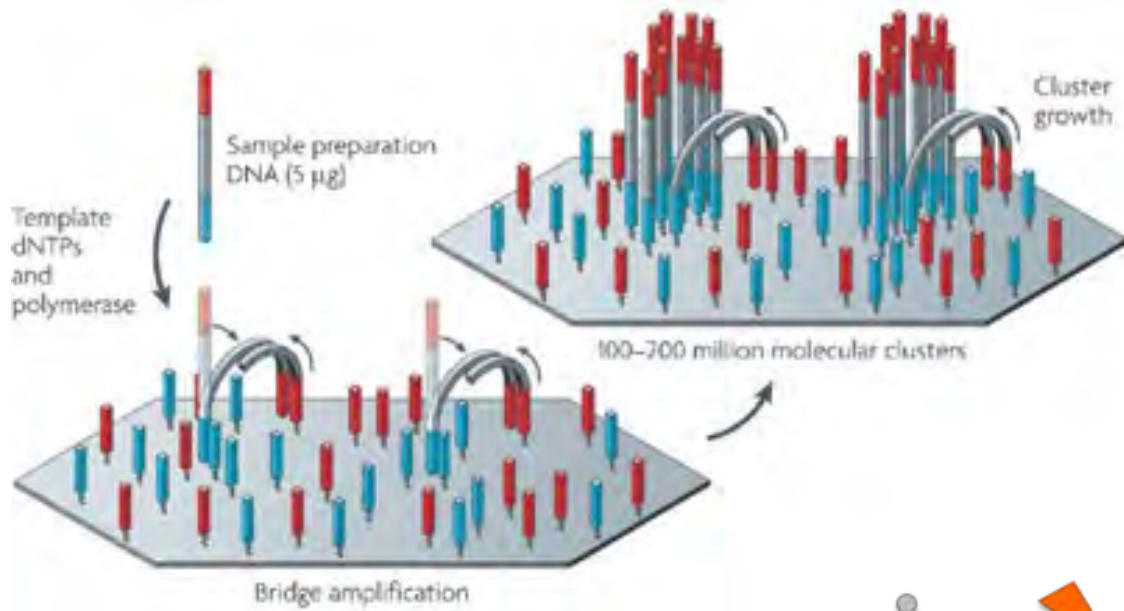
- 1 **Denaturation** at 94-96°C
- 2 **Annealing** at ~68°C
- 3 **Elongation** at ca. 72 °C

# Step 4: Pooling & Quality check

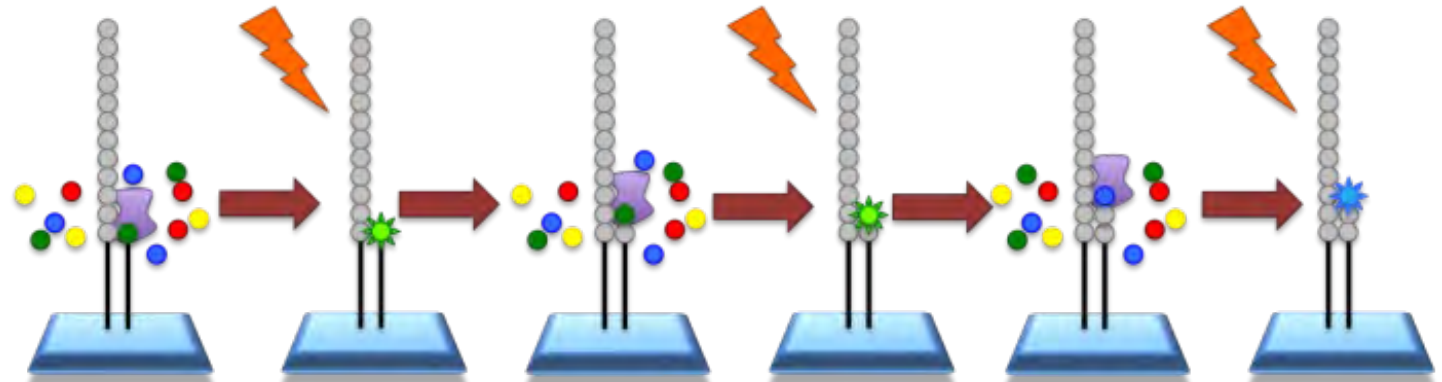


# Sequencing (e.g. Illumina MiSeq)

i.



ii.

