ACES: NGS Metagenomics

on the FASTER cluster

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21 March, 2023

High Performance
Research Computing
DIVISION OF RESEARCH

Introduction to Metagenomics

- Sequencing of communities of microorganisms
- No need for isolation and lab cultivation
- High depth short-read sequencing (Next Generation Sequencing - NGS)
- Long-read (third generation) sequencing
  - PacBio
  - Oxford Nanopore Technologies

Wooley et al. 2010 - https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1000667

Sequencing Strategies

- Whole genome sequencing (WGS)
- Marker gene
  - 16S Ribosomal RNA (rRNA)
    - Bacteria
    - Archaea
  - 18S Ribosomal RNA
    - Fungus
    - Eukaryotes

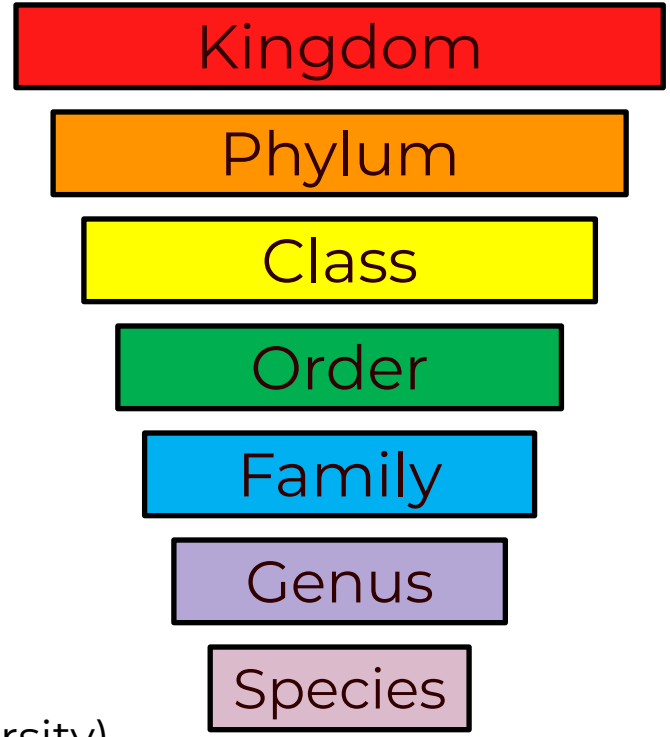
https://www.illumina.com/systems/sequencing-platforms/miseq.html

WGS Metagemomics

- Sequencing whole genomes of the microorganisms present in the sample
- Facilitates discovering gene functions and genome structures
- Steps involved:
  - Genome assembly (special software/considerations)
  - Binning
  - Predicting and annotating genes

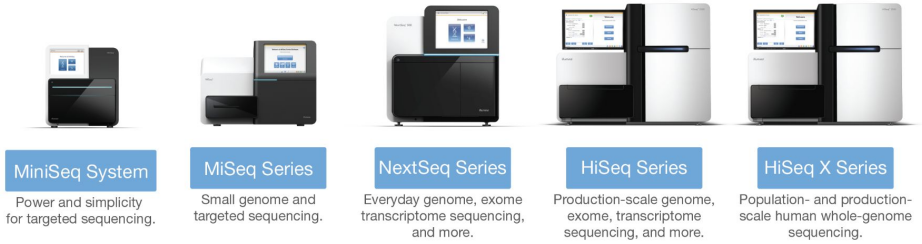
Marker Gene Metagenomics

- Usually based on 16S rRNA
  - Conserved within species
  - Varies greatly between species
  - Widely used for microbial ecology - many resources available
- Needs a reference database to match the Operational Taxonomic Units (OTUs)
  - Silva
  - Greengenes
- Steps:
  - Preprocessing (removing noise, QC)
  - OTU clustering and taxonomic assignment
  - Alpha diversity analysis (within sample diversity)
  - Beta diversity analysis (between sample diversity)



Sequencing Platforms

- Illumina
  - Short reads (up to 300 bp)
  - Highly accurate
  - High depth sequencing
- PacBio
  - Long reads (10-25kb)
  - Accurate (99.5%)
- Oxford Nanopore
  - Long reads (10-30 kb)
  - Less accurate (~95%)
  - Portable
  - Affordable



