ASV data intro manual

Data collection

DNA Extraction, 16S rRNA Gene Amplification, and Amplicon Sequencing was done at the Ziel NGS-Core Facility of the Technical University Muenchen (TUM).

Cite: Reitmeier S, Kiessling S, Clavel T, et al. Arrhythmic Gut Microbiome Signatures Predict Risk of Type 2 Diabetes. Cell Host Microbe. 2020;28(2):258-272.e6. doi:10.1016/j.chom.2020.06.004

Data transformation

The demulitplexed, per-sample, primer-free amplicon reads were processed with the DADA2 workflow https://benjjneb.github.io/dada2/tutorial.html.

See asv_creation_KORA_dada2.R script.

Result: 1. ASV table (seqtab2020.rds); 2. Taxonomic assignment (taxa2020.rds); 3. Phylogenetic tree (phylotree2020.phy).

Cite: Sommer et al. (TBD)

Intro to phyloseq data

Important Bioconductor tutorial to work with phyloseq data:
https://www.bioconductor.org/packages/devel/bioc/vignettes/phyloseq/inst/doc/phyloseq-analysis.html

Details about phyloseq structure: McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8(4):e61217. Published 2013 Apr 22. doi:10.1371/journal.pone.0061217

```
library(phyloseq)
library(ggplot2)
library(ggtree)
```

load the data

```
# set working directory
setwd('/Users/alicesommer/Desktop/Bureau/DOCTORATE/data_pipeline_microbiome')

# load microbiome data
ASV_table <- readRDS('dada2output/seqtab2020.rds')
taxon_assign <- readRDS('dada2output/taxa2020.rds')
# load phylogenetic information
load("dada2output/phylotree2020.phy")
```

create a phyloseq object

```
ps <- phyloseq(otu_table(ASV_table, taxa_are_rows=FALSE),
               #sample_data(sample_df), ## here you can add the KORA variables from your PV
               tax_table(taxon_assign),
               phy_tree(tGTR$tree))
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 15801 taxa and 2034 samples ]
## tax_table()   Taxonomy Table:    [ 15801 taxa by 7 taxonomic ranks ]
## phy_tree()    Phylogenetic Tree: [ 15801 tips and 15799 internal nodes ]
```

plot summary statistics of the ASV data

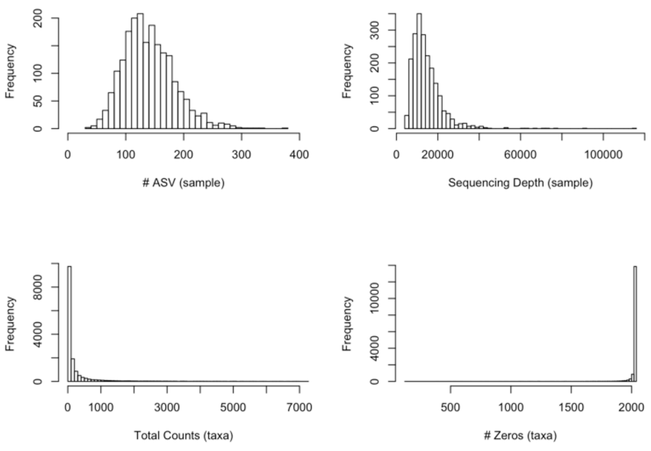
```
par(mfrow=c(2,2))

## nr. of ASV present
locate_ASV_in_sample <- apply(otu_table(ps), 1, function(x) sum(x != 0))
hist(locate_ASV_in_sample, breaks = 30, main = "", xlab = "# ASV (sample)", xlim = c(0,400))

## sequencing depth (count statistics accross each n)
hist(sample_sums(ps), breaks = 50, main = "", xlab = "Sequencing Depth (sample)")
# min(sample_sums(ps))

## count statistics accross each p
hist(taxa_sums(ps), breaks = 7000, main = "", xlab = "Total Counts (taxa)", xlim=c(1,7000))

## zero dist accross each p
zero_p <- apply(otu_table(ps), 2, function(x) sum(x == 0))
hist(zero_p, breaks = 100, main = "", xlab = "# Zeros (taxa)")
```



apply function on every row of taxa table

```
locate_NA_taxa <- apply(taxon_assign, 1, function(x) sum(is.na(x)))
table(locate_NA_taxa)
```

```
## locate_NA_taxa
##     0     1     2     3     4     5     6
##   468 12177  2573   538    26     9    10
```

plot a phylogenetic tree

```
tree_big <- ggtree(ps, aes(color=Phylum), branch.length='none', layout = "circular")
tree_big <- tree_big + theme(legend.position="bottom")

tree_big
```