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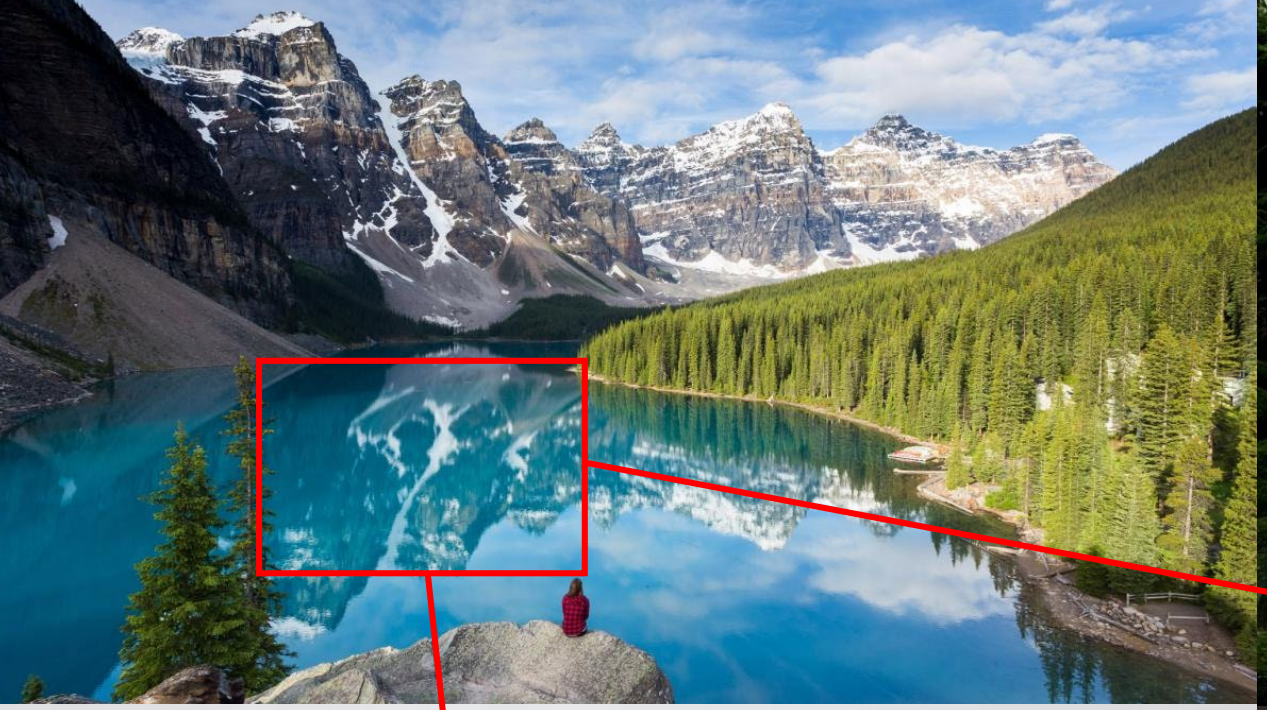
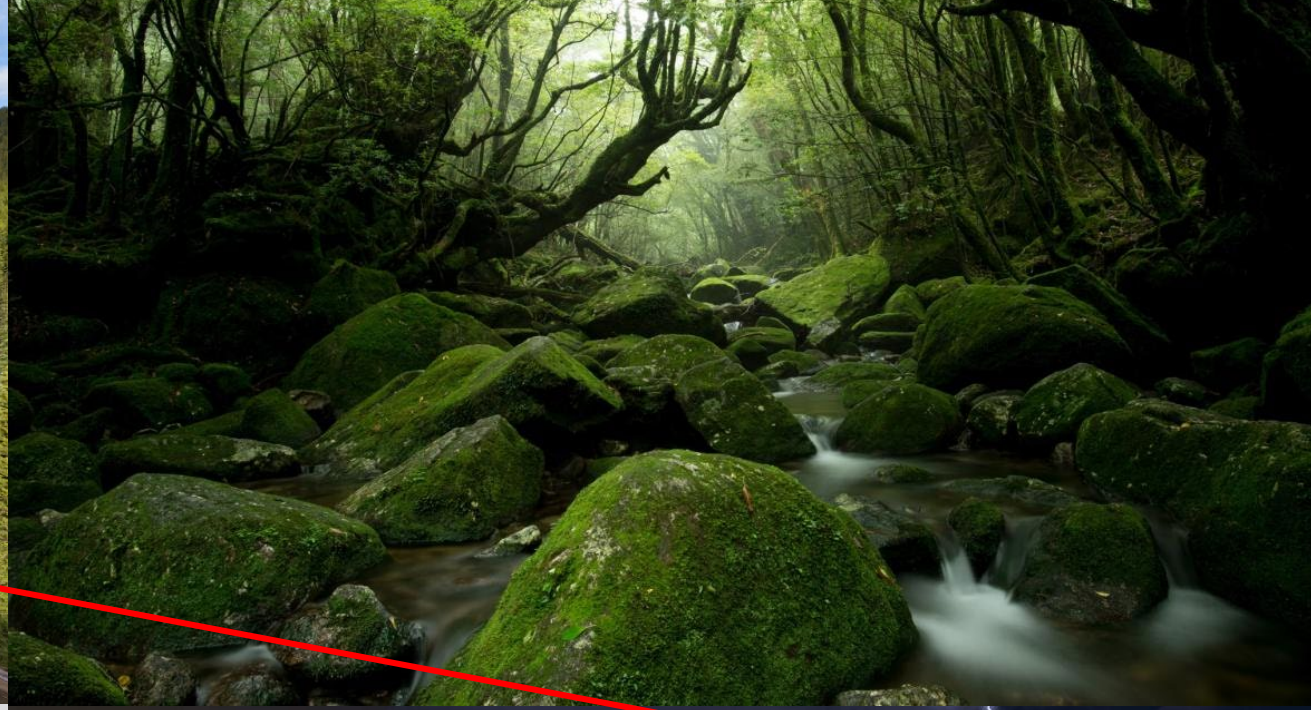
BIOL 432

Metabarcoding

What is a microbiome?





