Supplemental Document S2

Bioinformatic Scripts

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Associated Files

Ch1_*Kazachstania_slooffiae*_qPCR.xlsx

Kazachstania slooffiae qPCR results, components, and conditions[10].

Ch1_script_files.xlsx

Files for bioinformatic scripts.

Ch1_QIIME2_weighted_unifrac_PCOA_results.qza

QIIME2 weighted unifrax PCoA results.

Ch1_QIIME2_Faith's_phylogenetic_diversity_results.qza

Faith's phylogenetic diversity results.

Ch1_QIIME2_ENS_results.qza

Effective number of species (ENS) results.

Ch1_QIIME2_Shannon_diversity_index_results.qza

Shannon diversity index results.

Ch1_QIIME2_taxonomy_results.qzv

QIIME2 Silva 132 taxonomic results.

QIIME2

Associated files: Ch1_script_files.xlsx tabs: “Metadata_QIIME2_wNC”, “Metadata_QIIME2”, and “Metadata_QIIME2_NC”

```
#!/bin/bash -l
#SBATCH --job-name=swine_downstream

#SBATCH --mem-per-cpu=4G
#SBATCH --time=1-00:00:00
#SBATCH --ntasks=10
#SBATCH --nodes=1

#SBATCH --mail-user=qinghong@ksu.edu
#SBATCH --mail-type=ALL

module load QIIME2/2019.7
##Quality control on run20201112
cd /bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20201112
qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/00_RAWDATA/16S/run20201112 \
  --input-format CasavaOneEightSingleLanePerSampleDirFmt \
  --output-path demux-paired-end_run1.qza
qiime demux summarize \
  --i-data demux-paired-end_run1.qza \
  --o-visualization demux-paired-end_run1.qzv
qiime cutadapt trim-paired \
  --i-demultiplexed-sequences demux-paired-end_run1.qza \
  --p-front-f ^GTGCCAGCMGCCGCGGTAA \
  --p-front-r ^GGACTACHVGGGTWTCTAAT \
  --p-error-rate 0.1 \
  --p-discard-untrimmed True \
  --o-trimmed-sequences demux-primer-trimmed-end_run1.qza
qiime demux summarize \
  --i-data demux-primer-trimmed-end_run1.qza \
  --o-visualization demux-primer-trimmed-end_run1.qzv
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs demux-primer-trimmed-end_run1.qza \
  --p-trunc-len-f 225 \
  --p-trunc-len-r 225 \
  --p-trim-left-f 0 \
  --p-trim-left-r 0 \
  --p-n-threads 10 \
  --o-table table_run1.qza \
  --o-representative-sequences rep-seqs_run1.qza \
```

```
  --o-denoising-stats denoising-stats_run1.qza
qiime metadata tabulate \
  --m-input-file denoising-stats_run1.qza \
  --o-visualization denoising-stats_run1.qzv


##Quality control on run20210216
cd /bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210216
qiime tools import \
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210216/pe-32-
manifest \
  --input-format PairedEndFastqManifestPhred33V2 \
  --output-path demux-paired-end_run2.qza
qiime demux summarize \
  --i-data demux-paired-end_run2.qza \
  --o-visualization demux-paired-end_run2.qzv
qiime cutadapt trim-paired \
  --i-demultiplexed-sequences demux-paired-end_run2.qza \
  --p-front-f ^GTGCCAGCMGCCGCGGTAA \
  --p-front-r ^GGACTACHVGGGTWTCTAAT \
  --p-error-rate 0.1 \
  --p-discard-untrimmed True \
  --o-trimmed-sequences demux-primer-trimmed-end_run2.qza
qiime demux summarize \
  --i-data demux-primer-trimmed-end_run2.qza \
  --o-visualization demux-primer-trimmed-end_run2.qzv
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs demux-primer-trimmed-end_run2.qza \
  --p-trunc-len-f 225 \
  --p-trunc-len-r 225 \
  --p-trim-left-f 0 \
  --p-trim-left-r 0 \
  --p-n-threads 10 \
  --o-table table_run2.qza \
  --o-representative-sequences rep-seqs_run2.qza \
  --o-denoising-stats denoising-stats_run2.qza
qiime metadata tabulate \
  --m-input-file denoising-stats_run2.qza \
  --o-visualization denoising-stats_run2.qzv


###Quality control on run20210218
cd /bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210218
qiime tools import \
```

```
  --type 'SampleData[PairedEndSequencesWithQuality]' \
  --input-path
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210218/pe-32-
manifest \
  --input-format PairedEndFastqManifestPhred33V2 \
  --output-path demux-paired-end_run3.qza
qiime demux summarize \
  --i-data demux-paired-end_run3.qza \
  --o-visualization demux-paired-end_run3.qzv
qiime cutadapt trim-paired \
  --i-demultiplexed-sequences demux-paired-end_run3.qza \
  --p-front-f ^GTGCCAGCMGCCGCGGTAA \
  --p-front-r ^GGACTACHVGGGTWTCTAAT \
  --p-error-rate 0.1 \
