# 导入数据：生成qiime2要求的对象格式，time统计计算时间

time qiime tools import \

--type 'SampleData[PairedEndSequencesWithQuality]' \

--input-path manifest \

--output-path paired-end-demux.qza \

--input-format PairedEndFastqManifestPhred33V2

# 摘要可视化为表格

qiime demux summarize \

--i-data paired-end-demux.qza \

--o-visualization paired-end-demux.qzv

#dada2

mkdir 160-180

time qiime dada2 denoise-paired \

--i-demultiplexed-seqs paired-end-demux.qza \

--p-trim-left-f 25 \

--p-trim-left-r 26 \

--p-trunc-len-f 160 \

--p-trunc-len-r 180 \

--o-table ./160-180/table-160-180.qza \

--o-representative-sequences ./160-180/rep-seqs-160-180.qza \

--o-denoising-stats ./160-180/denoising-stats-160-180.qza

#对特征表统计进行进行可视化

qiime metadata tabulate \

--m-input-file ./160-180/denoising-stats-160-180.qza \

--o-visualization ./160-180/denoising-stats-160-180.qzv

#特征表汇总

time qiime feature-table summarize \

--i-table ./160-180/table-160-180.qza \

--o-visualization ./160-180/table-160-180.qzv \

#特征序列汇总

time qiime feature-table tabulate-seqs \

--i-data ./160-180/rep-seqs-160-180.qza \

--o-visualization ./160-180/rep-seqs-160-180.qzv

#构建进化树

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

--i-sequences ./160-180/rep-seqs-160-180.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

# Alpha和beta多样性分析

time qiime diversity core-metrics-phylogenetic \

--i-phylogeny rooted-tree.qza \

--i-table ./160-180/table-160-180.qza \

--p-sampling-depth 12111 \

--m-metadata-file sample-metadata.tsv \

--output-dir ./160-180/core-metrics-resul

#Alpha多样性组间显著性分析和可视化

qiime diversity alpha-group-significance \

--i-alpha-diversity ./160-180/core-metrics-resul/faith\_pd\_vector.qza \

--m-metadata-file sample-metadata.tsv \

--o-visualization ./160-180/core-metrics-resul/faith-pd-group-significance.qzv

qiime diversity alpha-group-significance \

--i-alpha-diversity ./160-180/core-metrics-resul/evenness\_vector.qza \

--m-metadata-file sample-metadata.tsv \

--o-visualization ./160-180/core-metrics-resul/evenness-group-significance.qzv

#Alpha稀疏曲线。

time qiime diversity alpha-rarefaction \

--i-table ./160-180/table-160-180.qza \

--i-phylogeny rooted-tree.qza \

--p-max-depth 50000 \

--m-metadata-file sample-metadata.tsv \

--o-visualization ./160-180/alpha-rarefaction.qzv

# green geen数据库物种注释

time qiime feature-classifier classify-sklearn \

--i-classifier ../gg-13-8-99-515-806-nb-classifier.qza \

--i-reads ./160-180/rep-seqs-160-180.qza \

--o-classification ./160-180/taxonomy.qza

#可视化

time qiime metadata tabulate \

--m-input-file ./160-180/taxonomy.qza \

--o-visualization ./160-180/taxonomy.qzv

#绘图堆叠柱状图

time qiime taxa barplot \

--i-table ./160-180/table-160-180.qza \

--i-taxonomy ./160-180/taxonomy.qza \

--m-metadata-file sample-metadata.tsv \

--o-visualization ./160-180/taxa-bar-plots.qzv

#silva数据库物种注释

time qiime feature-classifier classify-sklearn \

--i-classifier ../silva-138-99-515-806-nb-classifier.qza \

--i-reads ./160-180/rep-seqs-160-180.qza \

--o-classification ./160-180/silva\_taxonomy.qza

#可视化

time qiime metadata tabulate \

--m-input-file ./160-180/silva\_taxonomy.qza \

--o-visualization ./160-180/silva\_taxonomy.qzv

#绘图堆叠柱状图

time qiime taxa barplot \

--i-table ./160-180/table-160-180.qza \

--i-taxonomy ./160-180/silva\_taxonomy.qza \

--m-metadata-file sample-metadata.tsv \

--o-visualization ./160-180/silva\_taxa-bar-plots.qzv

#5.5.1 将qza格式转化为biom格式

time qiime tools export \

--input-path ./160-180/table-160-180.qza \

--output-path ./160-180/exported-feature-table

time qiime tools export \

--input-path ./160-180/taxonomy.qza \

--output-path ./160-180/exported-feature-table

#5.5.2 修改taxonomy的表头

sed 's/Feature ID\tTaxon/#Feature ID\ttaxonomy/' ./160-180/exported-feature-table/taxonomy.tsv >160-180/exported-feature-table/taxonomy\_add\_head.tsv

#5.5.3 合并，给feature表添加taxonomy

biom add-metadata -i ./160-180/exported-feature-table/feature-table.biom \

--observation-metadata-fp ./160-180/exported-feature-table/taxonomy\_add\_head.tsv \

--sc-separated taxonomy -o ./160-180/exported-feature-table/feature\_table\_tax.biom

#5.5.4 将导出的biom格式feature-table转化为可读的txt格式

biom convert -i ./160-180/exported-feature-table/feature\_table\_tax.biom -o ./160-180/exported-feature-table/feature\_table\_tax.txt --to-tsv --header-key taxonomy