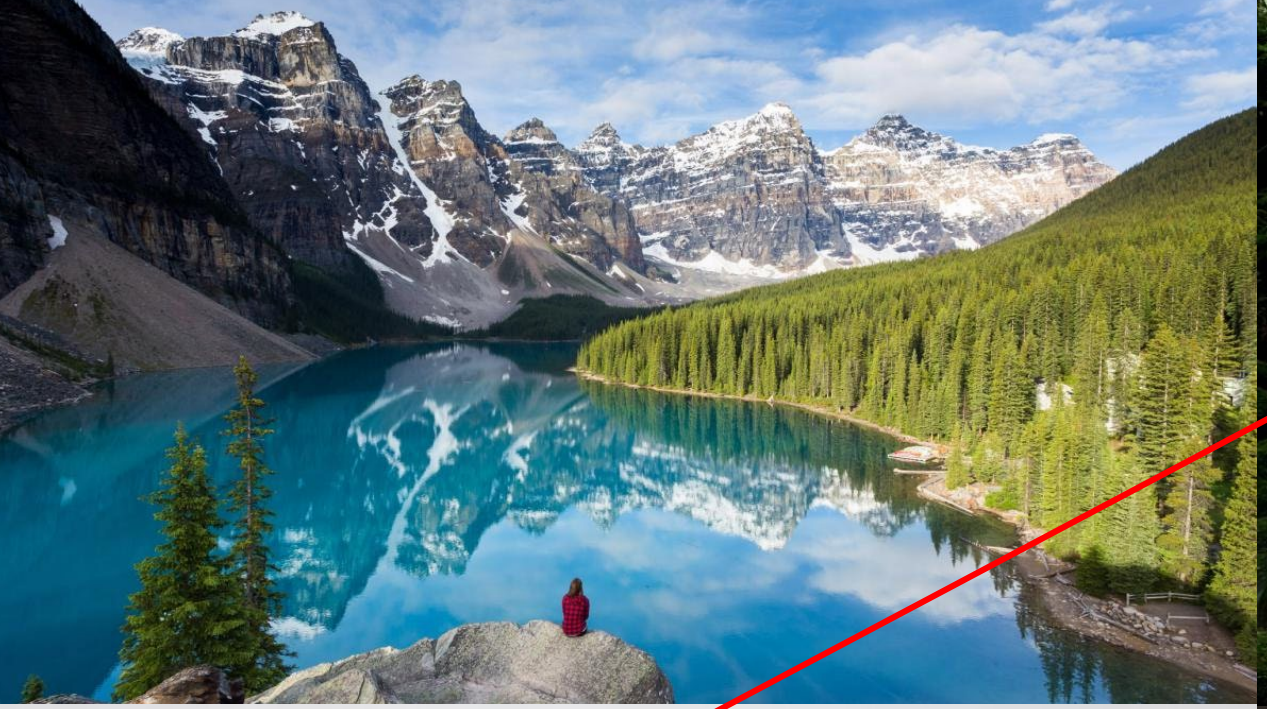
Molecular Ecology

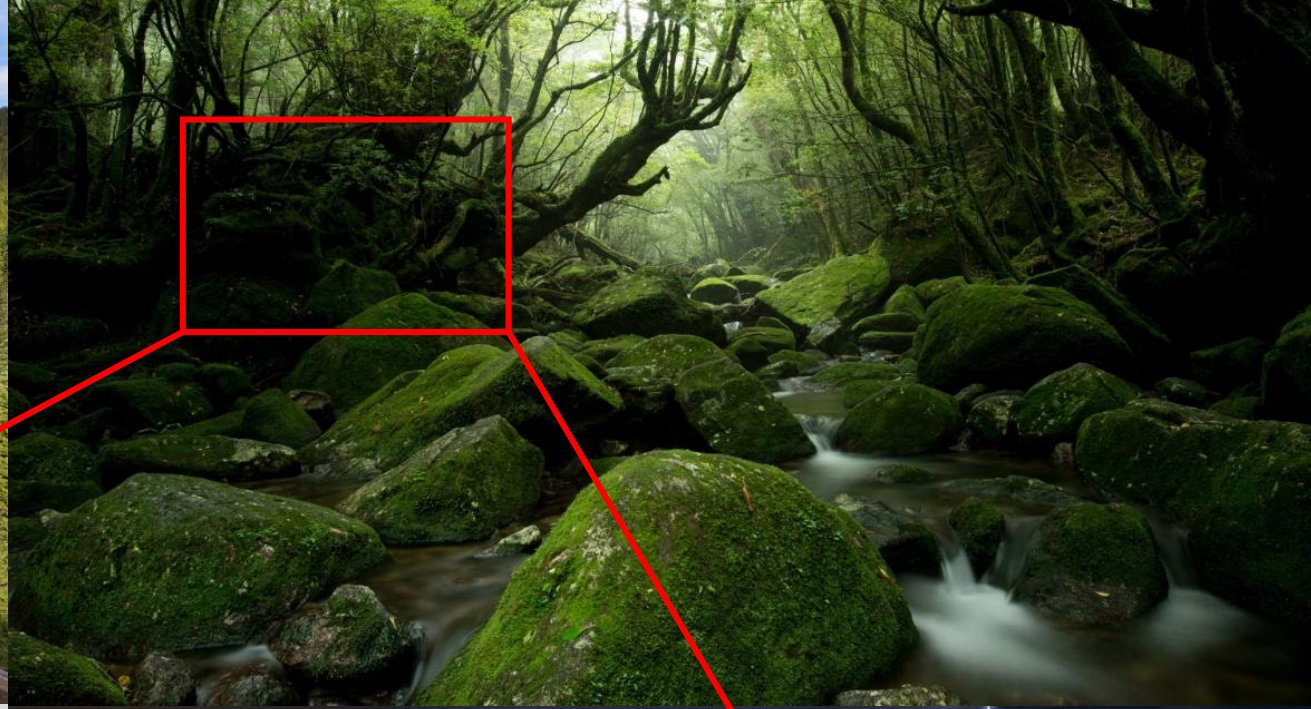
Original Article

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

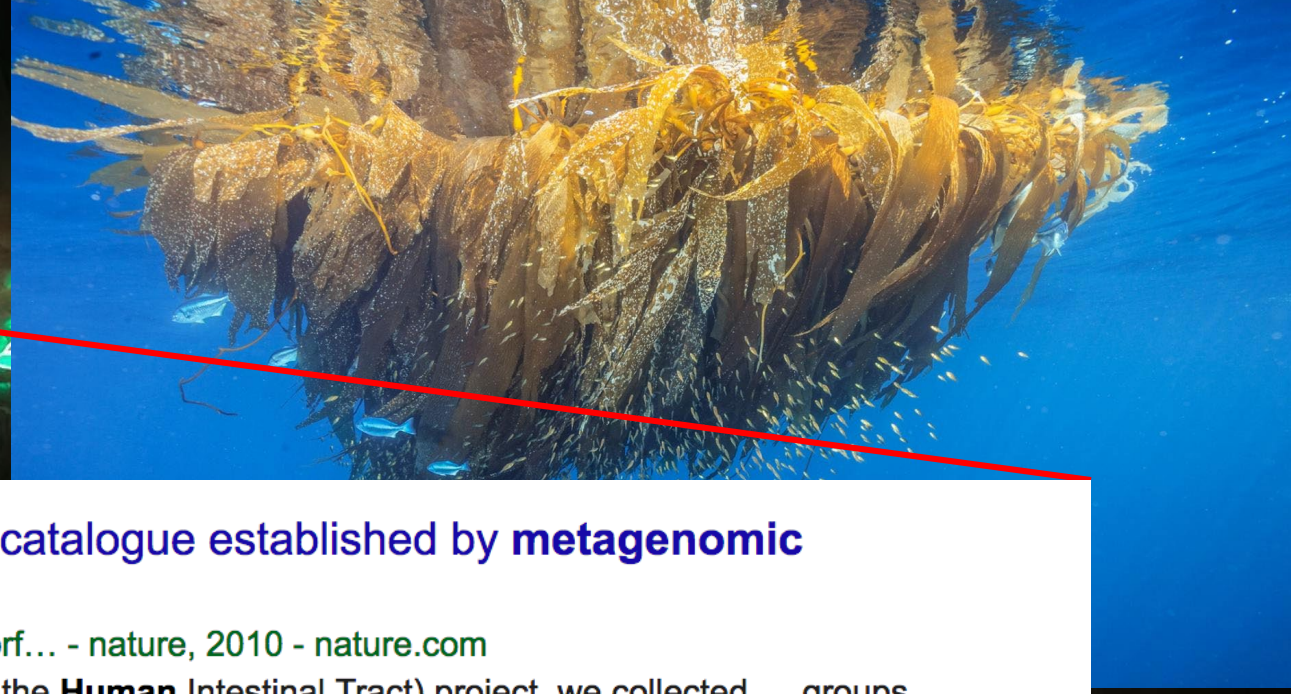
Bacterial populations and metabolites in the feces of free roaming and captive grizzly bears

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 …

☆ 99 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 …

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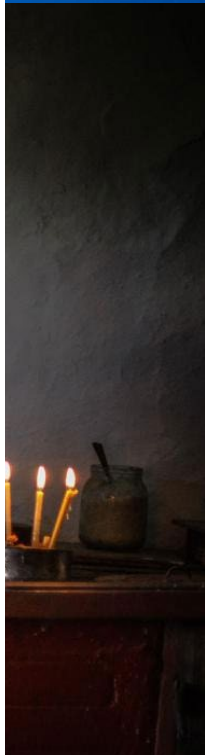
[HTML] Structure, function and diversity of the **healthy human** microbiome