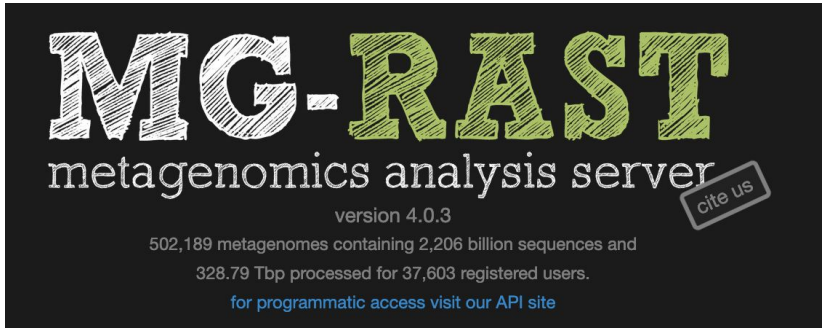
https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf
https://nanoporetech.com

Metagenomics Software

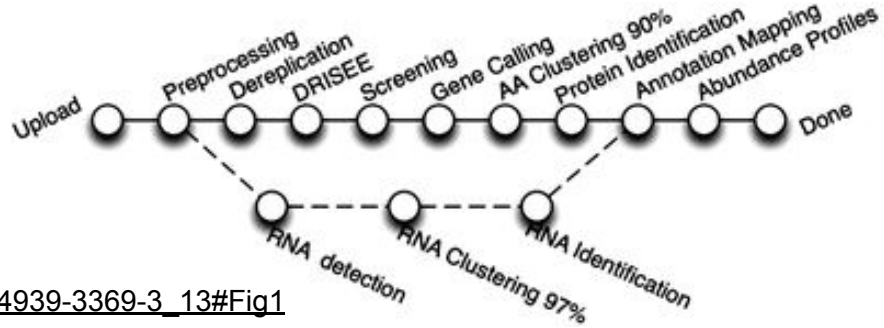
Commonly used software suites and pipelines

MG-RAST metagenomics analysis server

- Open-source web application for metagenomic analysis
- Large repository for metagenomic data
- Hosted by The University of Chicago and Argonne National Laboratory
- Full analysis pipeline



https://www.mg-rast.org



Keegan et al. 2016 -
https://link.springer.com/protocol/10.1007/978-1-4939-3369-3_13#Fig1

Bacterial and Viral Bioinformatics Resource Center

- Formerly known as PATRIC
- Designed to support research on bacterial and viral diseases
- Among other tools, provides some metagenomic functions:
  - Metagenomic read mapping
  - Taxonomic classification
  - Metagenomic binning

https://www.bv-brc.org

Mothur

- Open-source software for microbial ecology
- Single piece of software - many functions/commands
- Example data and protocols available
- Contains accelerated versions of the DOTUR and SONS programs
- Highly cited (> 17,700 citations)
- Schloss et al. 2009: https://journals.asm.org/doi/10.1128/aem.01541-09