  --p-discard-untrimmed True \
  --o-trimmed-sequences demux-primer-trimmed-end_run3.qza
qiime demux summarize \
  --i-data demux-primer-trimmed-end_run3.qza \
  --o-visualization demux-primer-trimmed-end_run3.qzv
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs demux-primer-trimmed-end_run3.qza \
  --p-trunc-len-f 225 \
  --p-trunc-len-r 225 \
  --p-trim-left-f 0 \
  --p-trim-left-r 0 \
  --p-n-threads 10 \
  --o-table table_run3.qza \
  --o-representative-sequences rep-seqs_run3.qza \
  --o-denoising-stats denoising-stats_run3.qza
qiime metadata tabulate \
  --m-input-file denoising-stats_run3.qza \
  --o-visualization denoising-stats_run3.qzv


##Merge all data and carry out analysis
cd /bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/Merge_Analysis
qiime feature-table merge \
  --i-tables
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20201112/table_r
un1.qza \
  --i-tables
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210216/table_r
un2.qza \
  --i-tables
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210218/table_r
un3.qza \
```

```
  --p-overlap-method 'sum' \
  --o-merged-table table.qza
qiime feature-table merge-seqs \
  --i-data
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20201112/rep-
seqs_run1.qza \
  --i-data
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210216/rep-
seqs_run2.qza \
  --i-data
/bulk/leet1/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/QC_run20210218/rep-
seqs_run3.qza \
  --o-merged-data rep-seqs.qza
qiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file Metadata_QIIME2_wNC.txt
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 11105 \
  --m-metadata-file Metadata_QIIME2_wNC.txt \
  --output-dir core-metrics-results
qiime diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-results/observed_otus_vector.qza \
  --m-metadata-file Metadata_QIIME2.txt \
  --o-visualization core-metrics-results/observed_otus_vector-group-significance.qzv
qiime diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-results/faith_pd_vector.qza \
  --m-metadata-file Metadata_QIIME2.txt \
  --o-visualization core-metrics-results/faith-pd-group-significance.qzv
qiime diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-results/shannon_vector.qza \
  --m-metadata-file Metadata_QIIME2.txt \
  --o-visualization core-metrics-results/shannon-group-significance.qzv
qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/bray_curtis_distance_matrix.qza \
```

```
  --m-metadata-file Metadata_QIIME2.txt \
  --m-metadata-column Stage \
  --o-visualization core-metrics-results/bray_curtis-Stage-significance.qzv
qiime diversity alpha-rarefaction \
  --i-table table.qza \
  --i-phylogeny rooted-tree.qza \
  --p-max-depth 11105 \
  --m-metadata-file Metadata_QIIME2_wNC.txt \
  --o-visualization core-metrics-results/alpha-rarefaction.qzv
qiime feature-classifier classify-sklearn \
  --i-classifier /bulk/qinghong/16S_Abby/silva-132-99-515-806-nb-classifier.qza \
  --i-reads rep-seqs.qza \
  --o-classification taxonomy_silva132.qza
qiime metadata tabulate \
  --m-input-file taxonomy_silva132.qza \
  --o-visualization taxonomy_silva132.qzv
qiime feature-table filter-samples \
  --i-table table.qza \
  --m-metadata-file Metadata_QIIME2_NC.txt \
  --p-exclude-ids True \
  --o-filtered-table table_NC_filtered.qza
qiime taxa barplot \
  --i-table table_NC_filtered.qza \
  --i-taxonomy taxonomy_silva132.qza \
  --m-metadata-file Metadata_QIIME2.txt \
  --o-visualization taxa-bar-plots_silva132.qzv

##Estimated number of species
cd
/bulk/leet1/Share_LeeLab/Swine_microbiome/KSU/Marker_genes/01_16S_Analysis/Merge_An
alysis
source /homes/qinghong/miniconda3/etc/profile.d/conda.sh
conda activate qiime2-2022.2
qiime feature-table filter-samples \
  --i-table table.qza \
  --m-metadata-file filtered_metadata_ENS.txt \
  --p-exclude-ids True \
  --o-filtered-table table_ENS_filtered.qza
qiime diversity alpha \
  --i-table table_ENS_filtered.qza \
  --p-metric enspie \
  --o-alpha-diversity ENS_vector.qza
qiime diversity alpha-group-significance \
  --i-alpha-diversity ENS_vector.qza \
  --m-metadata-file Swine_metadata_ENS.txt \
  --o-visualization ENS-group-significance.qzv
```