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

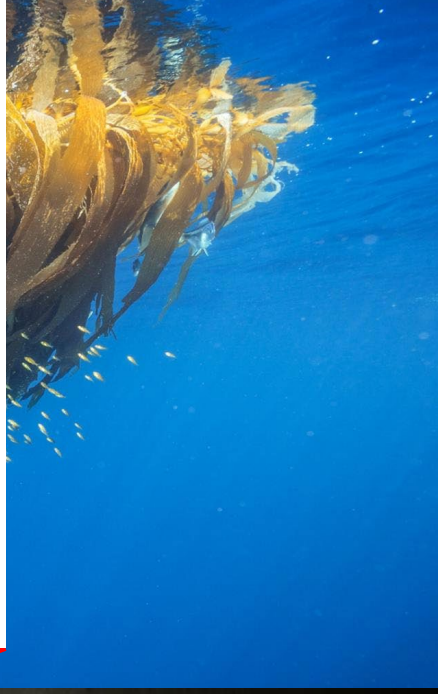
Published: 01 October 2007

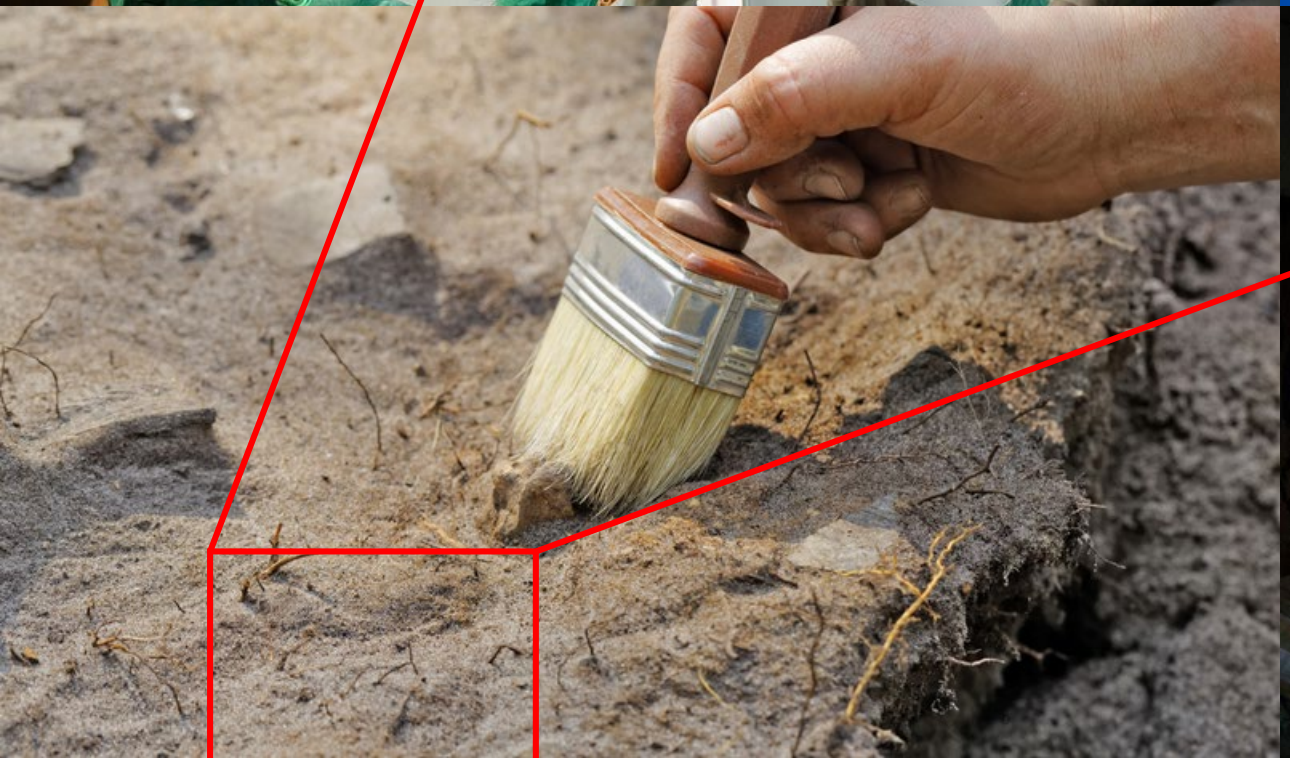
Sedimentary DNA from a submerged site reveals wheat in the British Isles 8000 years ago

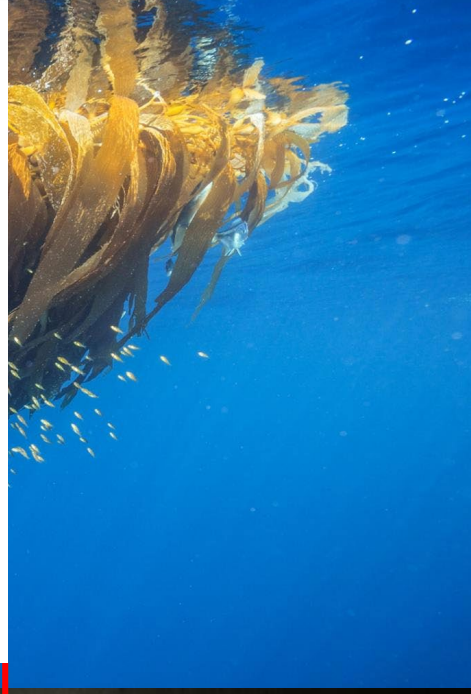
Oliver Smith[1], Garry Momber[2], Richard Bates[3], Paul Garwood[4], Simon Fitch[5], Mark Pallen[6,*], Vincent Gaffn…

+ 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

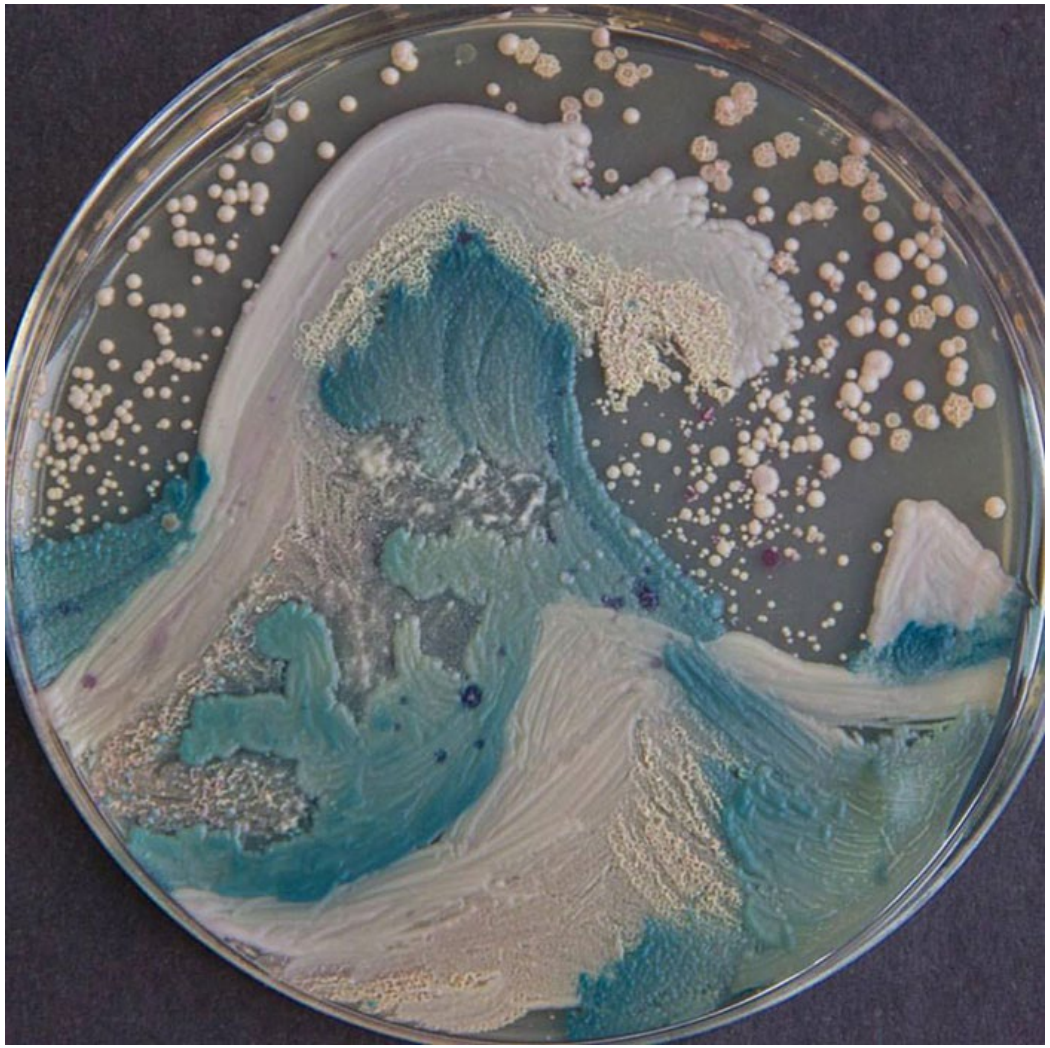
Who is eating what: diet assessment using next generation sequencing

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

Microbial cultures



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

Wilson, Weightman & Wade. 1997. Med Microbiol.

What is a species?