https://mothur.org

QIIME 2

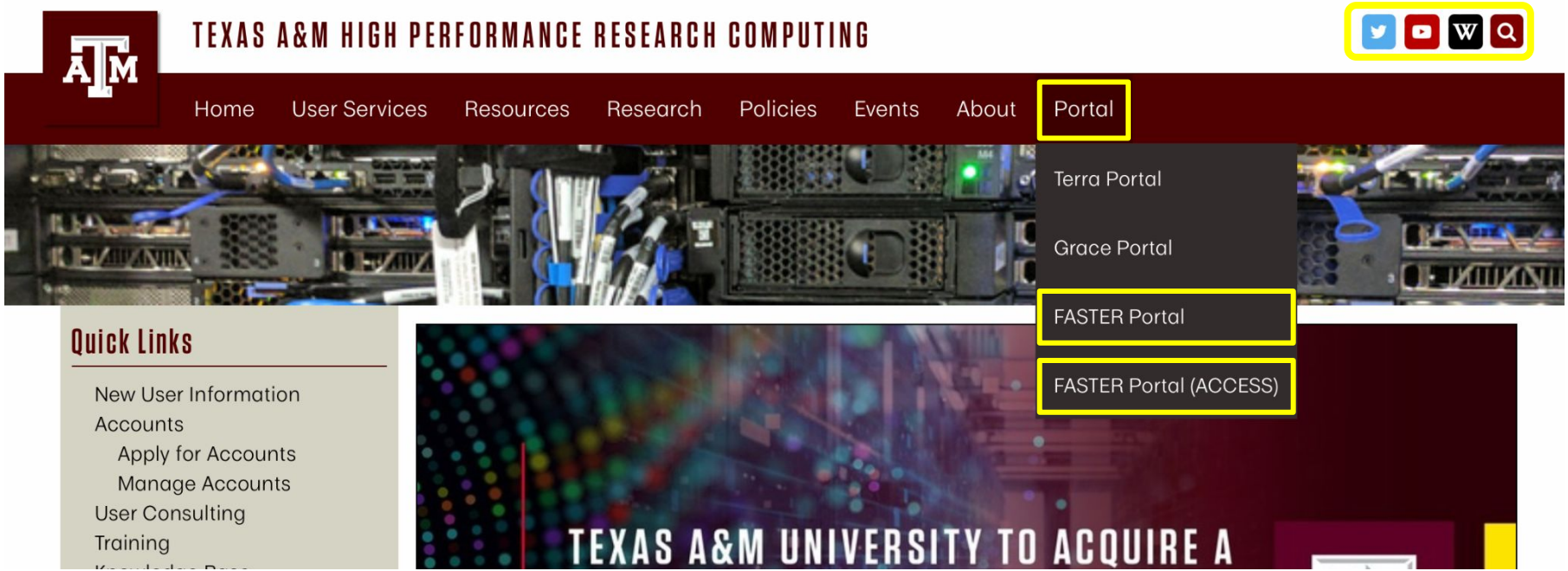
- Open-source pipeline for microbiome analysis
- Takes raw fastq data (multiplexed or demultiplexed)
- From demultiplexing to publication-ready figures
- Incorporates many other software packages (e.g. Mothur, FastTree)

