```
#Project 2 QIIME script
#Commands for ASV analysis
#Importing demultiplexed sequences with manifest file
qiime tools import \
--type 'SampleData[SequencesWithQuality]' \
--input-path /mnt/datasets/project_2/voles/voles_manifest.txt \
--output-path demux3.qza \
--input-format SingleEndFastqManifestPhred33

#Generating a feature table with DADA2
qiime dada2 denoise-single
--i-demultiplexed-seqs ./demux3.qza
--p-trunc-len 180
--o-table ./dada2_table180.qza
--o-representative-sequences ./dada2_rep_set180.qza
--o-denoising-stats ./dada2_stats180.qza

#Viewing the feature table
qiime feature-table summarize \
--i-table ./dada2_table180.qza \
--m-sample-metadata-file ./metadata.tsv \
--o-visualization ./dada2_table180.qzv

#Generating alpha rarefaction curves for ASV
qiime diversity alpha-rarefaction
--i-table ./dada2_table180.qza
--m-metadata-file ./voles_metadata.txt
--o-visualization ./alpha_rarefaction_curves180.qzv
--p-min-depth 100
```

```
--p-max-depth 85000

#Generating a tree for ASV phylogenetic diversity analyses
qiime phylogeny align-to-tree-mafft-fasttree \
--i-sequences dada2_rep_set180.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

#Generating diversity metrics for ASV
qiime diversity core-metrics-phylogenetic
--i-table ./dada2_table180.qza
--i-phylogeny ./rooted-tree.qza
--m-metadata-file ./voles_metadata.txt
--p-sampling-depth 18000
--output-dir ./core-metrics-results18000

#Visualizing alpha diversity metrics of ASV Faith's PD and Pielou's evenness
qiime diversity alpha-group-significance \
--i-alpha-diversity ./core-metrics-results18000/faith_pd_vector.qza \
--m-metadata-file ./voles_metadata.txt \
--o-visualization ./core-metrics-results18000/faiths_pd_statistics.qzv

qiime diversity alpha-group-significance \
--i-alpha-diversity ./core-metrics-results18000/evenness_vector.qza \
--m-metadata-file ./voles_metadata.txt \
--o-visualization ./core-metrics-results18000/evenness_statistics.qzv
```

```
#Getting taxonomy classifier: Silva 138 99% OTUs from 515F/806R region of sequences
wget   -O "silva-138-99-515-806-nb-classifier" “https://data.qiime2.org/2021.8/common/silva-138-99-515-806-nb-classifier.qza”
```

```
#ASV silva taxonomy assignment
qiime feature-classifier classify-sklearn \
--i-reads ./dada2_rep_set180.qza \
--i-classifier ./silva-138-99-515-806-nb-classifier.qza \
--o-classification ./taxonomy180silva.qza
```

```
#Exporting ASV feature table for analysis in R
qiime tools export \
--input-path dada2_table180.qza \
--output-path exported3
```

```
#Exporting ASV taxonomy180silva to tsv for analysis in R
qiime tools export \
--input-path taxonomy180silva.qza \
--output-path exported3
```

```
#Exporting rooted-tree.qza for analysis in R
qiime tools export \
--input-path rooted-tree.qza \
--output-path exported3
```

```
#Editing the column names of ASV taxonomy.tsv
sed \
'1 s/Feature ID/#OTUID/;1 s/Taxon/taxonomy/;1 s/Confidence/confidence/' \
exported3/taxonomy.tsv \
```

```
> exported3/biom-taxonomy.tsv

#combining taxonomy with BIOM data
biom add-metadata \
--i exported3/feature-table.biom \
--o exported3/table-with-taxonomy.biom \
--observation-metadata-fp exported3/biom-taxonomy.tsv \
--sc-separated taxonomy