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

-Ernst Mayr

The phylogenetic species concept

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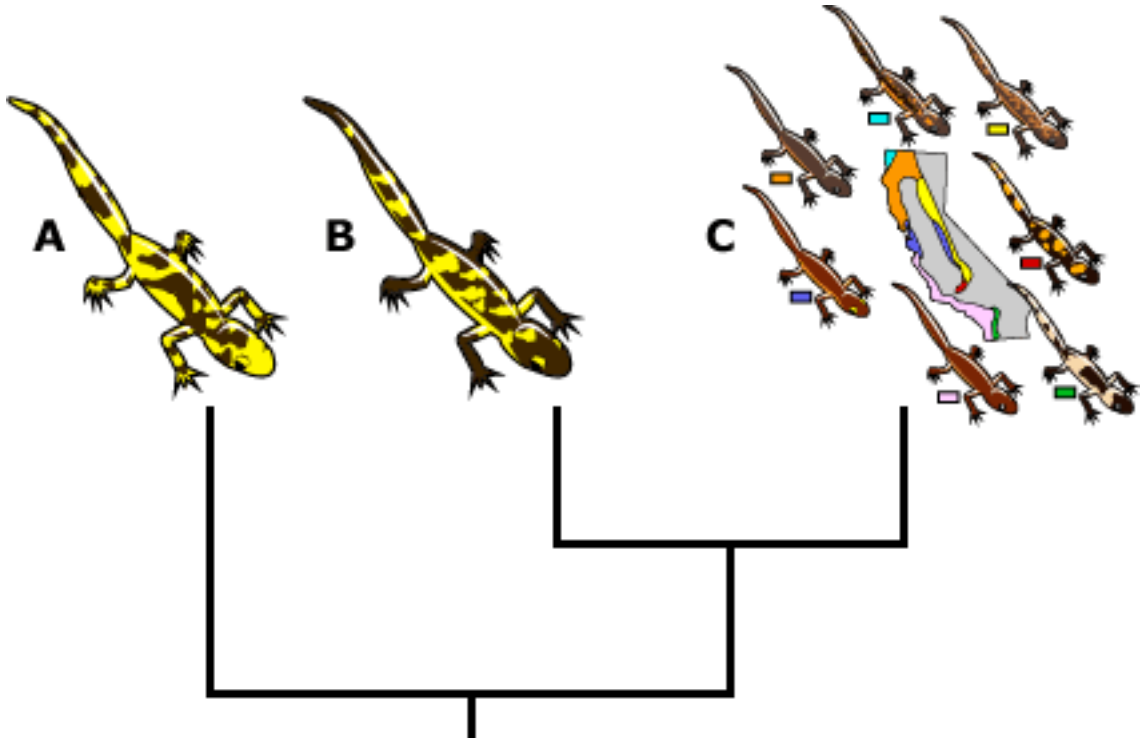

The phylogenetic species concept

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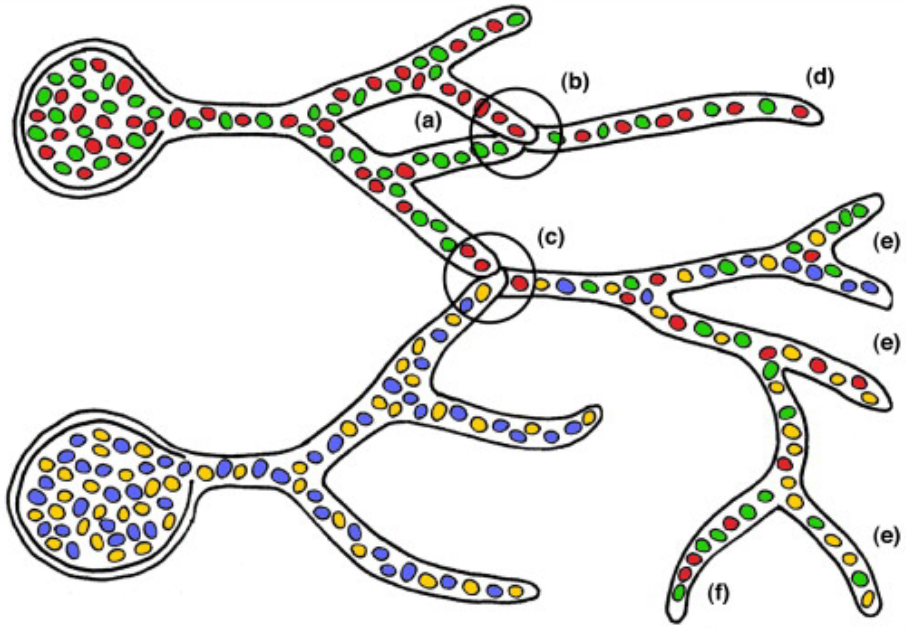
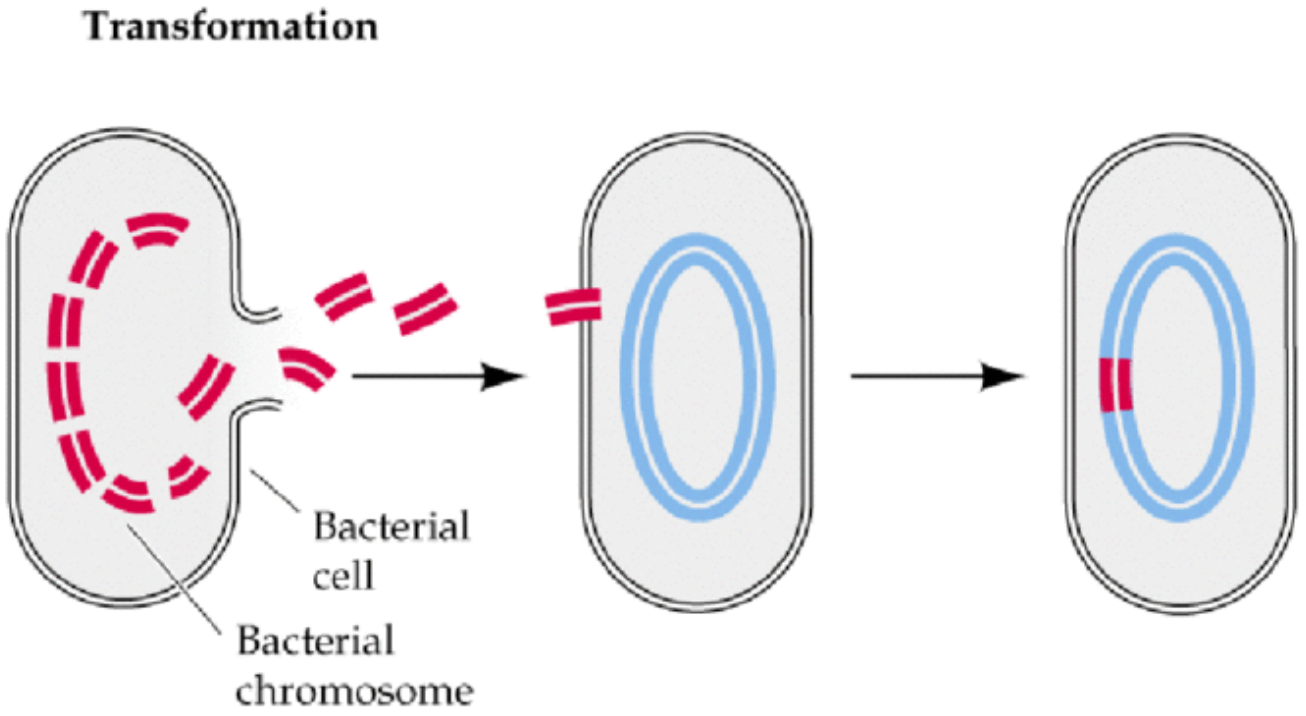
Fungal genetics:



Bacterial genetics:



Permission pending from Sinaur Associates, Inc.