Principal Coordinate Analysis (PCoA)

```
# Associated files: Ch1_QIIME2_weighted_unifrac_PCOA_results.qza and Ch1_script_files.xlsx tab “Metadata_PCoA_and_Effective_Number”
# Open RStudio

# Install applications, if not installed previously
install.packages("tidyverse")
install.packages("qiime2R")
install.packages("plyr")
install.packages("ggpubr")

# Set working directory
setwd("QIIME2_Merge_Analysis/")

# Load applications
library("tidyverse")
library("qiime2R")
library ("plyr")
library("ggpubr")

#Generate PCoA plot points file
wunifrac<-read_qza("Ch1_QIIME2_weighted_unifrac_PCOA_results.qza")
SampleID <- ldply(wunifrac$data$Vectors$SampleID, data.frame)
PC1 <- ldply(wunifrac$data$Vectors$PC1, data.frame)
PC2 <- ldply(wunifrac$data$Vectors$PC2, data.frame)
merge <- c %>% add_column(PC1) %>% add_column(PC2)
write.csv(merge,"PCoA.csv")

# Combine the metadata (“Metadata_QIIME2” tab in Ch1_script_files.xlsx) and PCoA plot points (PCoA.csv) into one file and save as Metadata_PCoA_Combined.csv (already performed and generated tab “Metadata_PCoA_Combined.csv” in Ch1_script_files.xlsx)

Data <- read.csv("Swine_16S_Metadata-PCoA.csv",header=T)

#Generate PCoA plot
        ggscatter(Data, x = "PCoA_Axis_1", y = "PCoA_Axis_2",
                fill = "Stage_Diet",
        shape = 21,
                size = 2,
                ellipse = T,
                color = "Stage_Diet",
                palette = c(P = rgb(0.86, 0.44, 0.43),
                        N.1 = rgb(0.49, 0.69, 0.92),
                 N.2 = rgb(0.40, 0.57, 0.75),
                 N.3 = rgb(0.20, 0.28,0.38),
                 G = rgb(0.41, 0.68, 0.34)),
```

```
                xlab = "Axis 1 (20%)", ylab = "Axis 2 (13%)")
# Exported with width x height: 1000 x 651
# Close RStudio
```

Faith and Effective Number of Species (ENS) Diversity Results

```
# Associated files: Ch1_QIIME2_Faith's_phylogenetic_diversity_results.qza and
Ch1_QIIME2_ENS_results.qza
# Open RStudio

# Install applications, if not installed previously
install.packages("qiime2R")

# Set working directory
setwd("QIIME2_Merge_Analysis/")

# Load applications
library("qiime2R")

# Generate alpha diversity result files
index_Faith<-read_qza("Ch1_QIIME2_Faith's_phylogenetic_diversity_results.qza")
index_Faith_values<-index_Faith$data
index_ENS<-read_qza("Ch1_QIIME2_ENS_results.qza")
index_ENS_values<-index_ENS$data

# Save files
write.csv(index_Faith_values,"Faith.csv")
write.csv(index_ENS_values,"ENS.csv")
```