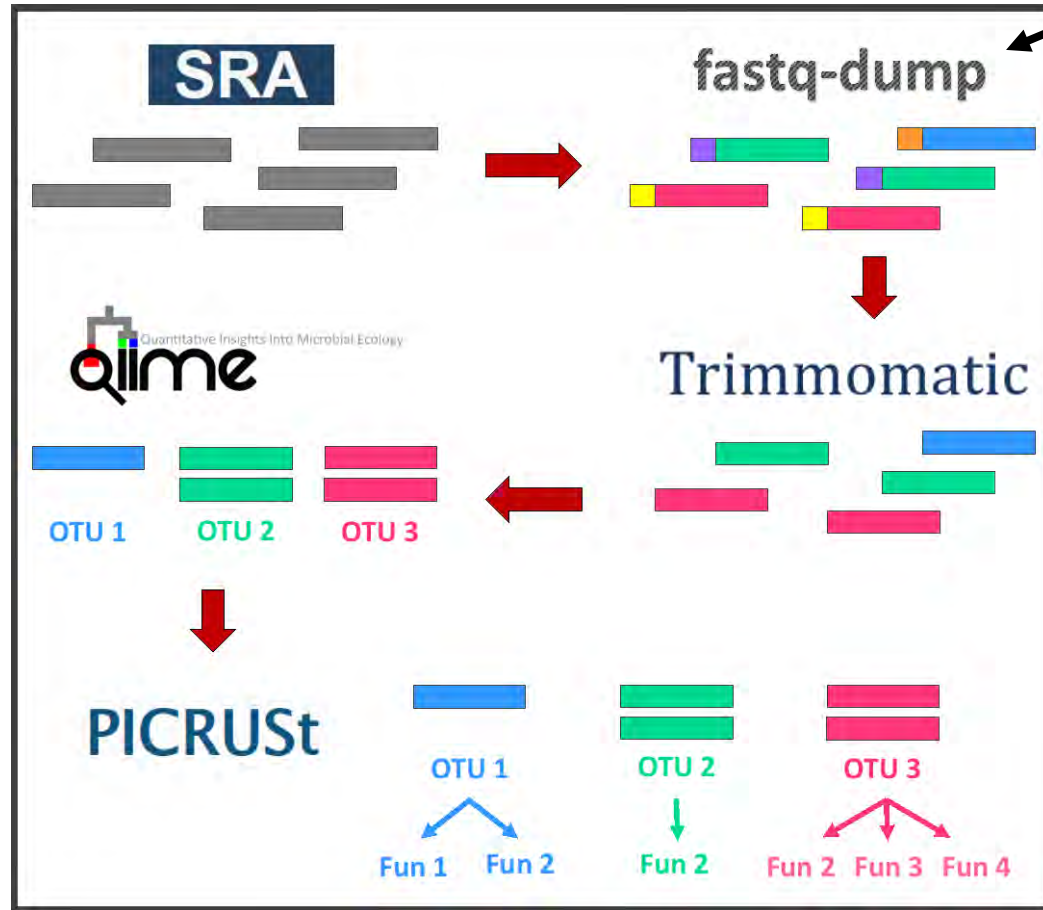




# How do we compare samples?

Analysis 'pipeline' overview

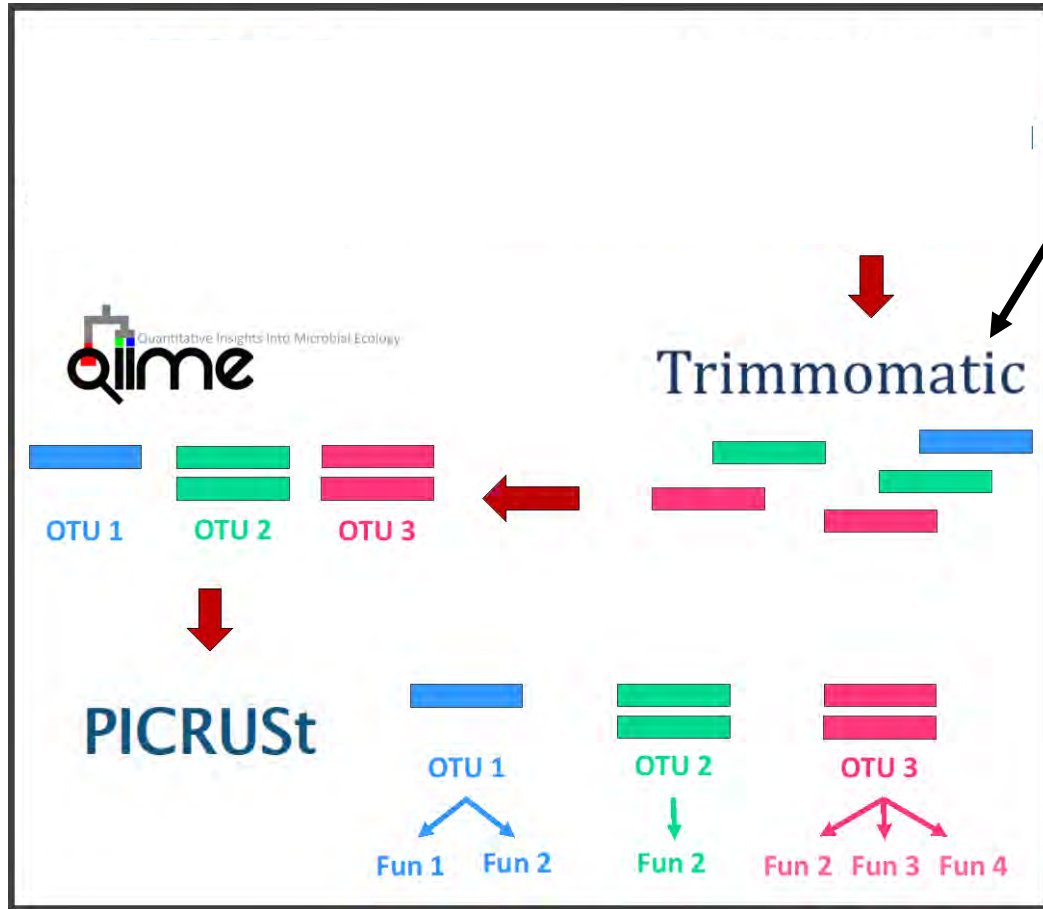
# Example of a pipeline



Steps 1 & 2:

- Find sequences from the NCBI SRA (sequence read archive)
- Download to your own computer

