

Activate the analysis environment

```
conda activate qiime2-amplicon-2024.2
```

Summarize the data from the demux file using QIIME commands**# Generate a visualization of demux summary**

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

QIIME will perform quality control on paired-end reads
It specifies to trim the left side of the forward read at position 0 and to truncate it at position 190. Subsequent commands will apply similar adjustments at different positions for reverse reads.

Following commands will instruct QIIME to generate three files having representative sequences, a data table, and statistics derived from the demux.qza data

```
qiime dada2 denoise-paired \  
  --i-demultiplexed-seqs demux.qza \  
  --p-trim-left-f 0 \  
  --p-trunc-len-f 190 \  
  --p-trim-left-r 0 \  
  --p-trunc-len-r 160 \  
  --o-representative-sequences rep-seqs.qza \  
  --o-table table.qza \  
  --o-denoising-stats stats.qza
```

This instruction compels QIIME to compile and format the metadata associated with our samples

This generates a visualization based on the stats.qza file produced earlier

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

This command mandates QIIME to aggregate all data in the feature table using the metadata.txt from our directory
Leveraging this data, it will generate visualizations for both the table and the sequences, utilizing the previously generated files

```
qiime feature-table summarize \  
  --i-table table.qza \  
  --o-visualization table.qzv \  
  --m-sample-metadata-file metadata.txt  
qiime feature-table tabulate-seqs \  
  --i-data rep-seqs.qza \  
  --o-visualization rep-seqs.qzv
```

This command initiates the download of a specific classifier file for taxonomy analysis

```
wget \  
  -O "gg-13-8-99-515-806-nb-classifier.qza" \  
  "https://data.qiime2.org/2024.2/common/gg-13-8-99-515-806-nb-  
classifier.qza"
```

This operation processes the data in the rep-seqs file to categorize the taxonomy based on the species identified in the subjects

```
qiime feature-classifier classify-sklearn \  
  --i-classifier gg-13-8-99-515-806-nb-classifier.qza \  
  --i-reads rep-seqs.qza \  
  --o-classification taxonomy.qza
```

This script constructs a visualization table displaying the taxonomic classifications derived from the earlier process

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

This script filters out mitochondria and chloroplast species from our taxonomy classification

It accomplishes this by manipulating data in the previously created table.qza file

```
qiime taxa filter-table \  
  --i-table table.qza \  
  --i-taxonomy taxonomy.qza \  
  --o-table filtered-table.qza
```

```
--p-exclude mitochondria,chloroplast \  
--o-filtered-table table.qza
```

Continuing to exclude mitochondria and chloroplast
This command directs QIIME to compile all data from the
feature table using the metadata.txt in our directory
It uses this compilation to generate visualizations for the table
and sequences, in conjunction with previously created files

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

This chunk generates a table showcasing the taxonomic
classification, now excluding mitochondria, based on previously
created files

```
qiime feature-classifier classify-sklearn \  
  --i-classifier gg-13-8-99-515-806-nb-classifier.qza \  
  --i-reads rep-seqs.qza \  
  --o-classification taxonomy.qza  
qiime metadata tabulate \  
  --m-input-file taxonomy.qza \  
  --o-visualization taxonomy.qzv
```

This command produces a taxonomy bar plot using the refined
table.qza, metadata file, and taxonomy file that exclude
mitochondria

```
qiime taxa barplot \  
  --i-table table.qza \  
  --i-taxonomy taxonomy.qza \  
  --m-metadata-file metadata.txt \  
  --o-visualization taxa-bar-plots.qzv
```

This command will eliminate highly variable positions
It then initiates the generation of multiple sequence
alignments

Using the representative sequences file, it will produce both an
unrooted and a rooted phylogenetic tree

```
qiime phylogeny align-to-tree-mafft-fasttree \  
  --i-sequences rep-seqs.qza \  
  --o-alignments rep-seqs-alignments.qza \  
  --o-rooted-tree rep-seqs-rooted-tree.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
```

Compute alpha and beta diversity metrics using a sample depth of 10000, ascertainable from the previously generated table.qzv
Utilizes the rooted tree file and the specified table, both mentioned above, along with the metadata file to generate comprehensive results in a folder named 'core metrics results'

```
qiime diversity core-metrics-phylogenetic \  
  --i-phylogeny rooted-tree.qza \  
  --i-table table.qza \  
  --p-sampling-depth 10000 \  
  --m-metadata-file metadata.txt \  
  --output-dir core-metrics-results
```

Examine the significance of alpha diversity

Utilizing the Shannon and Observed features files from the 'core metrics results' folder, it creates visualizations to discern any significant alpha diversity trends

```
qiime diversity alpha-group-significance \  
  --i-alpha-diversity core-metrics-results/shannon_vector.qza \  
  --m-metadata-file metadata.txt \  
  --o-visualization core-metrics-results/shannon_vector.qzv  
qiime diversity alpha-group-significance \  
  --i-alpha-diversity core-metrics-results/observed_features_vector.qza \  
  --m-metadata-file metadata.txt \  
  --o-visualization core-metrics-results/observed_features_vector.qzv
```

Generate visualizations of beta diversity significance for categories such as sex, population, and flock using the Bray Curtis dissimilarity matrix from the core metrics results

Separate commands are necessary for each different demographic category

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results/bray_curtis_distance_matrix.qza \  
  --m-metadata-file metadata.txt \  
  --m-metadata-column sex \  
  --o-visualization core-metrics-results/bray_curtis_distance_matrix.qzv
```

```
--o-visualization core-metrics-results/bray-curtis-sex-significance.qzv \  
--p-pairwise
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results/bray_curtis_distance_matrix.qza \  
  \  
  --m-metadata-file metadata.txt \  
  --m-metadata-column population \  
  --o-visualization core-metrics-results/bray-curtis-population-  
significance.qzv \  
  --p-pairwise
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results/bray_curtis_distance_matrix.qza \  
  \  
  --m-metadata-file metadata.txt \  
  --m-metadata-column flock \  
  --o-visualization core-metrics-results/bray-curtis-flock-significance.qzv \  
  --p-pairwise
```