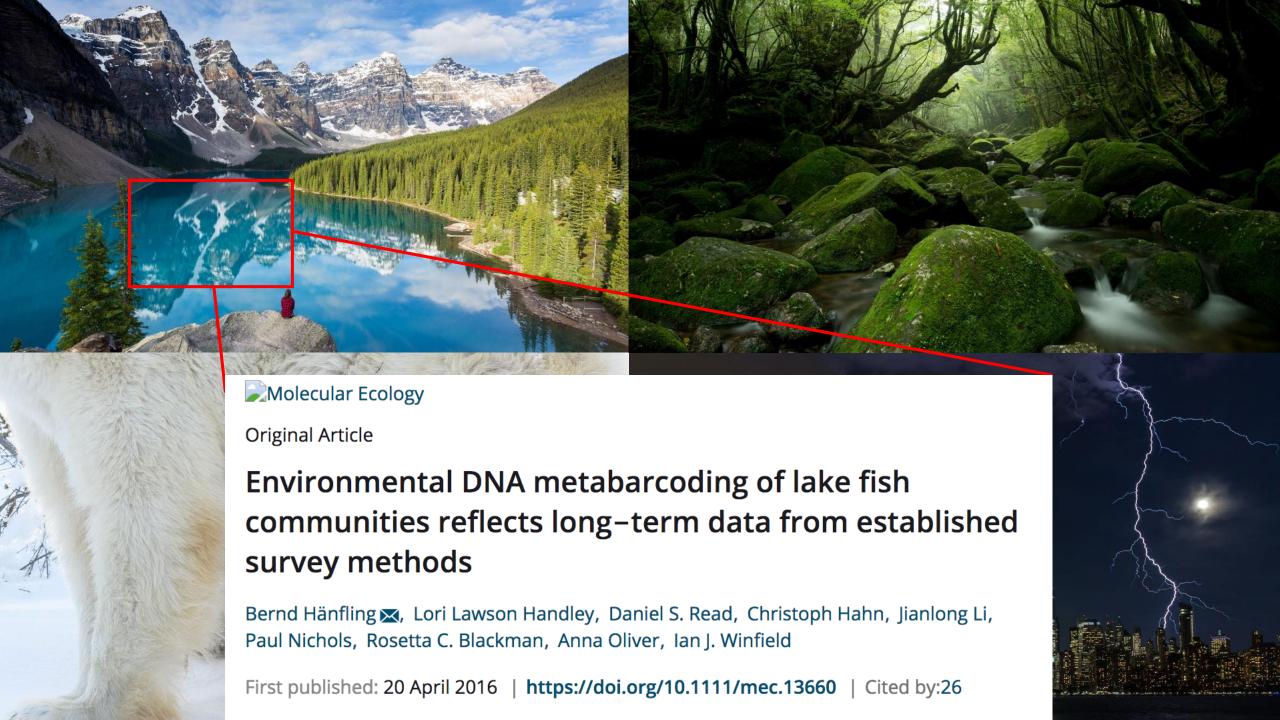# BIOL 432

Metabarcoding

What is a microbiome?