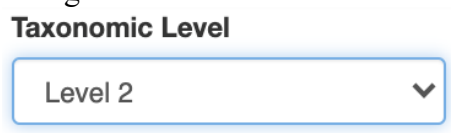
Shannon Transformation to Effective Number

```
# Associated files: Ch1_QIIME2_Shannon_diversity_index_results.qza and Ch1_script_files.xlsx tab “Metadata”
# Open RStudio

# Install applications, if not installed previously
install.packages("tidyverse")
install.packages("qiime2R")

# Set working directory
setwd("QIIME2_Merge_Analysis/")

# Load applications
library("tidyverse")
library("qiime2R")

# Generate Shannon diversity index plot points file
index<-read_qza("Ch1_QIIME2_weighted_unifrac_PCOA_results.qza")
index_values<-index$data

# Transform Shannon diversity index into effective number
index_values$shannon = exp(index_values$shannon)
write.csv(index_values,"Effective_Number.csv")

# Combine the metadata (“Metadata” tab in Ch1_script_files.xlsx) and effective number values ("Effective_Number.csv") into one file and save as Metadata-Effective_Number_Combined.csv (already performed and generated tab “Metadata-Effective_Number_Combined.csv” in Supplemental Table S7)

Data <- read.csv("Metadata-Effective_Number_Combined.csv",header=T)
Data$Day <- as.character(Data$Day)

# Generate box plot
Data %>%
mutate(name = fct_relevel(as.factor(Day), "30920", "31320", "31720", "32120", "32520", "32920", "40220", "40620", "41020", "41420", "41820", "42220", "42620", "43020", "50420", "50820", "51220", "51620", "81120", "52020", "52420", "52820", "60220", "60920","61620", "62320", "70720", "71420", "72120", "72820", "80420"))

ggplot(Data, aes(group=Day, y=Effective_Number)) +
    geom_boxplot(fill="slateblue", alpha=0.2) +
    theme(axis.text.x=element_blank(), axis.ticks.x=element_blank()) +
    ylab("Effective Number")
# Export as svg image
# Close RStudio
```

Heatmaps and DESeq2
Associated files: Ch1_QIIME2_taxonomy_results.qzv and Ch1_script_files.xlsx tabs: "Metadata", "leve_2", "level_6_RA>1%"

Phyla
Prepare Files for Heatmap and DESeq2

- Prepare count matrix
    - Use level 2 csv file from Ch1_QIIME2_taxonomy_results.qzv
        - Go to https://view.qiime2.org/ on your web browser
        - Upload taxa-bar-plots_silva152.qzv file
        - Navigate to level 2



        - Download csv file

    - Open a new Excel file and the csv file (level-2.csv)
    - Copy the contents of the csv and paste into the Excel file
    - Copy the Excel file contents, make a new tab in Excel and paste transposed (right click > "Paste Special" > "Transpose")
        - Taxa should now be in rows with one sample per column
    - Delete the first Excel tab (non-transposed data)
    - Delete the rows with "D_0__Bacteria;__" and "Unassigned;__" (both are unclassified bacteria)
    - Normalize data
        - Sum each column (total reads per sample)
        - Divide each cell by the total number of reads per sample and multiple by a multiple of 10
            - If total reads were 999,888,777, then multiply by 1,000,000,000
            - If total reads were 999,888,777,666, then multiply by 1,000,000,000,000
        - Use round =ROUND(CELL_NUMBER,0) Excel function to generate an integer for each cell
        - Copy and paste the last block of cells (rounded numbers) as values
        - Delete all other rows except first row and rounded/integers rows
            - Can check math by summing each column which should be a multiple of 10
            - Do not save the file with this summation
    - Save the Excel file as a csv file (level_2.csv)
    - Close web browser and Excel