http://qiime.org

Logging into FASTER via the HPRC Portal

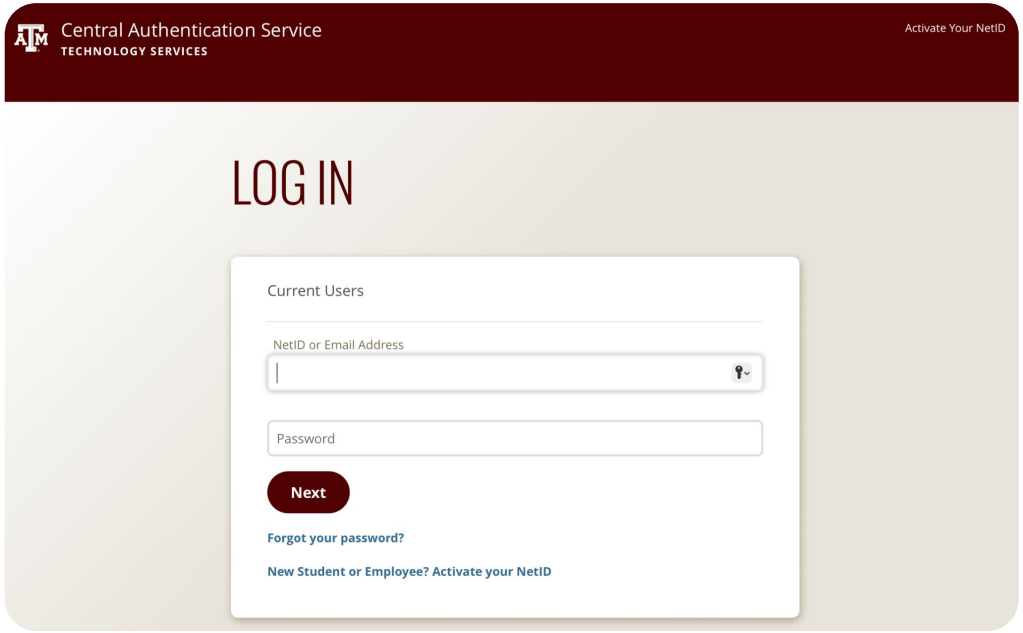
Accessing the HPRC Portal

- HPRC webpage: hprc.tamu.edu Portal dropdown menu



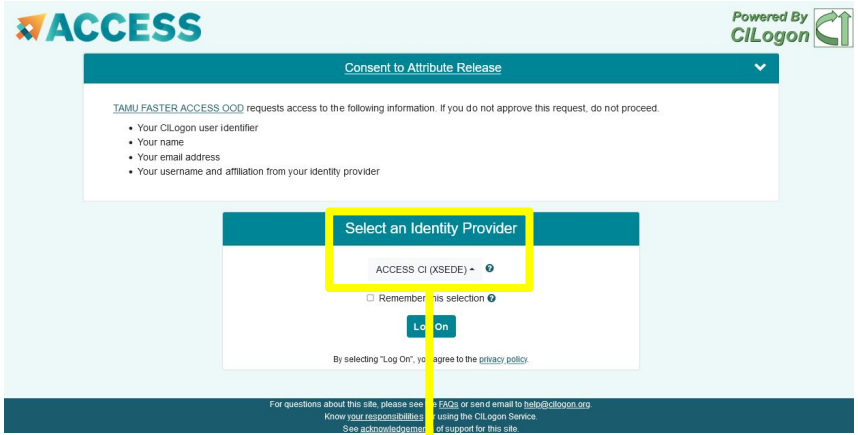
Accessing FASTER via the HPRC Portal (TAMU)

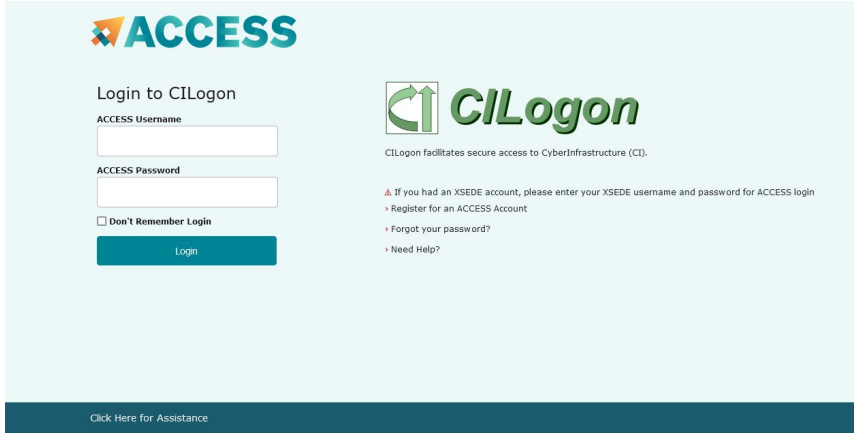
Log-in using your TAMU NetID credentials.

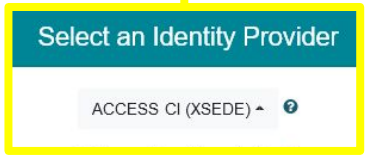


Accessing FASTER via the HPRC Portal (ACCESS)

Log-in using your ACCESS credentials.





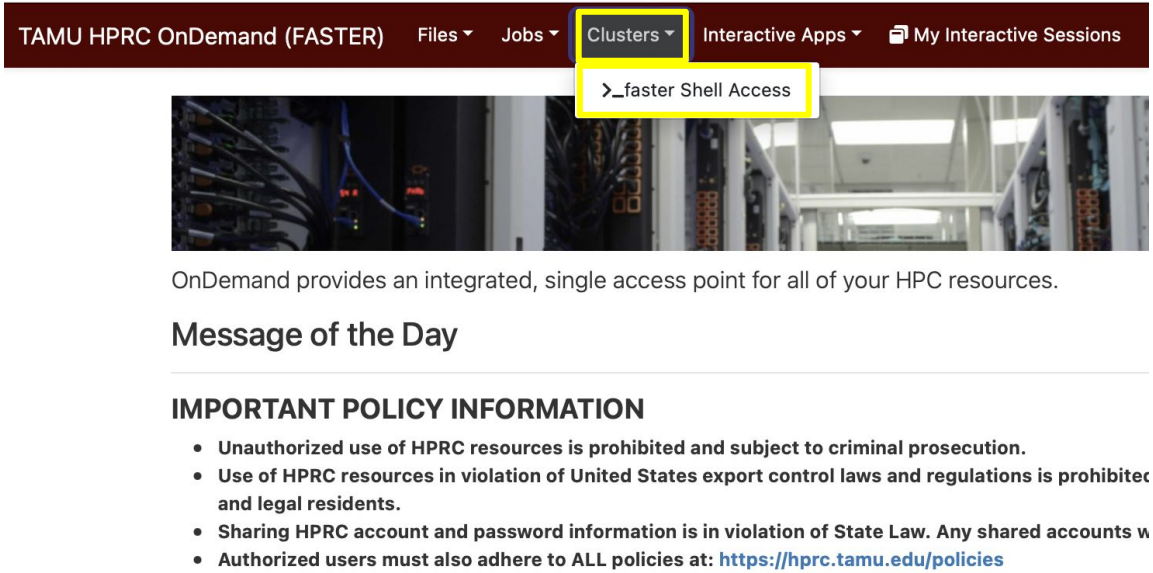


Select the Identity Provider appropriate for your account.

