

```
#===== Copying commands and scripts =====
```

```
mkdir -p $SCRATCH/NGS_metagenomics_Feb19/Scripts
cd $SCRATCH/NGS_metagenomics_Feb19/Scripts
cp /scratch/training/NGS_metagenomics/Feb19/*.sh .
```

```
#===== Setting the environment =====
```

```
module load Anaconda/3-5.0.0.1
source activate qiime2-2018.8
module load R_tamu/3.4.2-intel-2017A-Python-2.7.12-default-mt
```

```
#===== Importing Data =====
```

```
mkdir -p $SCRATCH/NGS_metagenomics_Feb19/Runs/FMT
cd $SCRATCH/NGS_metagenomics_Feb19/Runs/FMT

curl -sL "https://data.qiime2.org/2018.2/tutorials/fmt/sample_metadata.tsv" > "sample-metadata.tsv"

curl -sL "https://data.qiime2.org/2018.2/tutorials/fmt/fmt-tutorial-demux-1-1p.qza" > "fmt-tutorial-demux-1.qza"

curl -sL "https://data.qiime2.org/2018.2/tutorials/fmt/fmt-tutorial-demux-2-1p.qza" > "fmt-tutorial-demux-2.qza"
```

```
#===== Sequence QC + Making Feature Table using DADA2 =====
```

```
#===== Summarization =====
```

```
qiime demux summarize \
  --i-data fmt-tutorial-demux-1.qza \
  --o-visualization demux-summary-1.qzv
```

```
qiime demux summarize \
  --i-data fmt-tutorial-demux-2.qza \
  --o-visualization demux-summary-2.qzv
```

```
#For visualizing any *.qzv file, you can download it locally and view it online at: https://view.qiime2.org
#or run the command: "qiime tools view file.qzv" on HPRC portal > VNC session by logging into: portal.hprc.tamu.edu
```

```
#===== DADA2 =====
```

```
#Sample QC using DADA2 R Package. Only SE, thus, ran for R1 and R2 files separately and then results are merged.
```

```
qiime dada2 denoise-single \  
  --p-trim-left 13 \  
  --p-trunc-len 150 \  
  --i-demultiplexed-seqs fmt-tutorial-demux-1.qza \  
  --o-representative-sequences rep-seqs-1.qza \  
  --o-table table-1.qza \  
  --o-denoising-stats stats-1.qza
```

```
qiime dada2 denoise-single \  
  --p-trim-left 13 \  
  --p-trunc-len 150 \  
  --i-demultiplexed-seqs fmt-tutorial-demux-2.qza \  
  --o-representative-sequences rep-seqs-2.qza \  
  --o-table table-2.qza \  
  --o-denoising-stats stats-2.qza
```

```
#===== Merging =====
```

```
qiime feature-table merge \  
  --i-tables table-1.qza \  
  --i-tables table-2.qza \  
  --o-merged-table table.qza
```

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

```
qiime feature-table merge-seqs \  
  --i-data rep-seqs-1.qza \  
  --i-data rep-seqs-2.qza \  
  --o-merged-data rep-seqs.qza
```

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

```
#===== Creating tree for phylogenetic diversity analyses =====
```

```
qiime alignment mafft \  
  --i-sequences rep-seqs.qza \  
  --o-alignment aligned-rep-seqs.qza
```

```
qiime alignment mask \  
  --i-alignment aligned-rep-seqs.qza \  
  --o-masked-alignment masked-aligned-rep-seqs.qza
```

```
qiime phylogeny fasttree \  
  --i-alignment masked-aligned-rep-seqs.qza \  
  --o-tree unrooted-tree.qza
```

```
qiime phylogeny midpoint-root \  
  --i-tree unrooted-tree.qza \  
  --o-rooted-tree rooted-tree.qza
```

```
#===== Taxonomic analysis =====
```

```
curl -sL "https://data.qiime2.org/2018.2/common/gg-13-8-99-515-806-nb-  
classifier.qza" > "gg-13-8-99-515-806-nb-classifier.qza"
```

```
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
```

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

```
#===== Alpha Diversity Analysis and Visualization =====
```

```
qiime diversity core-metrics-phylogenetic \  
  --i-phylogeny rooted-tree.qza \  
  --i-table table.qza \  
  --p-sampling-depth 84 \  
  --m-metadata-file sample-metadata.tsv \  
  --output-dir core-metrics-results
```

```
qiime diversity alpha-group-significance \  
  --i-alpha-diversity core-metrics-results/faith_pd_vector.qza \  
  --m-metadata-file sample-metadata.tsv \  
  --o-visualization core-metrics-results/faith-pd-group-  
significance.qzv
```

```
qiime diversity alpha-group-significance \  
  --i-alpha-diversity core-metrics-results/faith_pd_vector.qza \  
  --m-metadata-file sample-metadata.tsv \  
  --o-visualization core-metrics-results/faith-pd-group-  
significance.qzv
```

```
--i-alpha-diversity core-metrics-results/evenness_vector.qza \  
--m-metadata-file sample-metadata.tsv \  
--o-visualization core-metrics-results/evenness-group-  
significance.qzv
```

Testing command:

```
qiime metadata tabulate \  
    --m-input-file core-metrics-results/faith_pd_vector.qza \  
    --o-visualization core-metrics-results/faith_pd_vector.qzv
```

#===== Beta Diversity Analysis and Visualization =====

```
qiime diversity beta-group-significance \  
    --i-distance-matrix core-metrics-results/  
unweighted_unifrac_distance_matrix.qza \  
    --m-metadata-file sample-metadata.tsv \  
    --m-metadata-column treatment-group \  
    --o-visualization core-metrics-results/unweighted-unifrac-treatment-  
group-significance.qzv \  
    --p-pairwise
```

```
qiime diversity beta-group-significance \  
    --i-distance-matrix core-metrics-results/  
unweighted_unifrac_distance_matrix.qza \  
    --m-metadata-file sample-metadata.tsv \  
    --m-metadata-column administration-route \  
    --o-visualization core-metrics-results/unweighted-unifrac-  
administration-route-significance.qzv \  
    --p-pairwise
```

#===== Alpha rarefaction plotting =====

```
qiime diversity alpha-rarefaction \  
    --i-table table.qza \  
    --i-phylogeny rooted-tree.qza \  
    --p-max-depth 380 \  
    --m-metadata-file sample-metadata.tsv \  
    --o-visualization alpha-rarefaction.qzv
```