Prepare for DESeq2
Open R studio
Install applications, if not already installed

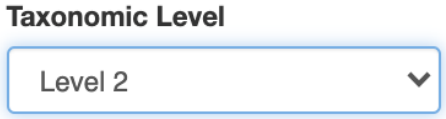
```
if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("DESeq2")

# Enter “a” when asked “Update all/some/none? [a/s/n]:”
# Enter “Yes” when asked “Do you want to install from sources the package which needs
compilation? (Yes/no/cancel)”

if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("pheatmap")

# Set working directory
setwd("DESeq2/")

# Load applications and files
library("DESeq2")
library("pheatmap")
coldata <- read.table("Metadata.csv", sep=",", header=TRUE)
cts_lvl2 <- read.table("level_2.csv", sep=",", header=TRUE, row.names=1)

# Perform differential analysis
dds_lvl2 <- DESeqDataSetFromMatrix(countData = cts_lvl2,
                                   colData = coldata,
                                   design= ~ Stage)
dds_lvl2 <- DESeq(dds_lvl2)

# Generate Heatmap
select_lvl2 <- order(rowMeans(counts(dds_lvl2,normalized=TRUE)),
                     decreasing=TRUE)[1:20]
df_lvl2 <- as.data.frame(colData(dds_lvl2)[,c("Stage")])
ntd_lvl2 <- normTransform(dds_lvl2)
rownames(df_lvl2) <- colnames(ntd_lvl2)
pheatmap(assay(ntd_lvl2), cluster_rows=TRUE, show_rownames=TRUE,
         cluster_cols=FALSE, annotation_col=df_lvl2)
pheatmap(assay(ntd_lvl2), cluster_rows=TRUE, show_rownames=T,
         cluster_cols=FALSE, annotation_col=df_lvl2, show_colnames = FALSE)
```

Genera

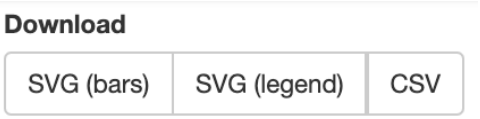
```
# Prepare Files for Heatmap and DESeq2
```

- Prepare count matrix
    - Use level 6 csv file from Ch1_QIIME2_taxonomy_results.qzv
        - Go to https://view.qiime2.org/
        - Upload taxa-bar-plots_silva152.qzv file

        - Navigate to level 6 (figure shows 2, but navigate to 6)



        - Download csv file



    - Open a new Excel file and the csv file (level-6.csv)
    - Copy the contents of the csv and paste into the Excel file
    - Copy the Excel file contents, make a new tab in Excel and paste transposed (right click > “Paste Special” > “Transpose”)
        - Taxa should not be in rows with one sample per column
    - Delete the first Excel tab (non-transposed data)
    - Delete the rows without a sixth level (only show D_4 or higher levels), rows with non-specific genera (i.e. D_5_uncultured bacterium), and rows with chloroplast, swine or mitochondria domains
    - Normalize data
        - Sum each column (total reads per sample)
        - Divide each cell by the total number of reads per sample and multiple by a multiple of 10
            - If total reads were 999,888,777, then multiply by 1,000,000,000
            - If total reads were 999,888,777,666, then multiply by 1,000,000,000,000
        - Use round =ROUND(CELL_NUMBER,0) Excel function to generate an integer for each cell
        - Copy and paste the last block of cells (rounded numbers) as values
        - Delete all other rows except first row and rounded/integers rows
            - Can check math by summing each column which should be a multiple of 10
            - Do not save the file with this summation
    - Combine all rows with less than 1% abundance into “other” category
    - Save the Excel file as a csv file (level-6.csv)

```
# Load file
cts_lvl6 <- read.table("level_6_RA>1%.csv", sep=",", header=TRUE, row.names=1)

# Perform differential analysis
dds_lvl6 <- DESeqDataSetFromMatrix(countData = cts_lvl6,
                                   colData = coldata,
                                   design= ~ Stage)
dds_lvl6 <- DESeq(dds_lvl6)

# Generate Heatmap
select_lvl6 <- order(rowMeans(counts(dds_lvl6,normalized=TRUE)),
            decreasing=TRUE)[1:20]
```

```
df_lvl6 <- as.data.frame(colData(dds_lvl6)[,c("Stage")])
ntd_lvl6 <- normTransform(dds_lvl6)
rownames(df_lvl6) <- colnames(ntd_lvl6)
pheatmap(assay(ntd_lvl6), cluster_rows=TRUE, show_rownames=TRUE,
        cluster_cols=FALSE, annotation_col=df_lvl6)
pheatmap(assay(ntd_lvl6), cluster_rows=TRUE, show_rownames=T,
        cluster_cols=FALSE, annotation_col=df_lvl6, show_colnames = FALSE)

# Close RStudio
```