Original Article

Environmental DNA metabarcoding of lake fish communities reflects long–term data from established survey methods
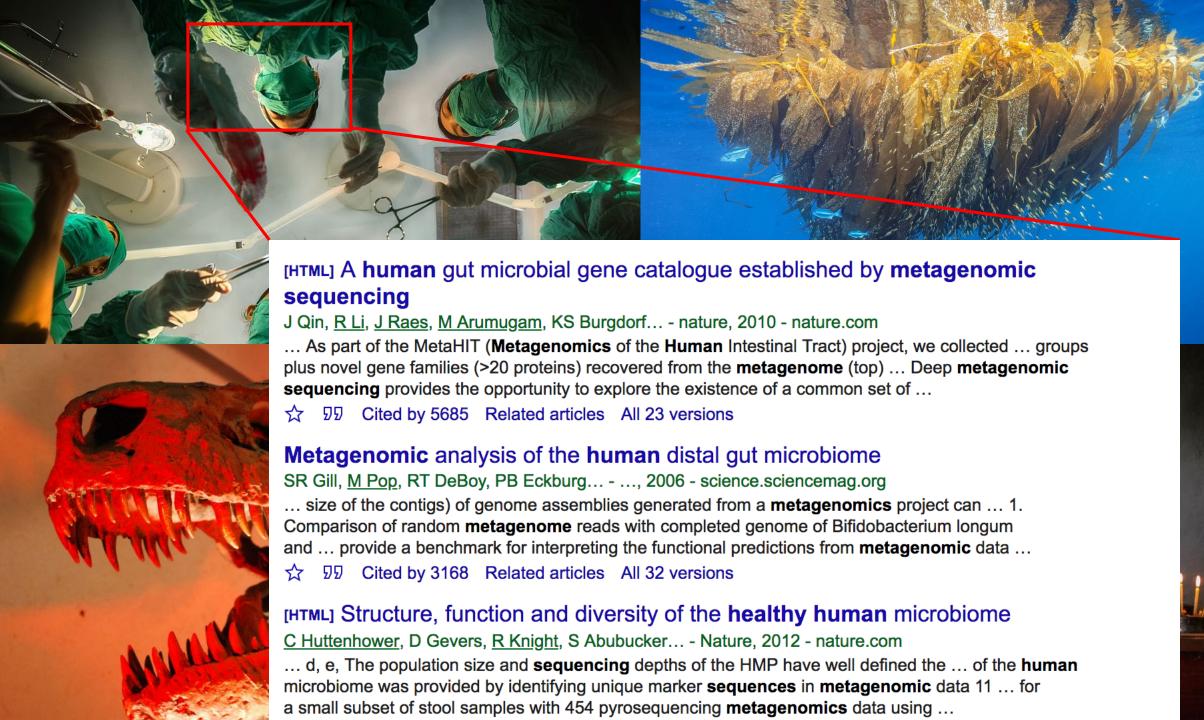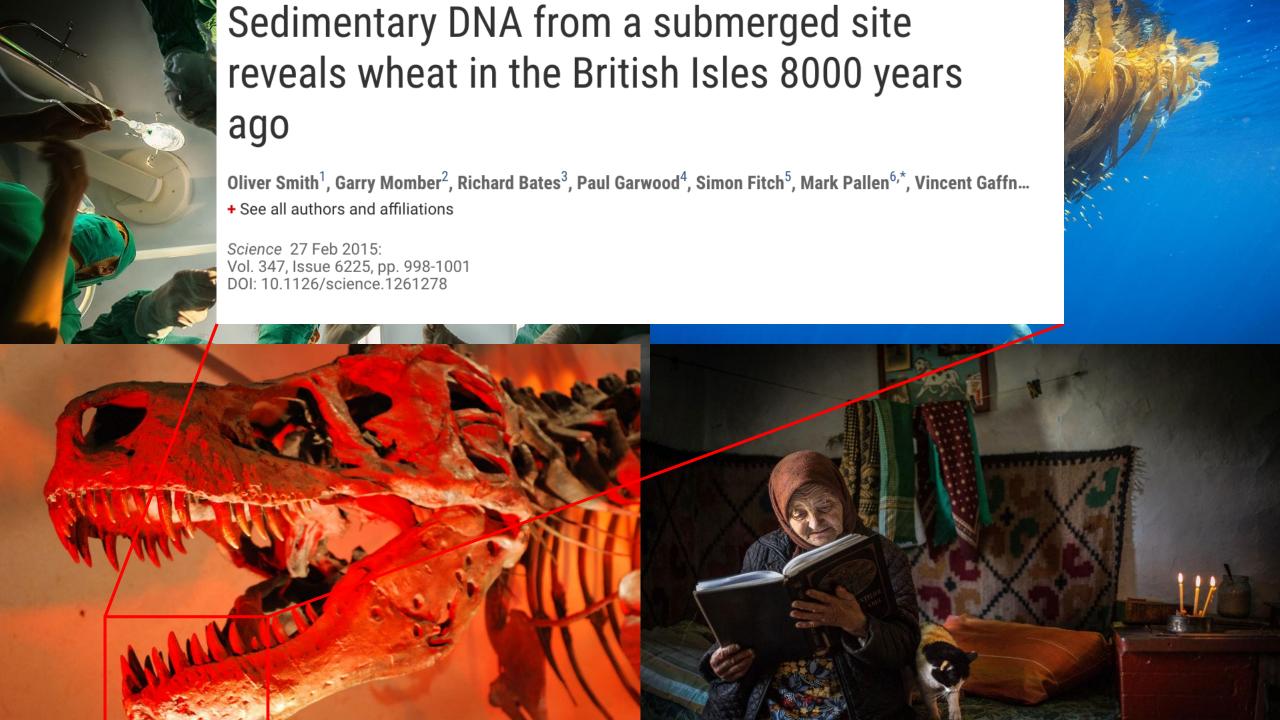
Bernd Hänfling ✉, Lori Lawson Handley, Daniel S. Read, Christoph Hahn, Jianlong Li, Paul Nichols, Rosetta C. Blackman, Anna Oliver, Ian J. Winfield

