Qiime2 analyzes the process code

qiime tools import \

 --type 'SampleData[PairedEndSequencesWithQuality]' \

 --input-path con-ova-manifest \

 --output-path con-ova-demux.qza \

 --input-format PairedEndFastqManifestPhred33V2

qiime demux summarize \

 --i-data con-ova-demux.qza \

 --o-visualization con-ova-demux.qzv

qiime dada2 denoise-paired \

 --i-demultiplexed-seqs con-ova-demux.qza \

 --p-trim-left-f 19 \

 --p-trim-left-r 20 \

 --p-trunc-len-f 290 \

 --p-trunc-len-r 270 \

 --p-n-threads 8 \

 --o-table con-ova-table.qza \

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

 --o-denoising-stats con-ova-denoising-stats.qza

qiime feature-table summarize \

 --i-table con-ova-table.qza \

 --o-visualization con-ova-table.qzv \

 --m-sample-metadata-file con-ova-metadata.tsv

qiime feature-table tabulate-seqs \

 --i-data con-ova-rep-seqs.qza \

 --o-visualization con-ova-rep-seqs.qzv

qiime metadata tabulate \

 --m-input-file con-ova-denoising-stats.qza \

 --o-visualization con-ova-denoising-stats.qzv

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

 --i-sequences con-ova-rep-seqs.qza \

 --o-alignment con-ova-aligned-rep-seqs.qza \

 --o-masked-alignment con-ova-masked-aligned-rep-seqs.qza \

 --o-tree con-ova-unrooted-tree.qza \

 --o-rooted-tree con-ova-rooted-tree.qza

qiime diversity core-metrics-phylogenetic \

 --i-phylogeny con-ova-rooted-tree.qza \

 --i-table con-ova-table.qza \

 --p-sampling-depth 6000 \

 --m-metadata-file con-ova-metadata.tsv \

 --output-dir con-ova-core-metrics-results

qiime diversity alpha-group-significance \

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

 --m-metadata-file con-ova-metadata.tsv \

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

qiime diversity alpha-group-significance \

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

 --m-metadata-file con-ova-metadata.tsv \

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

qiime diversity alpha-group-significance \

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

 --m-metadata-file con-ova-metadata.tsv \

 --o-visualization con-ova-core-metrics-results/shannon-group-significance.qzv

qiime diversity beta-group-significance \

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

 --m-metadata-file con-ova-metadata.tsv \

 --m-metadata-column group \

 --o-visualization con-ova-core-metrics-results/unweighted-unifrac-group-significance.qzv \

 --p-pairwise

qiime diversity beta-group-significance \

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

 --m-metadata-file con-ova-metadata.tsv \

 --m-metadata-column group \

 --o-visualization con-ova-core-metrics-results/weighted-unifrac-group-significance.qzv \

 --p-pairwise

qiime diversity beta-group-significance \

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

 --m-metadata-file con-ova-metadata.tsv \

 --m-metadata-column group \

 --o-visualization con-ova-core-metrics-results/bray-curtis-group-significance.qzv \

 --p-pairwise

qiime diversity alpha-rarefaction \

 --i-table con-ova-table.qza \

 --i-phylogeny con-ova-rooted-tree.qza \

 --p-max-depth 10000 \

 --m-metadata-file con-ova-metadata.tsv \

 --o-visualization alpha-rarefaction.qzv

qiime feature-classifier classify-sklearn \

 --i-classifier classifier-v3v4.qza \

 --i-reads con-ova-rep-seqs.qza \

 --o-classification con-ova-taxonomy.qza

qiime metadata tabulate \

 --m-input-file con-ova-taxonomy.qza \

 --o-visualization con-ova-taxonomy.qzv

qiime taxa barplot \

 --i-table con-ova-table.qza \

 --i-taxonomy con-ova-taxonomy.qza \

 --m-metadata-file con-ova-metadata.tsv \

 --o-visualization con-ova-taxa-bar-plots.qzv

Train the classifie-v3v4

qiime tools import \

 --type 'FeatureData[Sequence]' \

 --input-path gg\_13\_8\_otus/rep\_set/99\_otus.fasta \

 --output-path 99\_otus.qza

qiime tools import \

 --type 'FeatureData[Taxonomy]' \

 --input-format HeaderlessTSVTaxonomyFormat \

 --input-path gg\_13\_8\_otus/taxonomy/99\_otu\_taxonomy.txt \

 --output-path ref-taxonomy.qza

time qiime feature-classifier extract-reads \

 --i-sequences 99\_otus.qza \

 --p-f-primer ACTCCTACGGGAGGCAGCA \

 --p-r-primer GGACTACHVGGGTWTCTAAT \

 --o-reads ref-seqs.qza

time qiime feature-classifier fit-classifier-naive-bayes \

 --i-reference-reads ref-seqs.qza \

 --i-reference-taxonomy ref-taxonomy.qza \

 --o-classifier classifier\_gg\_13\_8\_99\_V3-V4.qza

Picrust related files

qiime tools import \

--input-path 13\_5\_97\_otus.fasta \

--output-path ref-sequences.qza \

--type 'FeatureData[Sequence]'

qiime vsearch cluster-features-closed-reference \

--i-sequences con-ova-rep-seqs.qza \

--output-dir closed-ref-otu/ \

--i-table con-ova-table.qza \

--i-reference-sequences ref-sequences.qza \

--p-perc-identity 0.97 \

--p-threads 8

qiime tools export \

--input-path closed-ref-otu/clustered\_table.qza \

--output-path closed-ref-otu/

biom convert \

--to-tsv -i closed-ref-otu/feature-table.biom -o closed\_reference\_otu\_table.tsv

Related file download method

wget \

 -O "gg\_13\_8\_otus.tar.gz" \

 " ftp://greengenes.microbio.me/greengenes\_release/gg\_13\_5/gg\_13\_8\_otus.tar.gz"

wget \

 -O "gg\_13\_5\_otus.tar.gz" \

 "ftp://greengenes.microbio.me/greengenes\_release/gg\_13\_5/gg\_13\_5\_otus.tar.gz"

wget \

 -O "STAMP\_2\_1\_3.exe" \

 "https://github.com/dparks1134/STAMP/releases/download/v2.1.3/STAMP\_2\_1\_3.exe"