SPIEC-EASI

Associated files: Ch1_Kazachstania_slooffiae_qPCR.xlsx “Results” tab, Ch1_script_files.xlsx tabs: “level_6_all_RA”, “Metadata”, “level_6_with_KS_PCR_P”, “level_6_Taxonomy_P”, “net1df_6_P”, “net1df_KS_6_P”, “vsize_6_P”, “net1df_KS_6_P”, “level_6_with_KS_PCR_N”, “level_6_Taxonomy_N”, “net1df_6_N”, “net1df_KS_6_N”, “vsize_6_N”, “net1df_KS_6_N”, “level_6_with_KS_PCR_G”, “level_6_Taxonomy_G”, “net1df_6_G”, “net1df_KS_6_G”, “vsize_6_G”, and “net1df_KS_6_G”

Prepare taxonomic files according to stage

- Utilize “level_6_all_RA” and “Metadata” tabs in in Ch1_script_files.xlsx to generate files which contain read counts and qPCR values (Ch1_*Kazachstania_slooffiae*_qPCR.xlsx; generate “level_6_with_KS_PCR” files) for taxa identified in each stage (P, N, G) (generate “Taxonomy” files)
- Include *Kazachstania slooffiae*
- Above steps already performed, and final files (which should be saved as csv files) are tabs in Ch1_script_files.xlsx : “level_6_with_KS_PCR_P”, “level_6_Taxonomy_P”, “level_6_with_KS_PCR_N”, “level_6_Taxonomy_N”, “level_6_with_KS_PCR_G”, and “level_6_Taxonomy_G”

```
# Open RStudio

# Install applications, if not installed previously
install.packages("devtools")
install.packages("SpiecEasi")
install.packages("phyloseq")
install.packages("igraph")
```

Set working directory

```
setwd("SPIEC-EASI/")
```

Load applications and file

```
library("devtools")
library("SpiecEasi")
library("phyloseq")
library("igraph")
sampledata <- read.csv("Metadata.csv", row.names=1)
```

Preweaning

```
# Load additional files for preweaning
otumat.6.P <-(read.csv("level_6_with_KS_PCR_P.csv", row.names=1))
taxmat.6.P <- as.matrix(read.csv("level_6_Taxonomy_P.csv", row.names=1))

# Prepare files for SPIEC-EASI
OTU.6.P = otu_table(otumat.6.P, taxa_are_rows = TRUE)
TAX.6.P = tax_table(taxmat.6.P)
abund.t.6.P <- t(OTU.6.P)
genus.6.P <- TAX.6.P[,6]
physeq.6.P = phyloseq(OTU.6.P, TAX.6.P, sampledata)

# Perform SPIEC-EASI
se.6.P <- spiec.easi(physeq.6.P, method='mb', lambda.min.ratio=1e-2, nlambda=20,
pulsar.params=list(rep.num=50))
net1.6.P <- adj2igraph(getRefit(se.6.P),  vertex.attr=list(name=taxa_names(physeq.6.P)))

# Adjust network layout
am.coord.6.P <- layout.fruchterman.reingold(net1.6.P)
net2.6.P <- adj2igraph(getRefit(se.6.P))
vsize.6.P <- rowMeans(clr(abund.t.6.P, 1))+10
write.csv(vsize.6.P,"vsize_6_P.csv", row.names = TRUE)
optbeta.6.P <- as.matrix(symBeta(getOptBeta(se.6.P)))
edge_cols.6.P <-  ifelse(optbeta.6.P>0, 'red', 'blue')[upper.tri(optbeta.6.P) &
optbeta.6.P!=0]
E(net2.6.P)$color=edge_cols.6.P
weights.6.P <- optbeta.6.P[upper.tri(optbeta.6.P) & optbeta.6.P!=0]*20+1
weights.6.P <- abs(weights.6.P)
E(net2.6.P)$weight <- weights.6.P
V(net2.6.P)$name <- genus.6.P

# Plot initial network (contains all connections)
plot(net2.6.P, layout = am.coord.6.P, vertex.size = vsize.6.P, edge.width =
E(net2.6.P)$weight, vertex.label.cex = 0.5, vertex.label.color = "black")
net2df.6.P <- get.data.frame(net2.6.P)
write.csv(net2df.6.P,"net1df_6_P.csv", row.names = FALSE)
#Manually remove any rows without Kazachstania slooffiae
#Edit vertex size file (vsize.csv) to contain only taxa and Kazachstania slooffiae in the
net2df file after removing rows without the fungus

# Import edited csv file containing only Kazachstania slooffiae connections
KS.df.6.P <-(read.csv("net1df_KS_6_P.csv"))
net3.6.P <- graph_from_data_frame(KS.df.6.P)

# Color vertices: bacteria green and fungi blue
V(net3.6.P)$color <- "seagreen1"
```

```
V(net3.6.P)$color[1] <- "skyblue1"

# Import file for vertex sizes
vsize.KS.6.P <- read.csv("vsize_KS_6_P.csv")

# Adjust layout of network
am.coord.KS.6.P <- layout.fruchterman.reingold(net3.6.P)
plot(net3.6.P,layout = am.coord.KS.6.P, vertex.size = vsize.KS.6.P$x, vertex.label.cex =
0.5, vertex.label.color = "black", edge.arrow.size=0.05, edge.width = KS.df.6.P$weight)

# Obtain stability
getStability(se.6.P)
# 0.03625496
```

