#datasets import

time qiime tools import \

 --type 'SampleData[SequencesWithQuality]' \

 --input-path manifest.txt \

 --output-path demux.qza \

 --input-format SingleEndFastqManifestPhred33V2

time qiime tools import \

 --type 'SampleData[PairedEndSequencesWithQuality]' \

 --input-format PairedEndFastqManifestPhred33V2 \

 --input-path manifest-2.txt \

 --output-path demux.qza \

#DADA2 denoise

qiime dada2 denoise-single \

 --i-demultiplexed-seqs demux.qza \

 --p-trim-left 10 \

 --p-trunc-len 150 \

 --o-representative-sequences rep-seqs.qza \

 --o-table table.qza \

 --o-denoising-stats stats.qza

qiime dada2 denoise-paired \

 --i-demultiplexed-seqs demux.qza \

 --p-trim-left-f 10 \

 --p-trim-left-r 10 \

 --p-trunc-len-f 150 \

 --p-trunc-len-r 150 \

 --o-table table.qza \

 --o-representative-sequences rep-seqs.qza \

 --o-denoising-stats stats.qza

#merging datasets

qiime feature-table merge \

 --i-tables table1.qza \

 --i-tables table6.qza \

 --i-tables table8.qza \

 --i-tables table9.qza \

 --i-tables table10.qza \

 --i-tables table17.qza \

 --i-tables table18.qza \

 --i-tables table20.qza \

 --i-tables table21.qza \

 --o-merged-table table.qza

qiime feature-table merge-seqs \

 --i-data rep-seqs1.qza \

 --i-data rep-seqs6.qza \

 --i-data rep-seqs8.qza \

 --i-data rep-seqs9.qza \

 --i-data rep-seqs10.qza \

 --i-data rep-seqs17.qza \

 --i-data rep-seqs18.qza \

 --i-data rep-seqs20.qza \

 --i-data rep-seqs21.qza \

 --o-merged-data rep-seqs.qza

#create OTUs

qiime vsearch cluster-features-closed-reference \

 --i-table table.qza \

 --i-sequences rep-seqs.qza \

 --i-reference-sequences 97\_otus.qza \

 --p-perc-identity 0.97 \

 --o-clustered-table table-97.qza \

 --o-clustered-sequences rep-seqs-97.qza \

 --o-unmatched-sequences unmatched-97.qza

#filter samples

qiime feature-table filter-samples \

 --i-table table-97.qza \

 --p-min-frequency 1000 \

 --p-min-features 4 \

 --o-filtered-table table-97-filter1000.qza

qiime feature-table summarize \

 --i-table table-97-filter1000.qza \

 --o-visualization table-97-filter1000.qzv \

 --m-sample-metadata-file Metadata-1-21.txt

#output

qiime tools export \

 --input-path table-97-filter1000.qza \

 --output-path export-table-97-filter1000

biom convert -i export-table-97-filter1000/feature-table.biom -o export-table-97-filter1000.txt --to-tsv

time qiime feature-classifier classify-sklearn \

 --i-classifier 97-gg-classifier.qza \

 --i-reads rep-seqs-97.qza \

 --o-classification rep-seqs-97-taxonomy.qza

qiime tools export \

 --input-path rep-seqs-97-taxonomy.qza \

 --output-path rep-seqs-97-taxonomy

qiime taxa barplot \

 --i-table table-97-filter1000.qza \

 --i-taxonomy rep-seqs-97-taxonomy.qza \

 --m-metadata-file Metadata-1-21.txt \

 --o-visualization Taxa-bar-plots-97-filter1000.qzv

qiime phylogeny align-to-tree-mafft-fasttree \

 --i-sequences rep-seqs-97.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

qiime tools export \

 --input-path rooted-tree.qza \

 --output-path exported-tree

#diversity analysis and visualization

qiime diversity core-metrics-phylogenetic \

 --i-phylogeny rooted-tree.qza \

 --i-table table-97-filter1000.qza \

 --p-sampling-depth 1000 \

 --m-metadata-file Metadata-1-21.txt \

 --output-dir core-metrics-results

qiime diversity alpha-rarefaction \

 --i-table table-97-filter1000.qza \

 --i-phylogeny rooted-tree.qza \

 --p-min-depth 10 \

 --p-max-depth 5000 \

 --m-metadata-file Metadata-1-21.txt \

 --o-visualization alpha-rarefaction.qzv

#p value-alpha-group-significance

qiime diversity alpha-group-significance \

 --i-alpha-diversity core-metrics-results/faith\_pd\_vector.qza \

 --m-metadata-file Metadata-1-21.txt \

 --o-visualization core-metrics-results/alpha-faith-pd-group-significance.qzv

qiime diversity alpha-group-significance \

 --i-alpha-diversity core-metrics-results/observed\_features\_vector.qza \

 --m-metadata-file Metadata-1-21.txt \

 --o-visualization core-metrics-results/alpha-observed\_features\_vector-group-significance.qzv

qiime diversity alpha-group-significance \

 --i-alpha-diversity core-metrics-results/evenness\_vector.qza \

 --m-metadata-file Metadata-1-21.txt \

 --o-visualization core-metrics-results/evenness-group-significance.qzv

qiime diversity alpha-group-significance \

 --i-alpha-diversity core-metrics-results/shannon\_vector.qza \

 --m-metadata-file Metadata-1-21.txt \

 --o-visualization core-metrics-results/alpha-shannon\_vector-group-significance.qzv

#p value-beta-group-significance

qiime diversity beta-group-significance \

 --i-distance-matrix core-metrics-results/bray\_curtis\_distance\_matrix.qza \

 --m-metadata-file Metadata-1-21.txt \

 --m-metadata-column description \

 --o-visualization core-metrics-results/bray\_curtis\_description-significance.qzv \

 --p-pairwise

qiime diversity beta-group-significance \

 --i-distance-matrix core-metrics-results/jaccard\_distance\_matrix.qza \

 --m-metadata-file Metadata-1-21.txt \

 --m-metadata-column description \

 --o-visualization core-metrics-results/jaccard\_description-significance.qzv \

 --p-pairwise

qiime diversity beta-group-significance \

 --i-distance-matrix core-metrics-results/weighted\_unifrac\_distance\_matrix.qza \

 --m-metadata-file Metadata-1-21.txt \

 --m-metadata-column description \

 --o-visualization core-metrics-results/weighted\_unifrac\_description-significance.qzv \

 --p-pairwise

qiime diversity beta-group-significance \

 --i-distance-matrix core-metrics-results/unweighted\_unifrac\_distance\_matrix.qza \

 --m-metadata-file Metadata-1-21.txt \

 --m-metadata-column description \

 --o-visualization core-metrics-results/unweighted-unifrac-description-significance.qzv \

 --p-pairwise