Two methods for microbiome sequencing

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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

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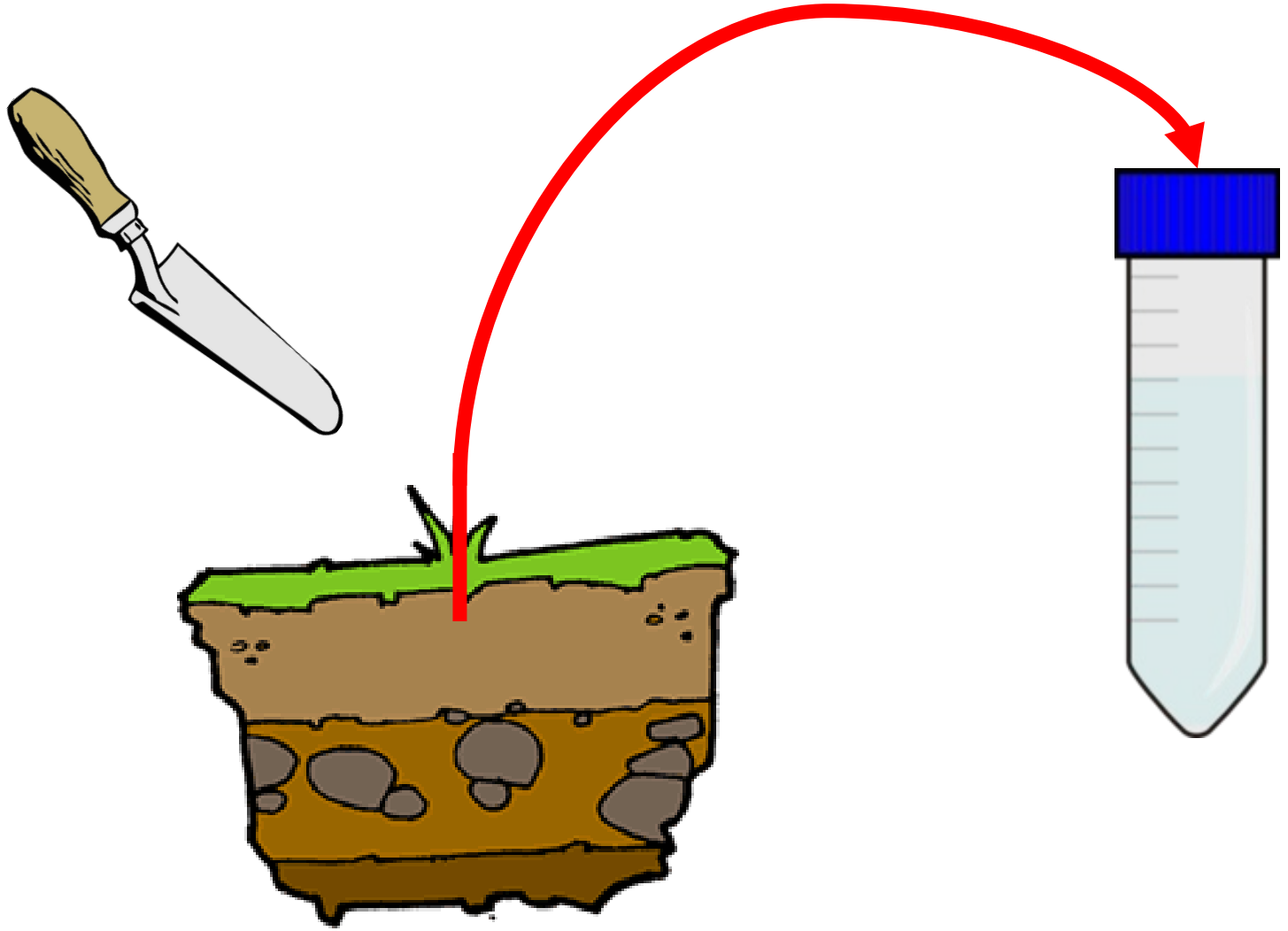
Metabarcoding

Microbiome analysis of high-throughput sequencing data

Step 1: collect the sample



Step 2: Extract DNA

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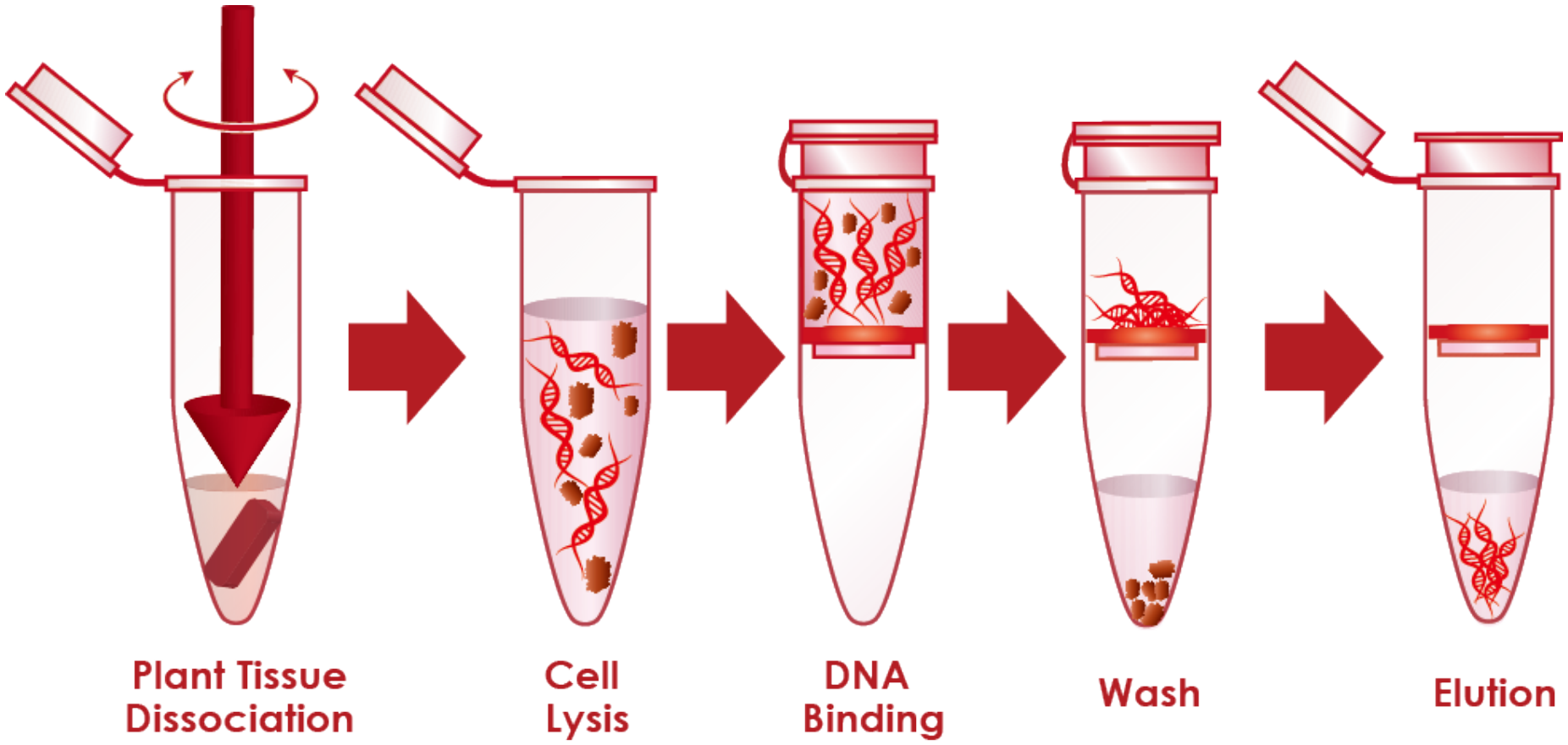

Step 3: PCR (add a barcode? An Illumina attachment?)