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

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

[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

Download Citation

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

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

Identifying fungi and bacteria

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

Wilson, Weightman & Wade. 1997. Med Microbiol.
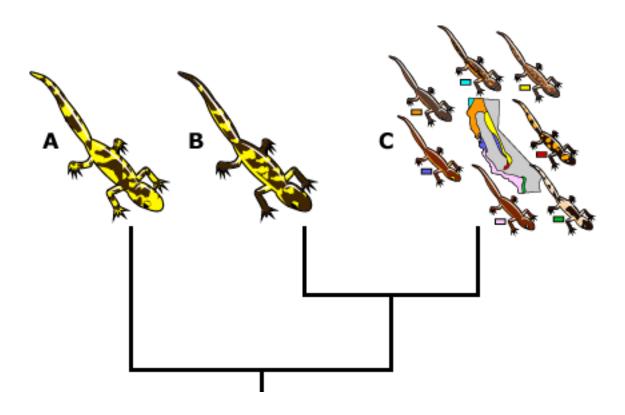
"Species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups."

-Ernst Mayr
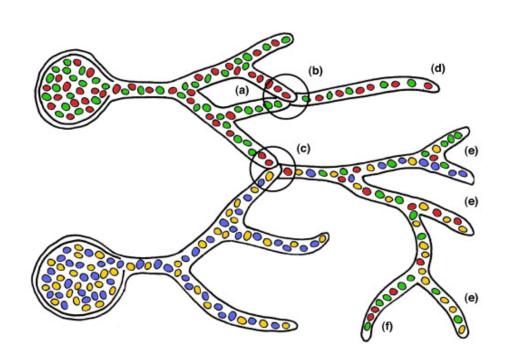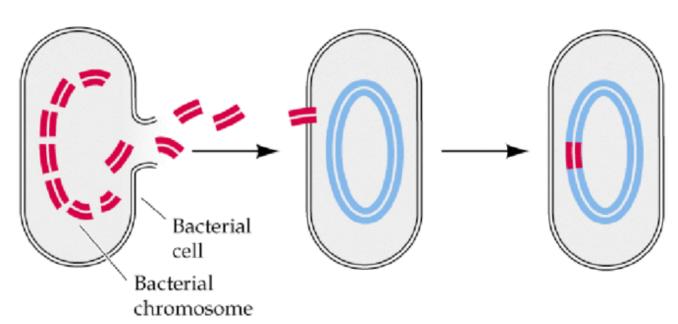
Fungal genetics:

Bacterial genetics:

Transformation

Bacterial cell

Bacterial chromosome

Permission pending from Sinaur Associates, Inc.