#Commands for OTU analysis
#downloading Greengenes 97% OTU database
wget -O "97_otus.fasta.gz" "https://github.com/biocore/qiime-default-reference/blob/master/qiime_default_reference/gg_13_8_otus/rep_set/97_otus.fasta.gz"

# Import Greengenes reference 97% OTU sequences
qiime tools import \
--type 'FeatureData[Sequence]' \
--input-path 97_otus.fasta \
--output-path 97_otus.qza


qiime vsearch cluster-features-open-reference
--i-table dada2_table180.qza
--i-sequences dada2_rep_set180.qza
--i-reference-sequences 97_otus.qza
--p-perc-identity 0.97
--o-clustered-table table-or-97.qza
--o-clustered-sequences rep-seqs-or-97.qza
--o-new-reference-sequences new-ref-seqs-or-97.qza
```

```
#Viewing the OTU feature table
qiime feature-table summarize \
--i-table ./table-or-97.qza \
--m-sample-metadata-file ./metadata.tsv \
--o-visualization ./table-or-97.qzv

#Generating alpha rarefaction curves for OTU
qiime diversity alpha-rarefaction
--i-table ./table-or-97.qza
--m-metadata-file ./voles_metadata.txt
--o-visualization ./alpha_rarefaction_curvesOTU.qzv
--p-min-depth 100
--p-max-depth 85000

#Generating a tree for OTU phylogenetic diversity analyses
qiime phylogeny align-to-tree-mafft-fasttree \
--i-sequences rep-seqs-or-97.qza \
--o-alignment aligned-rep-seqsOTU.qza \
--o-masked-alignment masked-aligned-rep-seqsOTU.qza \
--o-tree unrooted-treeOTU.qza \
--o-rooted-tree rooted-treeOTU.qza

#Generating diversity metrics for OTU
qiime diversity core-metrics-phylogenetic
--i-table ./table-or-97.qza
--i-phylogeny ./rooted-treeOTU.qza
--m-metadata-file ./voles_metadata.txt
--p-sampling-depth 18000
```

```
--output-dir ./core-metrics-resultsOTU

#Creating visualizations for alpha diversity metrics of OTU Faith's PD and Pielou's evenness
qiime diversity alpha-group-significance \
--i-alpha-diversity ./core-metrics-resultsOTU/faith_pd_vector.qza \
--m-metadata-file ./voles_metadata.txt \
--o-visualization ./core-metrics-resultsOTU/faiths_pd_statistics.qzv

qiime diversity alpha-group-significance \
--i-alpha-diversity ./core-metrics-resultsOTU/evenness_vector.qza \
--m-metadata-file ./voles_metadata.txt \
--o-visualization ./core-metrics-resultsOTU/evenness_statistics.qzv



#97% OTU SILVA taxonomy assignment
qiime feature-classifier classify-sklearn
--i-classifier silva-138-99-515-806-nb-classifier.qza
--i-reads rep-seqs-or-97.qza
--o-classification taxonomyOTUsilva.qza

#Exporting OTU feature table
qiime tools export \
--input-path table-or-97.qza\
--output-path exportedOTU2

#Exporting taxonomyOTUsilva to tsv for analysis in R
qiime tools export \
--input-path taxonomyOTUsilva.qza \
--output-path exportedOTU2
```

```
#Exporting rooted-treeOTU.qza for analysis in R
qiime tools export \
--input-path rooted-treeOTU.qza \
--output-path exportedOTU2

#Editing the column names of OTU taxonomy.tsv
sed \
'1 s/Feature ID/#OTUID/;1 s/Taxon/taxonomy/;1 s/Confidence/confidence/' \
exportedOTU2/taxonomy.tsv \
> exportedOTU2/biom-taxonomy.tsv

#combining taxonomy with BIOM data
biom add-metadata \
-i exportedOTU2/feature-table.biom \
-o exportedOTU2/table-with-taxonomy.biom \
--observation-metadata-fp exportedOTU2/biom-taxonomy.tsv \
--sc-separated taxonomy
```