Shell access via the HPRC Portal

Access through (most) web browsers

–Top Banner Menu “Clusters” -> “Shell Access”



Getting started with QIIME 2 - Terminology

- Artifacts
  - Contain data and metadata
  - .qza file extension
  - Allows QIIME to track type, format, and provenance of the data
- Visualization
  - Terminal output of an analysis (e.g. tables, graphs)
  - Also contain data and metadata
  - Can be viewed at https://view.qiime2.org

Getting started with QIIME 2 - Terminology

- Semantic types
  - Essentially the classification of an artifact
  - Helps users avoid using incorrect inputs for analyses

Common semantic types

Unless otherwise noted the following semantic types are defined by, and importable from, the q2-types plugin. It is also possible to define semantic types in any plugin, so the available semantic types are not limited to those defined in q2-types. Instructions will be added soon for how to accomplish this. In the meantime, you can refer to the q2-dummy-types repository for annotated examples.

`FeatureTable[Frequency]` : A feature table (e.g., samples by OTUs) where each value indicates the frequency of an OTU in the corresponding sample expressed as raw counts.

`FeatureTable[RelativeFrequency]` : A feature table (e.g., samples by OTUs) where each value indicates the relative abundance of an OTU in the corresponding sample such that the values for each sample will sum to 1.0.

`FeatureTable[PresenceAbsence]` : a feature table (e.g., samples by OTUs) where each value indicates whether an OTU is present or absent in the corresponding sample.

`FeatureTable[Composition]` : A feature table (e.g., samples by OTUs) where each value indicates the frequency of an OTU in the corresponding sample, and all frequencies are greater than zero.

`Phylogeny[Rooted]` : A rooted phylogenetic tree.

`Phylogeny[Unrooted]` : An unrooted phylogenetic tree.

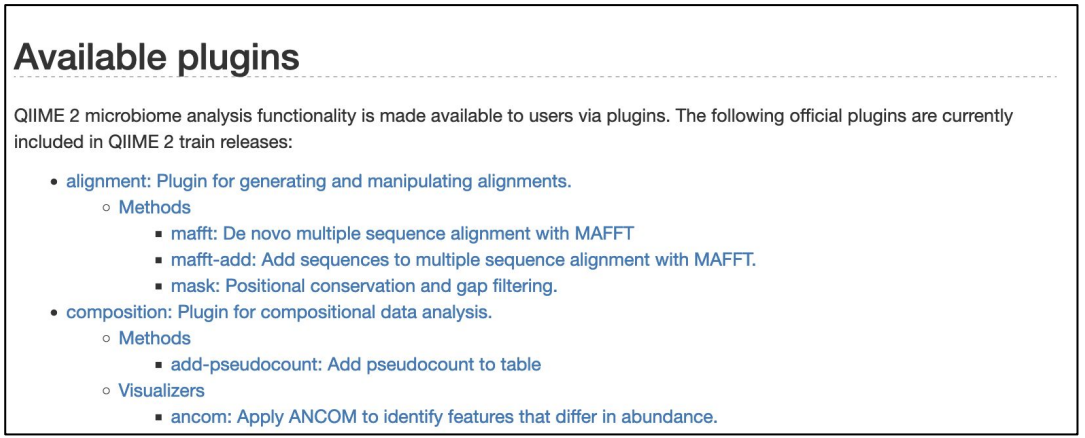
`DistanceMatrix` : A distance matrix.