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
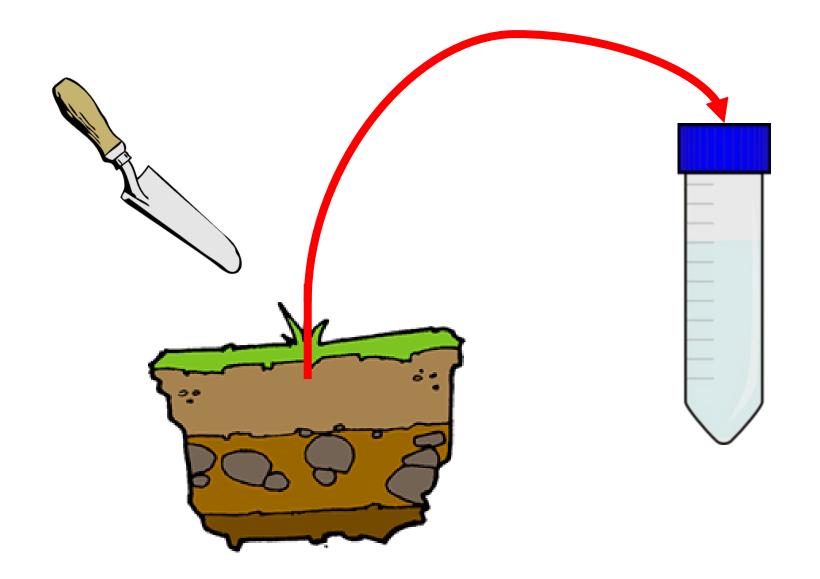
Metabarcoding

