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🌐 BIT 577 QIIME2 Tutorial

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ABSTRACT

Command line scripts for processing and analyzing 16S amplicon data using QIIME2.

OPEN  ACCESS



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Protocol status: Working
We use this protocol and it's working

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Keywords: metagenomics,
Nanopore sequencing, WGS

1 Download conda through the QIIME2 website or miniconda website. After installation, download and install QIIME2

2 Activate Qiime environment:

```
conda activate qiime2-amplicon-2023.9
```

```
# change out to whatever version has been downloaded
```

3 Import data

```
qiime tools import \  
  --type 'SampleData[SequencesWithQuality]' \  
  --input-path  
/path/to/your/sequencefiles \  
  --input-format CasavaOneEightSingleLanePerSampleDirFmt \  
  --output-path demux-single-end.qza
```

4 Check quality

```
qiime demux summarize --p-n 10000 --i-data demux-single-end.qza --o-visualization qual_viz
```

```
qiime tools view qual_viz.qzv
```

5 Denoise with DADA2

```
qiime dada2 denoise-single \  
  --i-demultiplexed-seqs demux-single-end.qza \  
  --p-trunc-len 200 \ #at what position you should trim your data based on the demux summary above  
  --o-table table.qza \ #ASV table  
  --o-representative-sequences rep-sets.qza \  
  --o-denoising-stats denoising-stats.qza
```

6 Filter Sequence Table

```
qiime feature-table summarize \  
> --i-table table.qza \  
> --o-visualization vis_table
```

```
qiime tools view vis_table.qzv
```

7 Align and Construct Phylogenetic Tree

```
qiime phylogeny align-to-tree-mafft-fasttree \ # program used for construction
--i-sequences rep-sets.qza \
--o-alignment aligned-rep-seqs.qza \
--o-masked-alignment masked-aligned-rep-seqs.qza \
--o-tree unrooted-tree.qza \
--o-rooted-tree rooted-tree.qza
```

8 Compute diversity measures (see QIIME2 for differences in alpha, beta, and emperor plots)

```
qiime diversity core-metrics-phylogenetic --i-table
table.qza --i-phylogeny rooted-tree.qza --p-sampling-depth 29761
--m-metadata-file /Users/isabellalivingston/Desktop/16S Sequence
Data/GSL_metadata.txt --o-rarefied-table rare_featuretable --o-faith-pd-vector
faithpd_vector --o-observed-features-vector observed_sample_features_vector
--o-shannon-vector shannon_sample_vector --o-evenness-vector
evenness_sample_vector --o-unweighted-unifrac-distance-matrix
unweighted_distance_matrix --o-weighted-unifrac-distance-matrix
weighted_distance_matrix --o-jaccard-distance-matrix jaccard_matrix
--o-bray-curtis-distance-matrix bc_matrix --o-unweighted-unifrac-pcoa-results
unweighted_pcoa --o-weighted-unifrac-pcoa-results weighted_pcoa
--o-jaccard-pcoa-results jaccard_pcoa --o-bray-curtis-pcoa-results bc_pcoa
--o-unweighted-unifrac-emperor unweighted_emperor --o-weighted-unifrac-emperor
weighted_emperor --o-jaccard-emperor jaccard_emperor --o-bray-curtis-emperor
bc_emperor
```

9 Test for differences in diversity using metadata

Alpha Diversity

```
qiime diversity alpha-group-significance --i-alpha-diversity faithpd_vector.qza
--m-metadata-file /path/to/metadata/txt file
--o-visualization faith-pd-group-significance.qzv
```

```
qiime diversity alpha-group-significance --i-alpha-diversity
evenness_sample_vector.qza --m-metadata-file
/Users/isabellalivingston/Desktop/16S\ Sequence\ Data\GSL_metadata.txt
--o-visualization evenness-group-significance.qzv
```

```
qiime diversity alpha-group-significance --i-alpha-diversity
shannon_sample_vector.qza --m-metadata-file
```

```
/Users/isabellalivingston/Desktop/Metadata\ for\ GSL.tsv --o-visualization  
alpha_diversity
```

```
# Emperor plots
```

```
qiime emperor plot --i-pcoa unweighted_pcoa.qza  
--m-metadata-file /Users/isabellalivingston/Desktop/16S\ Sequence\  
Data/GSL_metadata.txt --o-visualization unweighted-unifrac-emperor-sex.qzv
```

```
qiime emperor plot --i-pcoa bc_pcoa.qza --m-metadata-file  
/Users/isabellalivingston/Desktop/16S\ Sequence\ Data/GSL_metadata.txt  
--p-custom-axes sex --o-visualization bc-sex-sex.qzv
```

```
# Beta diversity
```

```
qiime diversity beta-group-significance --i-distance-matrix  
unweighted_distance_matrix.qza --m-metadata-file  
/Users/isabellalivingston/Desktop/16S\ Sequence\ Data/GSL_metadata.txt  
--m-metadata-column sex --o-visualization unweighted-unifrac-sex-significance.qzv  
--p-pairwise
```

10 Taxonomic Classification

```
wget https://data.qiime2.org/2018.2/common/gg-13-8-99-515-806-nb-classifier.qza #download  
classifier of choice
```

```
qiime feature-classifier classify-sklearn \  
--i-classifier /path/to/classifier.qza \  
--i-reads rep-sets.qza \  
--o-classification {TAXONOMY}.qza
```

```
qiime metadata tabulate \  
--m-input-file {TAXONOMY}.qza --o-visualization {TAXONOMY_VIZ}.qzv
```

```
qiime taxa barplot \  
--i-table table.qza  
\  
--i-taxonomy  
{TAXONOMY}.qza \  
--m-metadata-file  
/path/to/metadata/txtfile\  
--o-visualization  
{TAXA-BAR-PLOTS_VIZ}.qzv
```

qiime tools view {TAXA-BAR-PLOTS_VIZ}.qzv

11 Export Data

```
Qiime tools export \  
--input-path taxtable.qza \  
--output-path exported-taxa-table
```

Should get a confirmation like this: Exported taxtable.qza as BIOMV210DirFmt to directory
exported-taxa-table

convert from biom to tsv for future analysis

```
Biom convert -i  
/path/to/biom/file -o table.tsv  
--to-tsv
```