https://docs.qiime2.org/2022.2/semantic-types

Getting started with QIIME 2 - Terminology

- Plugins
  - Software packages that perform specific analyses (e.g. q2-demux, q2-diversity)
  - Can be written by third-party developers
  - Plugins available for all steps necessary for complete pipeline

https://docs.qiime2.org/2022.2/plugins/available

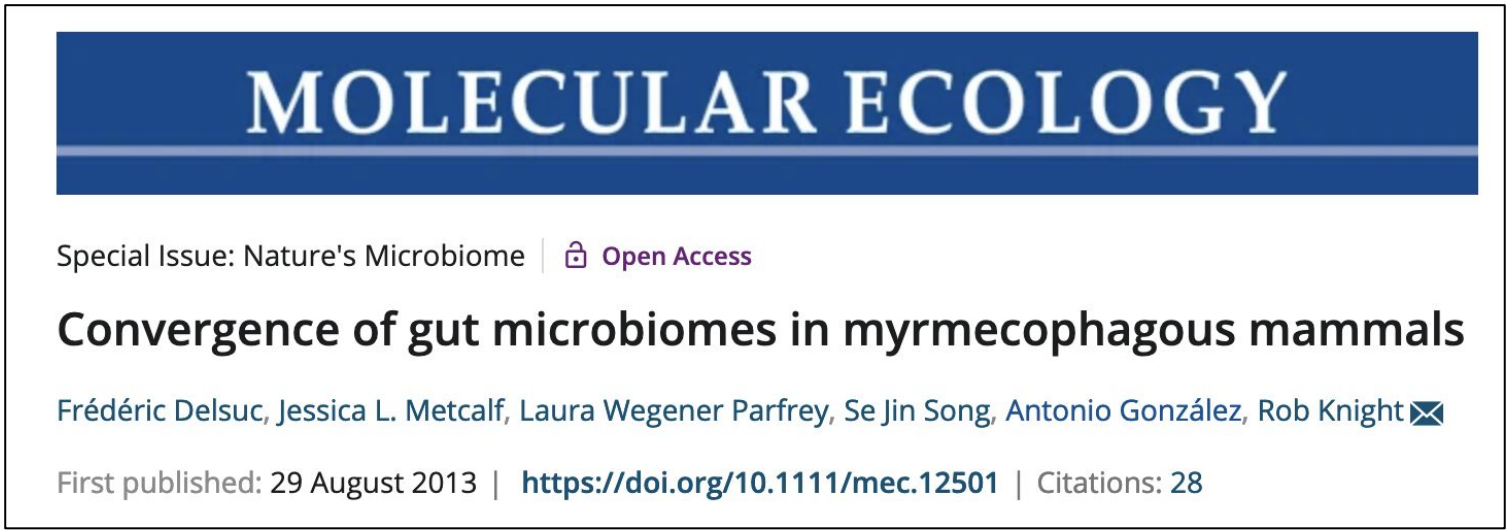


Available plugins

QIIME 2 microbiome analysis functionality is made available to users via plugins. The following official plugins are currently included in QIIME 2 train releases:

- alignment: Plugin for generating and manipulating alignments.
  - Methods
    - mafft: De novo multiple sequence alignment with MAFFT
    - mafft-add: Add sequences to multiple sequence alignment with MAFFT.
    - mask: Positional conservation and gap filtering.
- composition: Plugin for compositional data analysis.
  - Methods
    - add-pseudocount: Add pseudocount to table
  - Visualizers
    - ancom: Apply ANCOM to identify features that differ in abundance.

Example data



MOLECULAR ECOLOGY

Special Issue: Nature's Microbiome | Open Access

Convergence of gut microbiomes in myrmecophagous mammals

Frédéric Delsuc, Jessica L. Metcalf, Laura Wegener Parfrey, Se Jin Song, Antonio González, Rob Knight

First published: 29 August 2013 | https://doi.org/10.1111/mec.12501 | Citations: 28

https://onlinelibrary.wiley.com/doi/10.1111/mec.12501

Getting started with QIIME 2 on FASTER

Set up the environment and working directory:

```
$ module purge
```

```
$ module load QIIME2/2022.8
```

```
$ mkdir $SCRATCH/metagenomics
```

```
$ cd $SCRATCH/metagenomics
```

```
$ cp -r /scratch/training/bio/metagenomics/* .
```

Import fastq reads to QIIME artifact

```
$ qiime tools import \
   --type EMPSingleEndSequences \
   --input-path fastqs \
   --output-path reads.qza
```