Microbiome analysis of high-throughput sequencing data

Plant Tissue Dissociation

Cell Lysis

DNA Binding

Wash

Elution
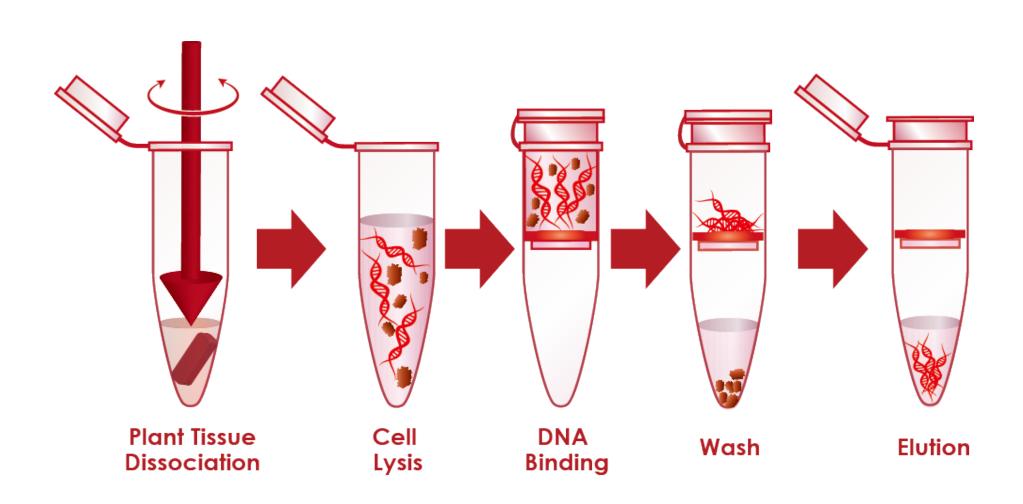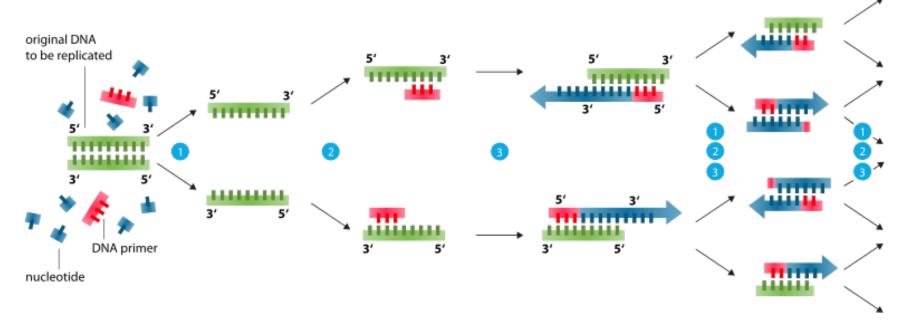
Polymerase chain reaction - PCR
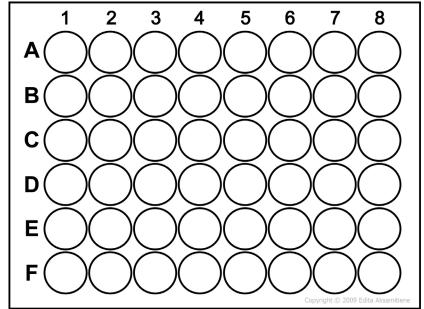
Step 4: Pooling & Quality check
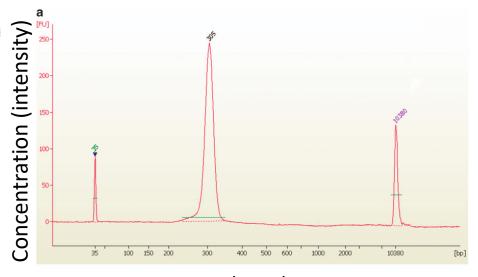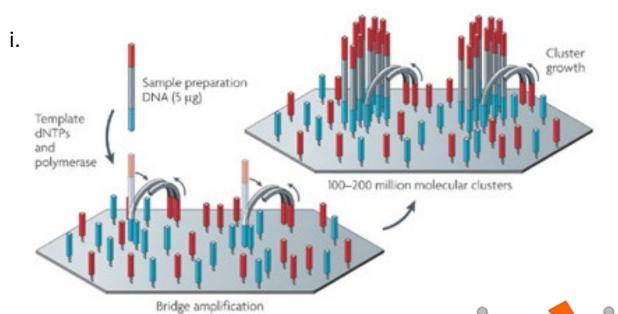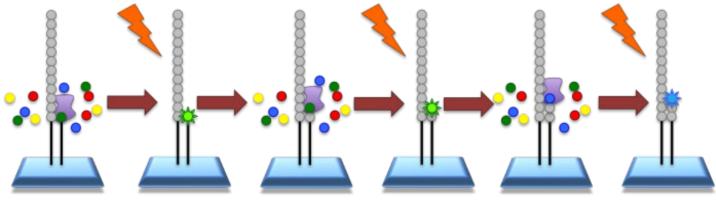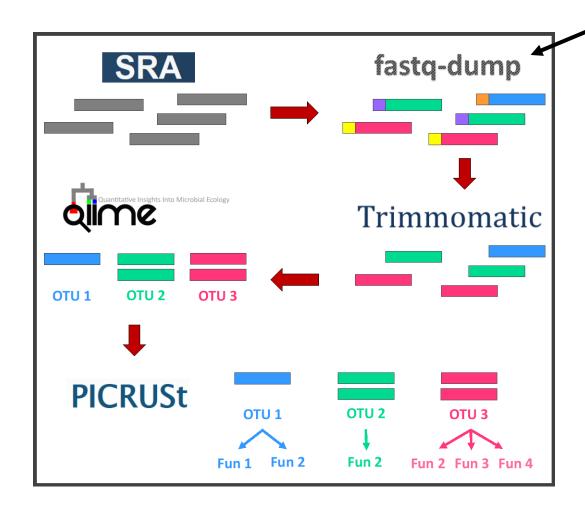
How do we compare samples?
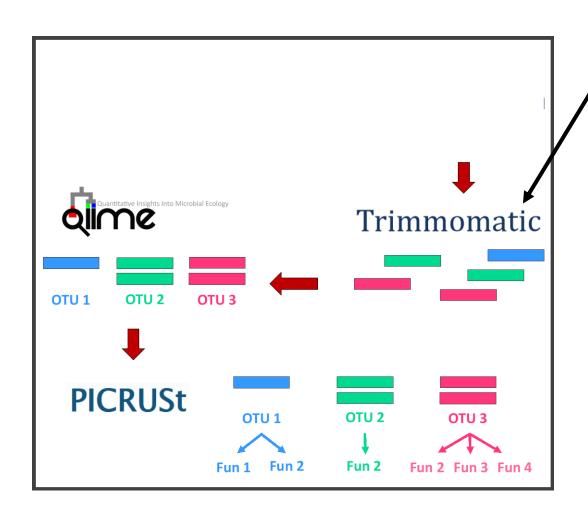
Analysis 'pipeline' overview

