#### 1. Unix crash course

- a. Getting a Unix environment
- b. Working with files and directories
- c. Six incredible commands / Redirectors and wildcards
- d. Some Powerful everyday examples
  - i. Renaming 1,000 files
  - ii. Making a barcode fasta file to demultiplex samples
  - iii. Converting NCBI taxon IDs to organism lineages
- b. Variables and For loops
- c. Version control (github)
- d. Accessing data from NCBI at the command line
- e. Help installing tools / An introduction to Conda
- f. The amazing power of EMACS / org-mode

# 3. Marker Gene Sequencing overview

a. Background of sequencing technology

## 4. Intro to FASTA/FASTQ file formats

- a. Compression formats and tools (zip, gzip, pbzip, bzip2, pigz)
- b. Demultiplexing example (Sabre / QIIME demux)
- c. Trimming / QA/QC (Trimmomatic, cutadapt, FASTQC/MultiQC)

## 5. Microbiome Sequencing Processing Pipelines

- a. Introduction to R
  - i. Installing R packages
  - ii. R- DADA2 Processing pipeline
  - iii. Going deeper with indexing
- b. Processing with DADA2 using the QIIME2-cli / R 3.6.1 environments
  - i. Setting up our working environment
  - ii. Quality trimming/filtering
  - iii. Generating an error model of our data
  - iv. Dereplication
  - v. a conversation about OTUs vs ASVs
  - vi. read pair merging
  - vii. Generating a count table
  - viii. Chimera identification (chimeraslayer)
  - ix. Overview of counts throughout
  - x. Assigning taxonomy (silva132, rdp, greengenes)
  - xi. Extracting the standard goods from DADA2
  - xii. Removing likely contaminants (decontam)

#### 6. Microbiome Analysis in R

- a. Taxonomic summaries
- b. Diversity Indexes (Alpha and Beta)
- c. Betadisper and permutational ANOVA
- d. Differential abundance analysis with DESeq2

### 7. Other Analysis / Visualization tools

- a. Galaxy
- b. LEFSE
- c. Metacoder
- d. Docker for Bioinformatics
- 8. Pipeline containerization using nextflow/nf-core
- 9. A brief overview of shotgun metagenomic sequencing



# **BIOCONDA**°