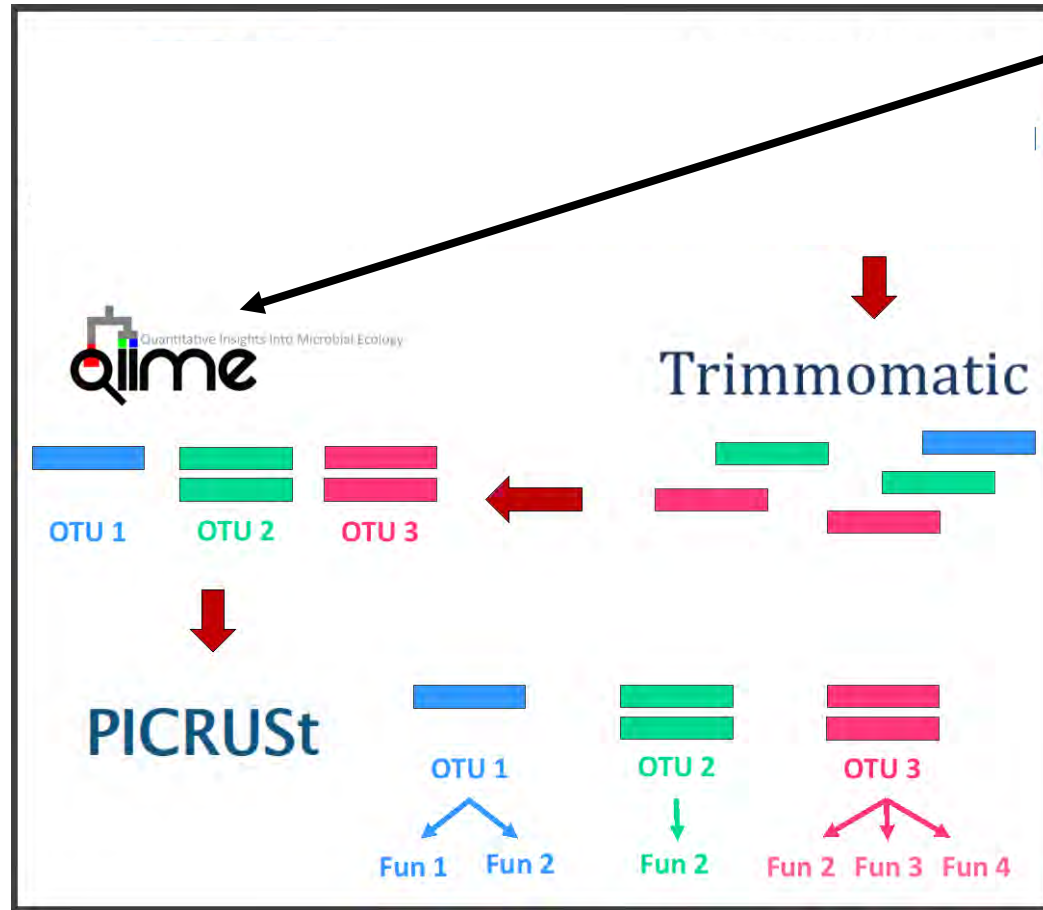
# Example of a pipeline



Step 3:

- Cut adapter sequences (sequencing primers)
- Cut low-quality reads (i.e. sequences)  
OR
- Cut all reads to a specified length
- Remove low-quality reads

# Example of a pipeline



Step 4:

- Assign Operational Taxonomic Units (OTUs) based on sequence similarity. Grouped by sequence similarity cutoff (e.g. 95, 98, 99%)