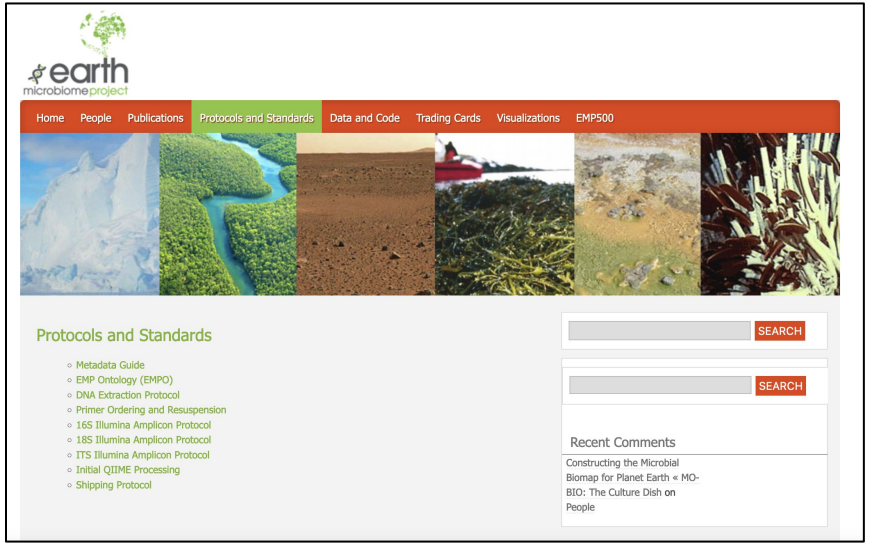
*This step will take several minutes

Importing Data

- QIIME 2 can import many types of data:
  - Fastq (single and paired-end)
  - Fasta
  - Feature tables
  - Phylogenetic trees
- Importing data creates QIIME 2 artifacts (with specific semantic types)
- Semantic types for raw fastq files:
  - EMPSingleEndSequences
  - EMPPairedEndSequences
  - MultiplexedSingleEndBarcodeInSequence
  - MultiplexedPairedEndBarcodeInSequence …

Importing Data

- Example data is in the EMP single end format
- Data is still multiplexed (single fastq file)
- Directory with two fastq files
  - Sequences
  - Barcodes



Demultiplexing the example data

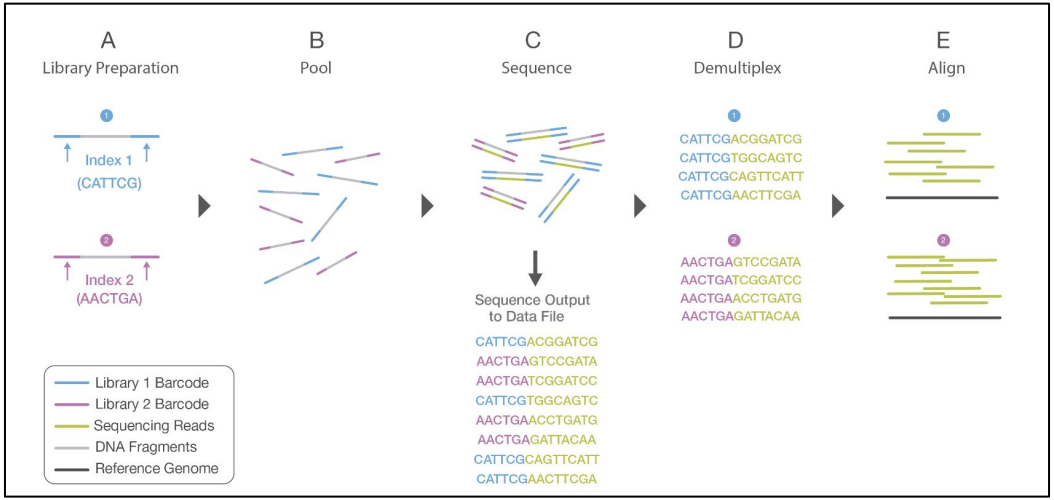
```
$ qiime demux emp-single --i-seqs reads.qza \
   --m-barcodes-file myrme-sample-data.txt \
   --m-barcodes-column barcode-sequence \
   --o-per-sample-sequences demux.qza \
   --o-error-correction-details demux-details.qza \
   --p-rev-comp-mapping-barcodes
```

```
$ qiime demux summarize --i-data demux.qza \
   --o-visualization demux.qzv
```

Upload and view demux.qzv file here: https://view.qiime2.org

Demultiplexing and Quality Control

- Example data (and most amplicon/targeted metagenomics datasets) are pooled for sequencing
- Unique barcodes (short oligos) applied to each sample
- Barcodes used to sort reads after sequencing



https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

Preprocessing Sequence Data

- Use the following commands to denoise and filter the example data
- Remember the interactive quality plot to choose the right values for the trimming options

```
$ qiime dada2 denoise-single \
   --i-demultiplexed-seqs demux.qza \
   --p-trim-left 8 --p-trunc-len 148 \
   --o-representative-sequences rep-seqs.qza \
   --o-table table.qza --o-denoising-stats stats.qza \
   --p-n-threads 8
```