Nursery

```
# Load additional files for nursery
otumat.6.N <-(read.csv("level_6_with_KS_PCR_N.csv", row.names=1))
taxmat.6.N <- as.matrix(read.csv("level_6_Taxonomy_N.csv", row.names=1))

# Prepare files for SPIEC-EASI
OTU.6.N = otu_table(otumat.6.N, taxa_are_rows = TRUE)
TAX.6.N = tax_table(taxmat.6.N)
abund.t.6.N <- t(OTU.6.N)
genus.6.N <- TAX.6.N[,6]
physeq.6.N = phyloseq(OTU.6.N, TAX.6.N, sampledata)

# Perform SPIEC-EASI
se.6.N <- spiec.easi(physeq.6.N, method='mb', lambda.min.ratio=1e-2, nlambda=20,
pulsar.params=list(rep.num=50))
net1.6.N <- adj2igraph(getRefit(se.6.N),  vertex.attr=list(name=taxa_names(physeq.6.N)))

# Adjust network layout
am.coord.6.N <- layout.fruchterman.reingold(net1.6.N)
net2.6.N <- adj2igraph(getRefit(se.6.N))
vsize.6.N <- rowMeans(clr(abund.t.6.N, 1))+10
write.csv(vsize.6.N,"vsize_6_N.csv", row.names = TRUE)
optbeta.6.N <- as.matrix(symBeta(getOptBeta(se.6.N)))
edge_cols.6.N <-  ifelse(optbeta.6.N>0, 'red', 'blue')[upper.tri(optbeta.6.N) &
optbeta.6.N!=0]
E(net2.6.N)$color=edge_cols.6.N
weights.6.N <- optbeta.6.N[upper.tri(optbeta.6.N) & optbeta.6.N!=0]*20+1
weights.6.N <- abs(weights.6.N)
E(net2.6.N)$weight <- weights.6.N
V(net2.6.N)$name <- genus.6.N

# Plot initial network (contains all connections)
plot(net2.6.N, layout = am.coord.6.N, vertex.size = vsize.6.N, edge.width =
E(net2.6.N)$weight, vertex.label.cex = 0.5, vertex.label.color = "black")
net2df.6.N <- get.data.frame(net2.6.N)
write.csv(net2df.6.N,"net1df_6_N.csv", row.names = FALSE)
#Manually remove any rows without Kazachstania slooffiae
#Edit vertex size file (vsize.csv) to contain only taxa and Kazachstania slooffiae in the
net2df file after removing rows without the fungus

# Import edited csv file containing only Kazachstania slooffiae connections
KS.df.6.N <-(read.csv("net1df_KS_6_N.csv"))
net3.6.N <- graph_from_data_frame(KS.df.6.N)


# Color vertices: bacteria green and fungi blue
```

```
V(net3.6.N)$color <- "seagreen1"
V(net3.6.N)$color[1] <- "skyblue1"

# Import file for vertex sizes
vsize.KS.6.N <- read.csv("vsize_KS_6_N.csv")

# Adjust layout of network
am.coord.KS.6.N <- layout.fruchterman.reingold(net3.6.N)
plot(net3.6.N,layout = am.coord.KS.6.N, vertex.size = vsize.KS.6.N$x, vertex.label.cex =
0.5, vertex.label.color = "black", edge.arrow.size=0.05, edge.width =KS.df.6.N$weight)

# Obtain stability
getStability(se.6.N)
#0.04480403
```

