QIIME2 for bacteria

time qiime tools import \

--type 'SampleData[PairedEndSequencesWithQuality]' \

--input-path pe-64-manifest \

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

--input-format PairedEndFastqManifestPhred33V2

time qiime demux summarize \

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

--o-visualization demux.qzv

time qiime dada2 denoise-paired \

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

--p-trunc-len-f 0 \

--p-trunc-len-r 0 \

--o-table table.qza \

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

--o-denoising-stats denoising-stats.qza

qiime feature-table summarize \

--i-table table.qza \

--o-visualization table.qzv

qiime feature-table tabulate-seqs \

--i-data rep-seqs.qza \

--o-visualization rep-seqs.qzv

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

--i-sequences rep-seqs.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 diversity core-metrics-phylogenetic \

--i-phylogeny rooted-tree.qza \

--i-table table.qza \

--p-sampling-depth 4000 \

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

--output-dir core-metrics-results

qiime feature-classifier classify-sklearn \

--i-classifier 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

QIIME2 for fungi

time qiime tools import \

--type 'SampleData[PairedEndSequencesWithQuality]' \

--input-path pe-64-manifest \

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

--input-format PairedEndFastqManifestPhred33V2

time qiime demux summarize \

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

--o-visualization demux.qzv

time qiime dada2 denoise-paired \

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

--p-trunc-len-f 0 \

--p-trunc-len-r 0 \

--o-table table.qza \

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

--o-denoising-stats denoising-stats.qza

qiime feature-table summarize \

--i-table table.qza \

--o-visualization table.qzv

qiime feature-table tabulate-seqs \

--i-data rep-seqs.qza \

--o-visualization rep-seqs.qzv

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

--i-sequences rep-seqs.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 diversity core-metrics-phylogenetic \

--i-phylogeny rooted-tree.qza \

--i-table table.qza \

--p-sampling-depth 4000 \

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

--output-dir core-metrics-results

Taxonomy was assigned to ASVs using against Warcup Database (rdp.cme.msu.edu/classifier/detail.jsp?root=1&depth=10&confidence=0.8&cncorrected=no) for fungi.

PCoA

library(vegan)

library(ggplot2)

library(RColorBrewer)

gene <- read.csv(file.choose(),header=T,row.names = 1)

meta<-read.csv(file.choose())

gene<-t(gene)

dis\_bray <- vegdist(gene, method = 'bray')

pcoa <- cmdscale(dis\_bray, k = 2, eig = TRUE, add = TRUE)

pcoa\_eig <- (pcoa$eig)[1:2] / sum(pcoa$eig)

sample\_site <- data.frame({pcoa$point})[1:2]

names(sample\_site)[1:2] <- c('PCoA1', 'PCoA2')

sample\_site$name <- rownames(gene)

sample\_site <- merge(sample\_site, meta, by = 'name', all.x = TRUE)

sample\_site$group<-as.factor(sample\_site$infection)

#plot\_color <- c(brewer.pal(9,"Paired"))

fig3<-ggplot(sample\_site) +

aes(x = PCoA1, y = PCoA2, colour = infection) +scale\_color\_manual(values=c(plot\_color))+

geom\_point(size = 4) +theme\_classic() + theme(panel.background = element\_blank(),

panel.grid.major = element\_blank(),

panel.grid.minor = element\_blank(),

legend.text = element\_text(size=12),

legend.position = 'right',

axis.text = element\_text(size=12),

axis.title = element\_text(size=12),

aspect.ratio = 1)+guides(color = guide\_legend(title="",ncol = 1))+

labs(x = paste('PCoA axis1: ', round(100 \* pcoa\_eig[1], 2),'%'), y = paste('PCoA axis2: ', round(100 \* pcoa\_eig[2], 2),'%'))+stat\_ellipse(aes(x = PCoA1, y = PCoA2,color=group),level = 0.95, show.legend = F)

PERMANOVA

library(vegan)

adonis\_result\_dis = adonis(dis\_bray, sample\_site, permutations = 999)

adonis\_result\_dis

Spearman correlation

library(WGCNA)

gene<-read.csv(file.choose(),row.names = 1)

gene <- t(gene)

gene2 <- t(gene2)

gene2<-read.csv(file.choose(),row.names = 1)

occor =corAndPvalue(gene, gene2 use = "pairwise.complete.obs", method="spearman")

occor.r = occor$cor

occor.p = occor$p

occor.r[occor.p>0.05] = 0

write.csv(gene,file="LC50-gene.csv")

Mantel tests

library(vegan)

phylum <- read.csv(file.choose(),header=T,row.names = 1)

gene <- read.csv(file.choose(),header=T,row.names = 1)

otu\_bray<-vegdist(phylum, method='bray')

gene1<-vegdist(gene, method='bray')

m=mantel(otu\_bray, gene1, method="spear", permutations=999)

m