Steps 1 & 2:

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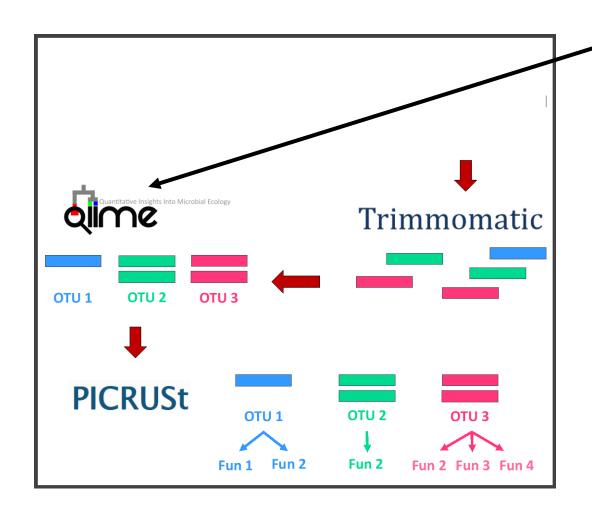- Download to your own computer

Step 3:

- Cut adapter sequences (sequencing primers)

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

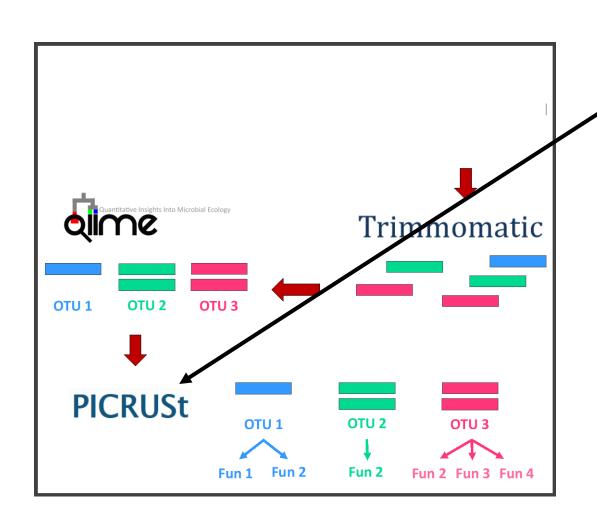- Remove low-quality reads

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)
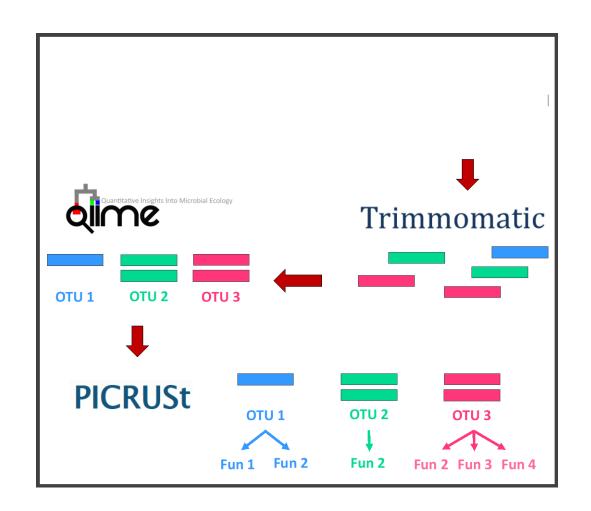
Step 5:

Assign a "function" to the OTU

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!