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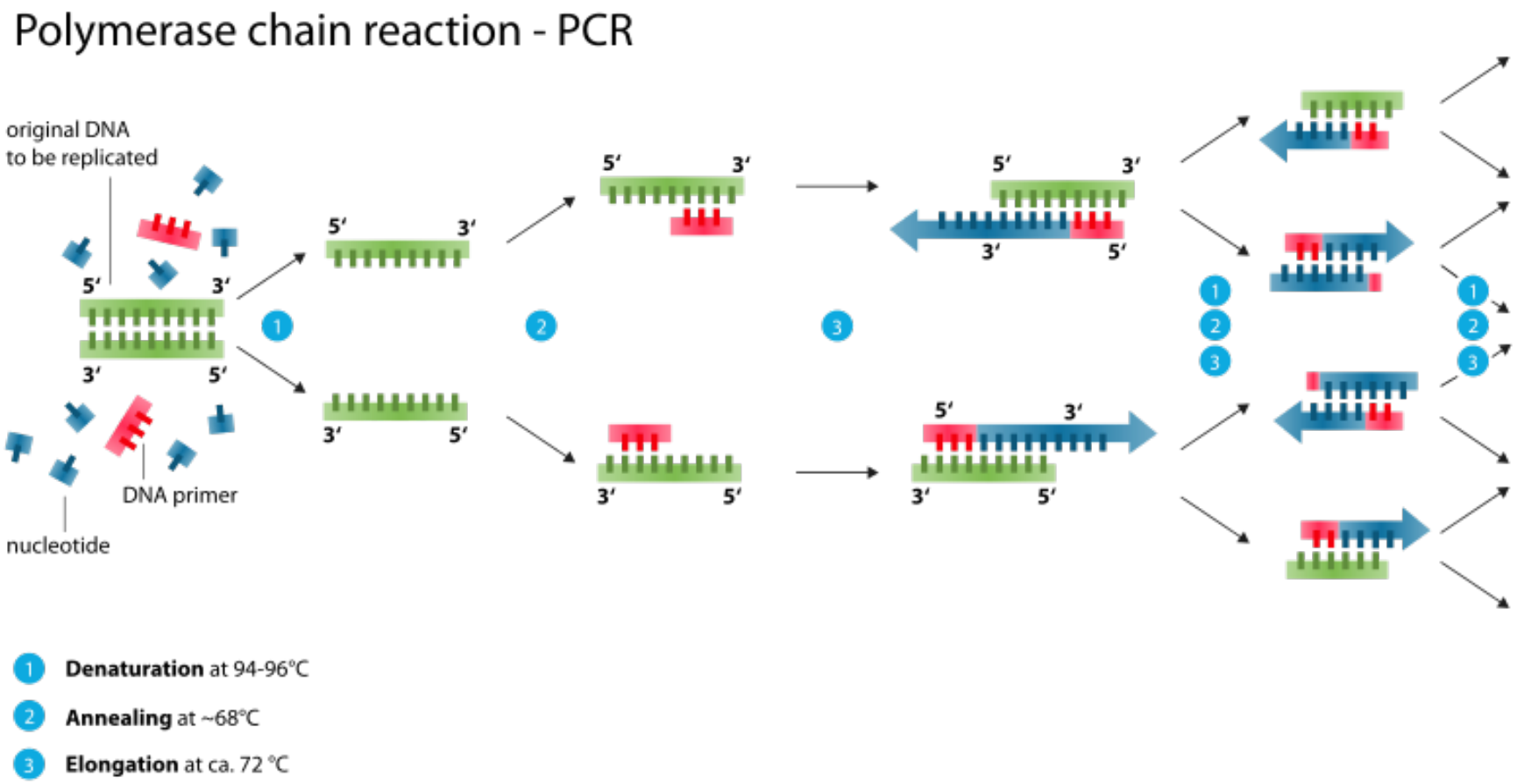

Step 4: Pooling & Quality check

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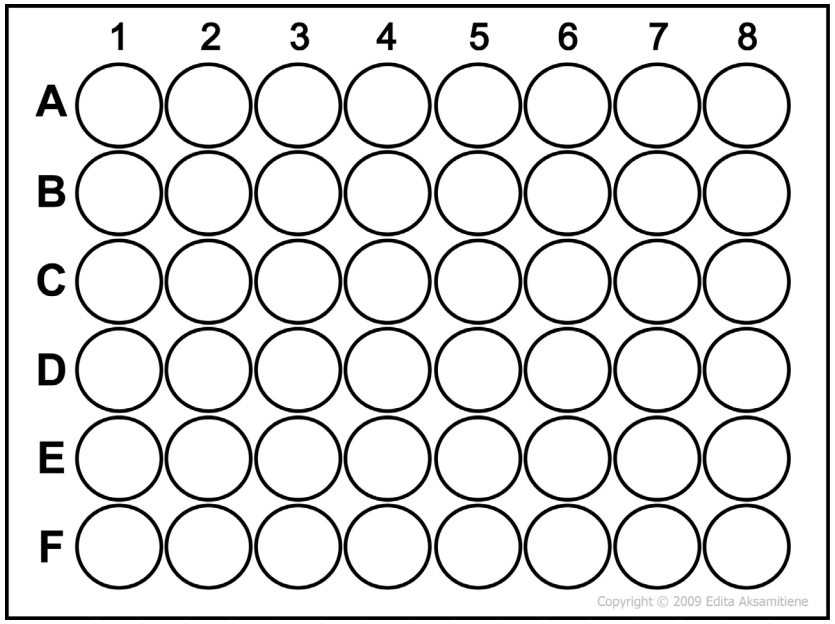
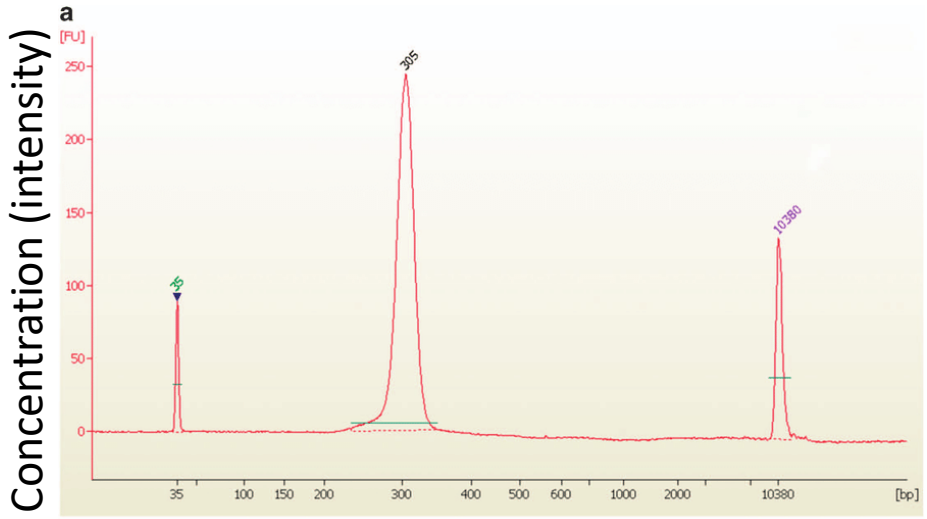
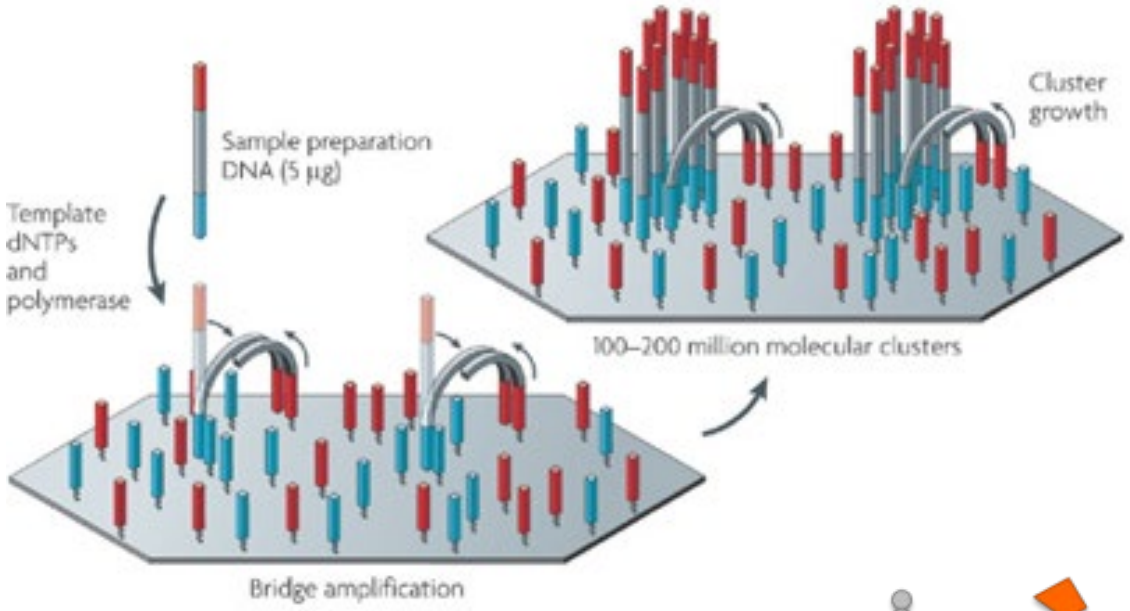


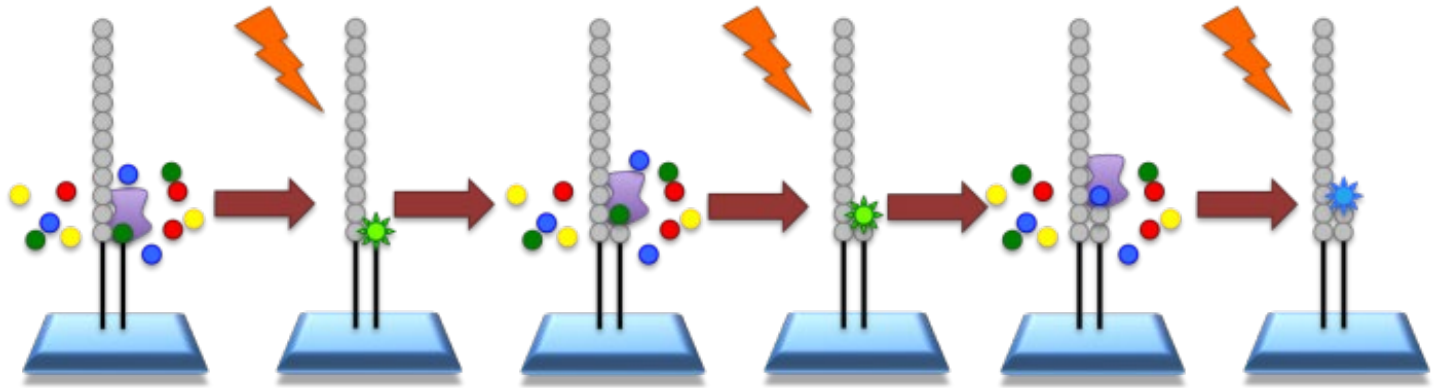


Sequencing (e.g. Illumina MiSeq)

i.



ii.