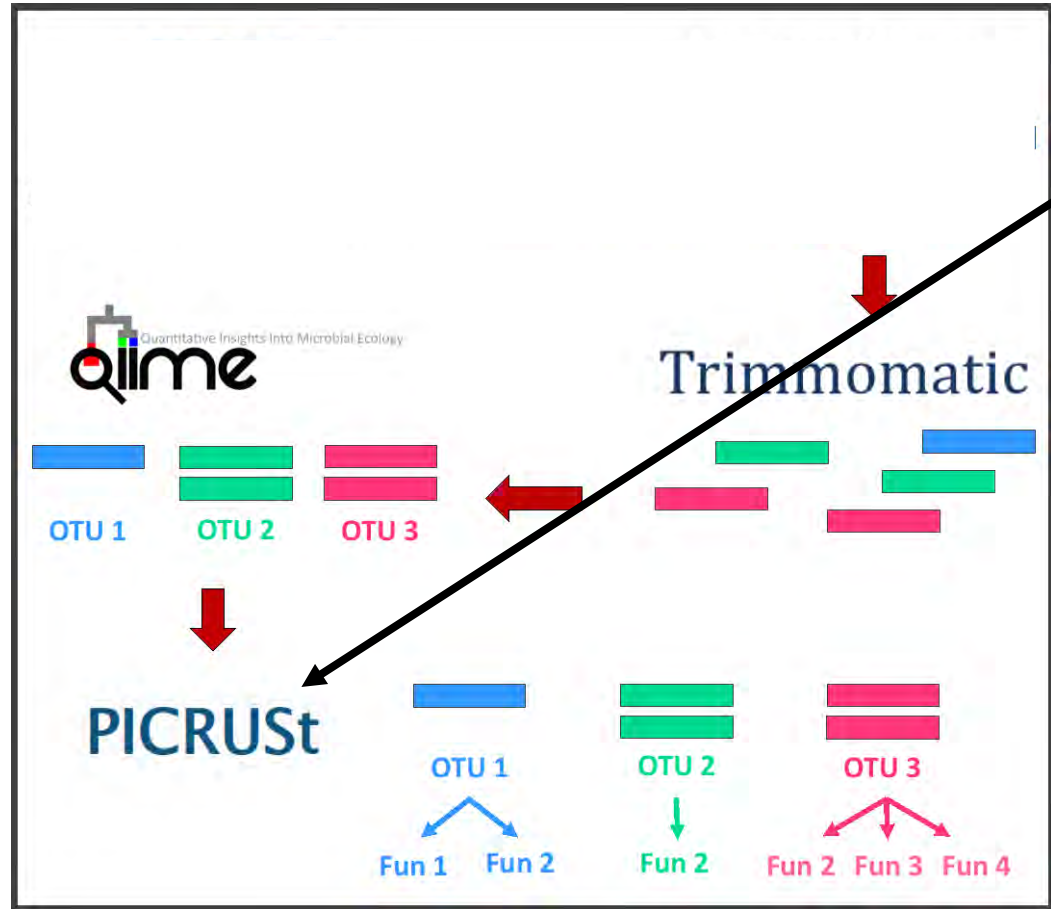
OR

- Amplicon Sequence Variants (ASVs). Each unique sequence is retained.
- Assign a “species” by comparing the sequence to a database (e.g. NCBI BLAST)

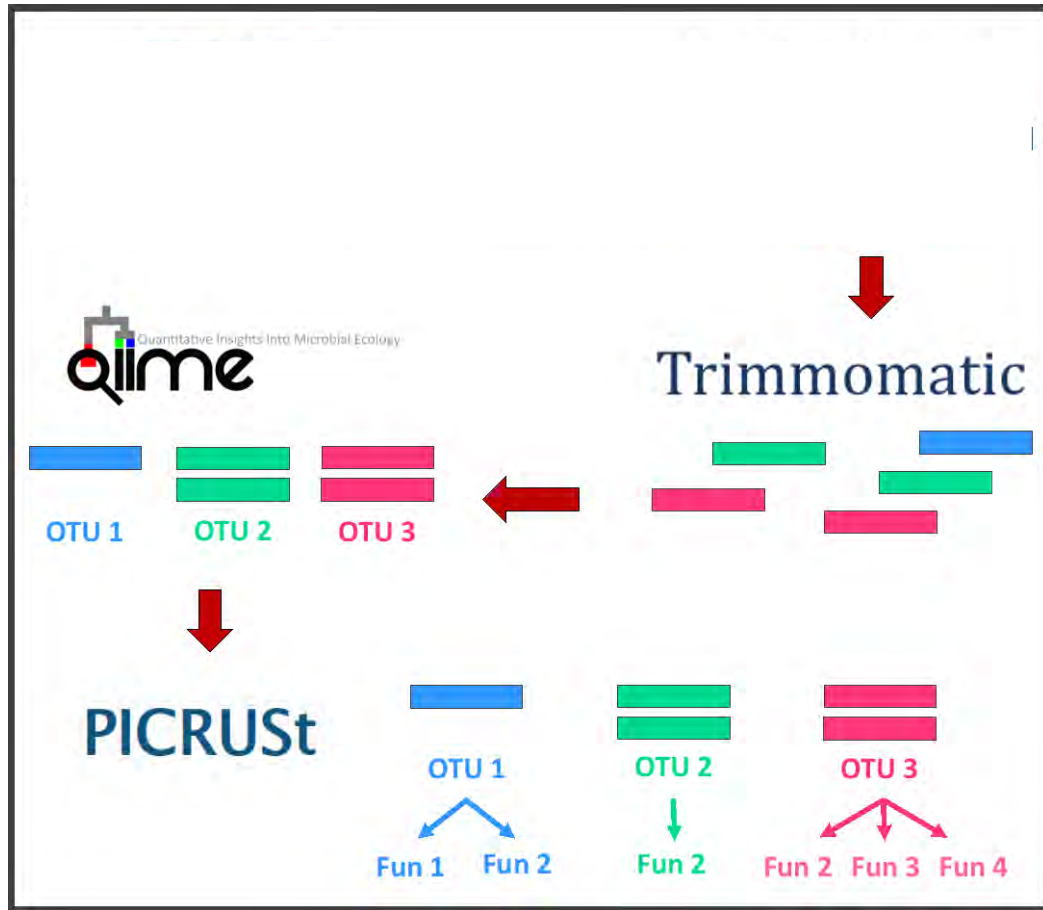
# Example of a pipeline

Step 5:

Assign a "function" to the OTU



# Example of a pipeline



Step 6:

Analyze the species communities!

Questions:

How similar/different are the samples?

What are the main taxa that are shared or different?

To answer these questions, use R!



```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("dada2", version = "3.10")
```

Dada2 Main Page:

<https://benjjneb.github.io/dada2/index.html>

Dada2 Tutorial:

<https://benjjneb.github.io/dada2/tutorial.html>