Growth Adult

```
# Load additional files for growth adult
otumat.6.G <-(read.csv("level_6_with_KS_PCR_G.csv", row.names=1))
taxmat.6.G <- as.matrix(read.csv("level_6_Taxonomy_G.csv", row.names=1))

# Prepare files for SPIEC-EASI
OTU.6.G = otu_table(otumat.6.G, taxa_are_rows = TRUE)
TAX.6.G = tax_table(taxmat.6.G)
abund.t.6.G <- t(OTU.6.G)
genus.6.G <- TAX.6.G[,6]
physeq.6.G = phyloseq(OTU.6.G, TAX.6.G, sampledata)

#Perform SPIEC-EASI
se.6.G <- spiec.easi(physeq.6.G, method='mb', lambda.min.ratio=1e-2, nlambda=20,
pulsar.params=list(rep.num=50))
net1.6.G <- adj2igraph(getRefit(se.6.G),  vertex.attr=list(name=taxa_names(physeq.6.G)))

#Adjust network layout
am.coord.6.G <- layout.fruchterman.reingold(net1.6.G)
net2.6.G <- adj2igraph(getRefit(se.6.G))
vsize.6.G <- rowMeans(clr(abund.t.6.G, 1))+10
write.csv(vsize.6.G,"vsize_6_G.csv", row.names = TRUE)
optbeta.6.G <- as.matrix(symBeta(getOptBeta(se.6.G)))
edge_cols.6.G <-  ifelse(optbeta.6.G>0, 'red', 'blue')[upper.tri(optbeta.6.G) &
optbeta.6.G!=0]
E(net2.6.G)$color=edge_cols.6.G
weights.6.G <- optbeta.6.G[upper.tri(optbeta.6.G) & optbeta.6.G!=0]*20+1
weights.6.G <- abs(weights.6.G)
E(net2.6.G)$weight <- weights.6.G
V(net2.6.G)$name <- genus.6.G

#Plot initial network (contains all connections)
plot(net2.6.G, layout = am.coord.6.G, vertex.size = vsize.6.G, edge.width =
E(net2.6.G)$weight, vertex.label.cex = 0.5, vertex.label.color = "black")
net2df.6.G <- get.data.frame(net2.6.G)
write.csv(net2df.6.G,"net1df_6_G.csv", row.names = FALSE)
#Manually remove any rows without Kazachstania slooffiae
#Edit vertex size file (vsize.csv) to contain only taxa and Kazachstania slooffiae in the
net2df file after removing rows without the fungus

# Import edited csv file containing only Kazachstania slooffiae connections
KS.df.6.G <-(read.csv("net1df_KS_6_G.csv"))
net3.6.G <- graph_from_data_frame(KS.df.6.G)


# Color vertices: bacteria green and fungi blue
```

```
V(net3.6.G)$color <- "seagreen1"
V(net3.6.G)$color[1] <- "skyblue1"


# Import file for vertex sizes
vsize.KS.6.G <- read.csv("vsize_KS_6_G.csv")

# Adjust layout of network
am.coord.KS.6.G <- layout.fruchterman.reingold(net3.6.G)
plot(net3.6.G,layout = am.coord.KS.6.G, vertex.size = vsize.KS.6.G$x, vertex.label.cex =
0.5, vertex.label.color = "black", edge.arrow.size=0.05,  edge.width=KS.df.6.G$weight)

# Obtain stability
getStability(se.6.G)
#0.03548979

# Close RStudio
```