Denoising and Filtering

- Identify and correct sequenced amplicons
- Filter chimeric reads
- Filter PhiX reads
- Multiple options for denoising and filtering in QIIME 2
  - DADA2
  - Deblur

Preprocessing Sequence Data

- Generate feature/sequence tables for visualization:

```
$ qiime metadata tabulate --m-input-file stats.qza \
   --o-visualization stats.qzv
```

```
$ qiime feature-table summarize --i-table table.qza \
   --o-visualization table.qzv \
   --m-sample-metadata-file myrme-sample-data.txt
```

```
$ qiime feature-table tabulate-seqs \
   --i-data rep-seqs.qza --o-visualization rep-seqs.qzv
```

- Download and view the qzv files at https://view.qiime2.org

Diversity Analysis

- Alpha Diversity - within sample
  - Shannon’s Diversity Index
  - Observed Features
  - Faith’s Phylogenetic Diversity
  - Evenness
- Beta Diversity - between samples
  - Jaccard Distance
  - Bray-Curtis Distance
  - Unweighted UniFrac Distance
  - Weighted UniFrac Distance

Diversity Analysis

- Use the following command to generate a phylogenetic tree for the diversity analyses

```
$ qiime phylogeny align-to-tree-mafft-fasttree \
   --i-sequences rep-seqs.qza \
   --o-alignment aligned-rep-seqs.qza \
   --o-masked-alignment masked-aligned-reps-seqs.qza \
   --o-tree unrooted-tree.qza \
   --o-rooted-tree rooted-tree.qza
```

Diversity Analysis

- Use the following command to generate the alpha and beta diversity metrics, as well as the PCA plots for the beta diversity metrics

```
$ qiime diversity core-metrics-phylogenetic \
   --i-phylogeny rooted-tree.qza --i-table table.qza \
   --p-sampling-depth 10590 \
   --m-metadata-file myrme-sample-data.txt \
   --output-dir diversity-analysis
```

- Upload and view the beta diversity qzv files at https://view.qiime2.org

Diversity Analysis

- Use the following commands to test for significance of alpha-level diversity

```
$ qiime diversity alpha-group-significance \
    --i-alpha-diversity diversity-analysis/faith_pd_vector.qza \
    --m-metadata-file myrme-sample-data.txt \
    --o-visualization diversity-analysis/faith_pd_vector-sig.qzv
```

```
$ qiime diversity alpha-group-significance \
    --i-alpha-diversity diversity-analysis/evenness_vector.qza \
    --m-metadata-file myrme-sample-data.txt \
    --o-visualization diversity-analysis/evenness_vector-sig.qzv
```

- Upload and view the alpha diversity qzv files at https://view.qiime2.org

Diversity Analysis

- Use the following commands to test for significance of beta-level diversity

```
$ qiime diversity beta-group-significance \
    --i-distance-matrix diversity-analysis/unweighted_unifrac_distance_matrix.qza \
    --m-metadata-file myrme-sample-data.txt --m-metadata-column diet \
    --o-visualization diversity-analysis/unweighted_unifrac_diet-significance.qzv \
    --p-pairwise
```

```
$ qiime feature-table filter-samples --i-table table.qza \
    --m-metadata-file myrme-sample-data.txt \
    --p-where "[species]!='panda' AND [species]!='pink-fairy-armadillo'" \
    --o-filtered-table only-multi-species.qza
```

```
$ qiime diversity core-metrics-phylogenetic --i-phylogeny rooted-tree.qza \
    --i-table only-multi-species.qza --p-sampling-depth 10590 \
    --m-metadata-file myrme-sample-data.txt \
    --output-dir diversity-analysis-only-multi-species
```

Diversity Analysis

- Use the following commands to test for significance of beta-level diversity

```
$ qiime diversity beta-group-significance \
    --i-distance-matrix \
    diversity-analysis-only-multi-species/unweighted_unifrac_distance_matrix.qza \
    --m-metadata-file myrme-sample-data.txt --m-metadata-column species \
    --o-visualization \
    diversity-analysis-only-multi-species/unweighted_unifrac_species-sig.qzv \
    --p-pairwise
```

- Upload and view the qzv file at https://view.qiime2.org