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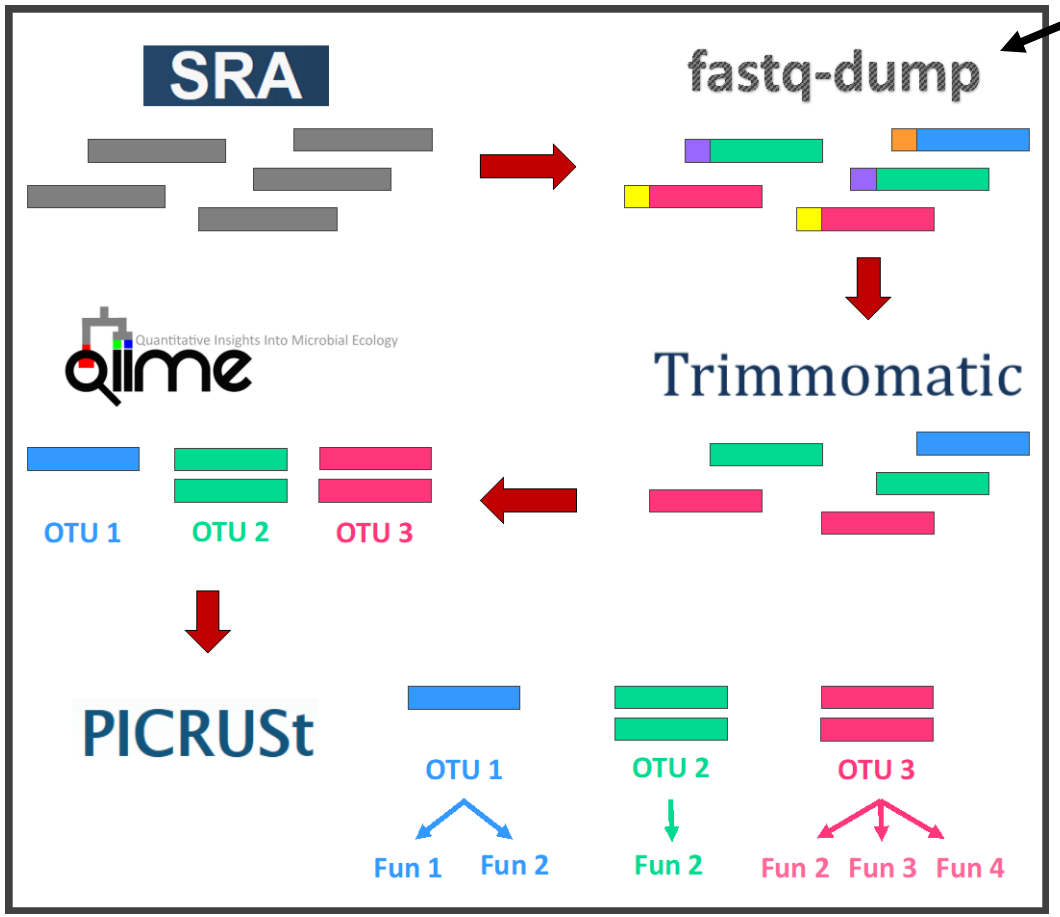
How do we compare samples?

Analysis ‘pipeline’ overview

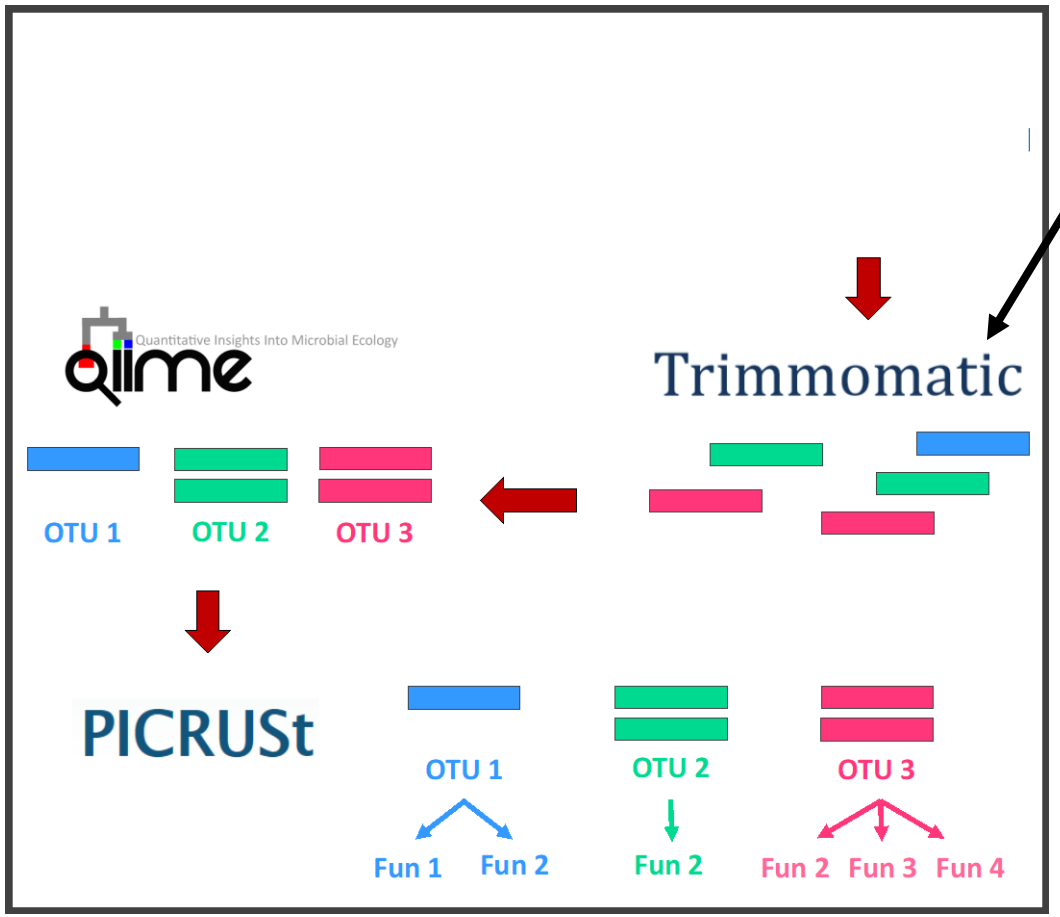
Example of a pipeline



Steps 1 & 2:

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

Example of a pipeline

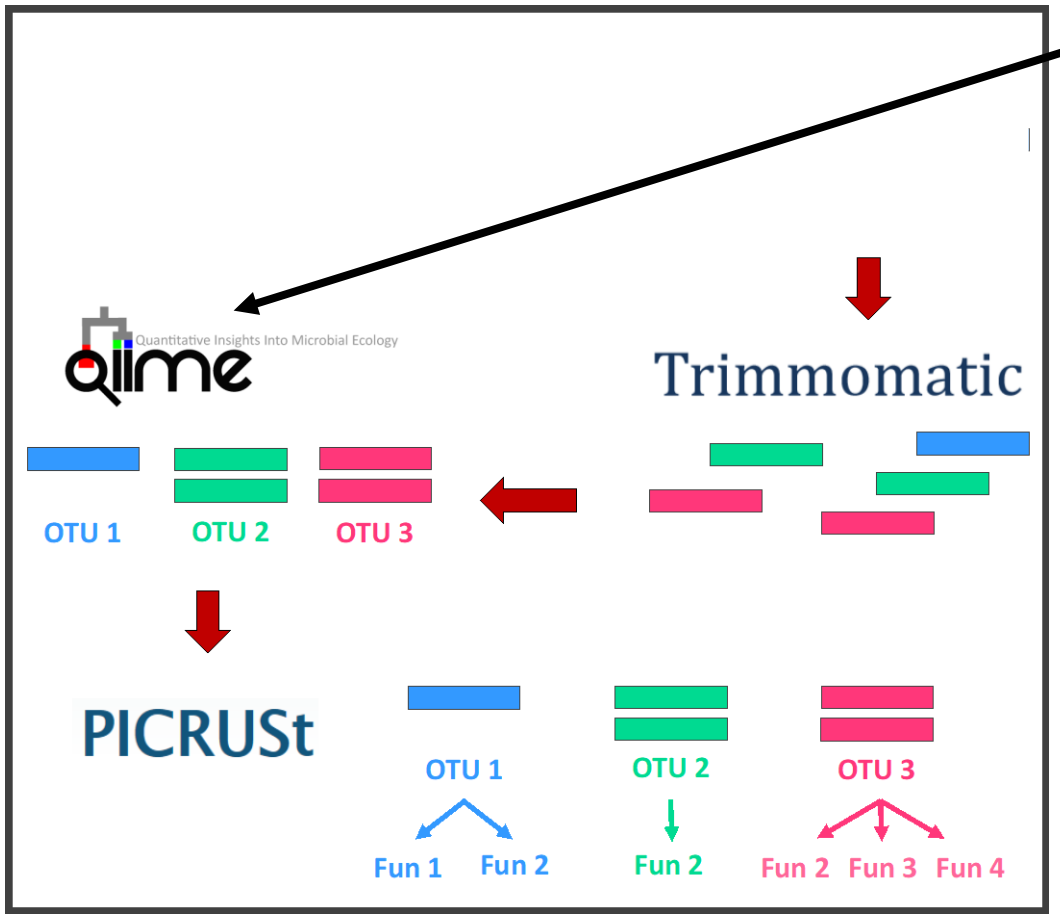


Step 3:

- 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

OR

- Assign a “species” by comparing the sequence to a database (e.g. NCBI BLAST)

Example of a pipeline

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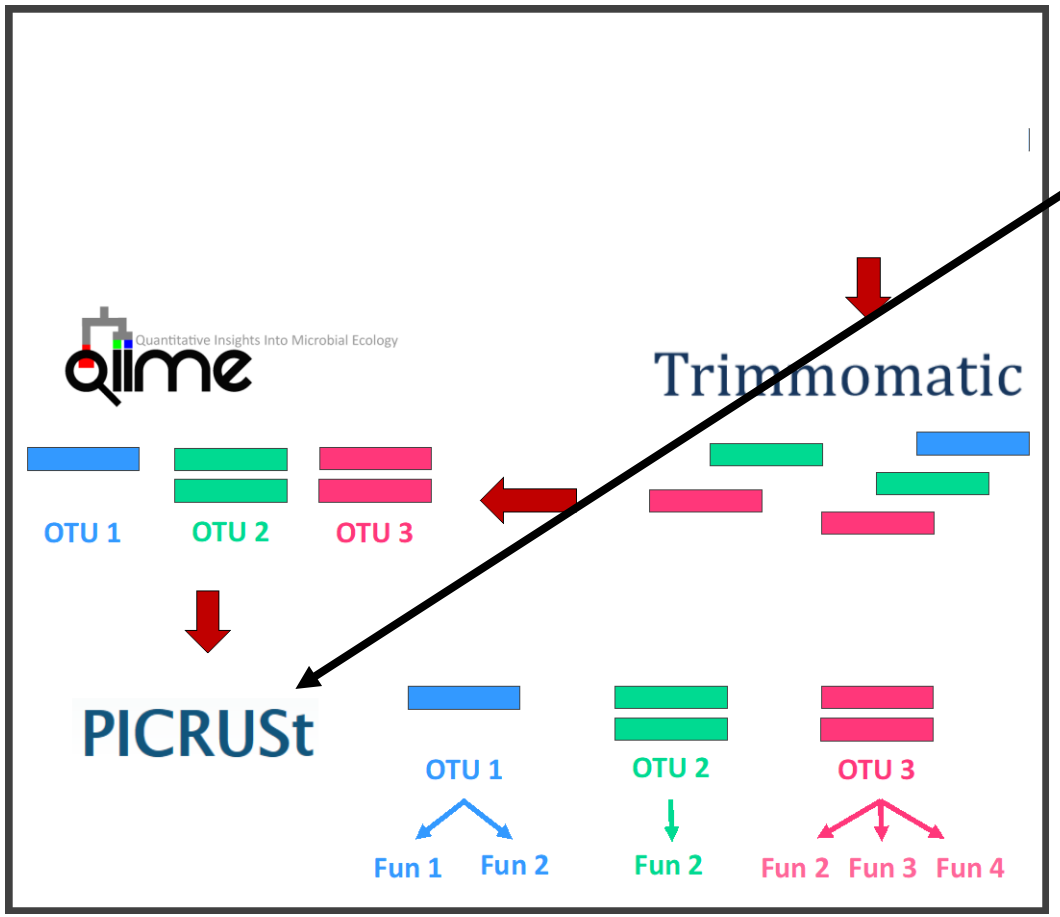
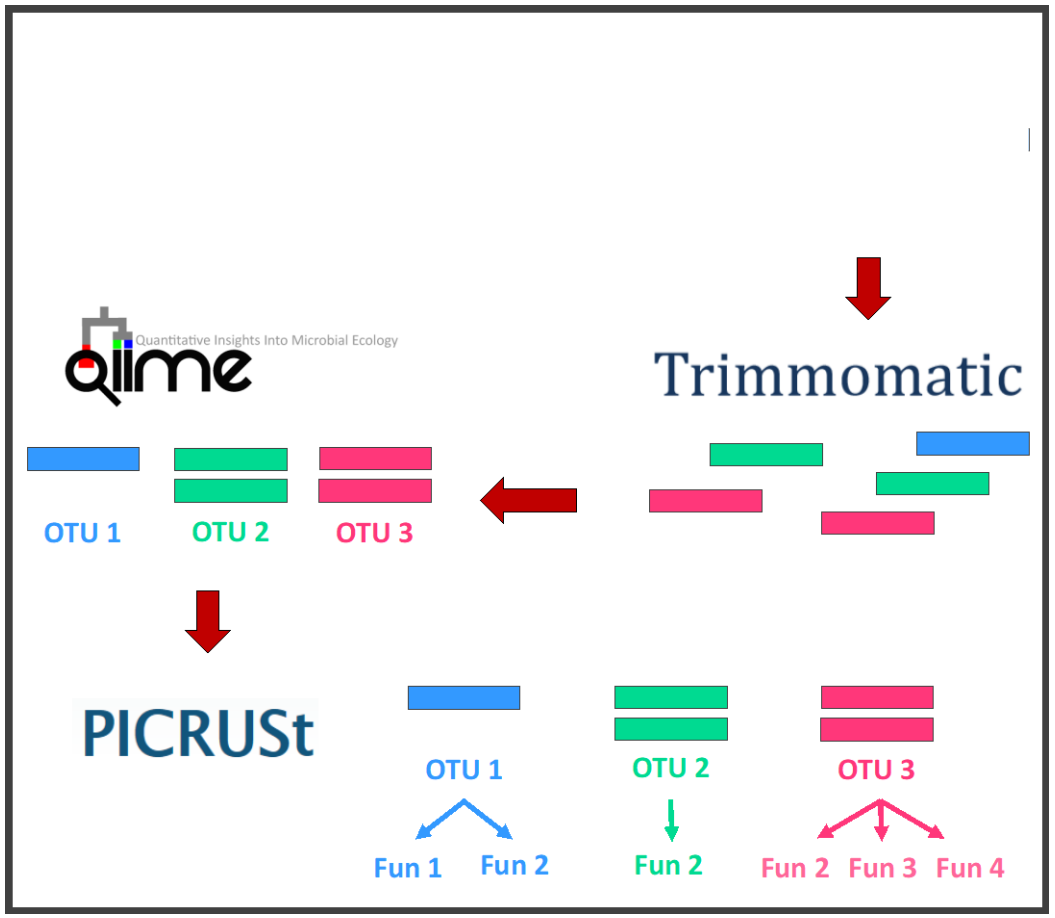


Step 5:

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!

Post-its

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Download: https://ColauttiLab.github.io/Data/OTU_files.zip

Pink (improve) Similar to other courses	Green (keep) Similar to other courses
Different from other courses	Different from other courses