Lab_10_Metagenomics_MBI3100A_2022_Assignment_Answers

2022-11-12

R Markdown

```
# clear the R environment
rm(list = ls())
```

Install required libraries

Installing these libraries may take some time. Try to update all other dependencies when prompted (type "a" and enter).

```
if (!require("BiocManager")) install.packages("BiocManager")
```

```
## Loading required package: BiocManager
```

```
if (!require("phyloseq")) BiocManager::install("phyloseq")
```

```
## Loading required package: phyloseq
```

```
if (!require("microbiomeMarker")) BiocManager::install("microbiomeMarkera")
```

```
## Loading required package: microbiomeMarker
```

```
## Registered S3 method overwritten by 'gplots':
##   method         from
##   reorder.factor DescTools
```

```
##
## Attaching package: 'microbiomeMarker'
```

```
## The following object is masked from 'package:phyloseq':
##
##     plot_heatmap
```

```
if (!require("tidyverse")) install.packages("tidyverse")
```

```
## Loading required package: tidyverse
```

```
## Found more than one class "atomicVector" in cache; using the first, from namespace 'Matrix'
## Also defined by 'Rmpfr'
## Found more than one class "atomicVector" in cache; using the first, from namespace 'Matrix'
## Also defined by 'Rmpfr'
## Found more than one class "atomicVector" in cache; using the first, from namespace 'Matrix'
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## Also defined by 'Rmpfr'
## Found more than one class "atomicVector" in cache; using the first, from namespace 'Matrix'
## Also defined by 'Rmpfr'
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.2 --
## v ggplot2 3.4.0      v purrr   0.3.5
## v tibble  3.1.8      v dplyr   1.0.10
## v tidyr   1.2.1      v stringr 1.4.1
## v readr   2.1.3      v forcats 0.5.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
if (!require("dendextend")) install.packages("dendextend")
```

```
## Loading required package: dendextend
## Registered S3 method overwritten by 'dendextend':
##   method     from
##   rev.hclust vegan
##
## ---------------------
## Welcome to dendextend version 1.16.0
## Type citation('dendextend') for how to cite the package.
##
## Type browseVignettes(package = 'dendextend') for the package vignette.
## The github page is: https://github.com/talgalili/dendextend/
##
## Suggestions and bug-reports can be submitted at: https://github.com/talgalili/dendextend/issues
## You may ask questions at stackoverflow, use the r and dendextend tags:
##   https://stackoverflow.com/questions/tagged/dendextend
##
##  To suppress this message use:  suppressPackageStartupMessages(library(dendextend))
## ---------------------
##
##
## Attaching package: 'dendextend'
##
## The following object is masked from 'package:stats':
##
##     cutree
```

Load Libraries

```
library(phyloseq)
library(ggplot2)
library(dplyr)
library(dendextend)
library(microbiomeMarker)
```

Data import

Question 1: Import the three files named as 'GP_sp_assignment_otu_table_df.csv', 'GP_sp_assignment_sample_data_df.csv', and 'GP_sp_assignment_tax_table_df.csv' and make a phyloseq object named 'asgmt_physeq'? (2 points)

Please provide the correct file/folder path

```
GP_sp_assignment_otu_table = read.table("./assignment_files/GP_sp_assignment_otu_table_df.csv",
           sep = "\t",
           header =  T,
           row.names = "otus")
my_OTU_table = otu_table(GP_sp_assignment_otu_table, taxa_are_rows = TRUE)
GP_sp_assignment_sample_data = read.table("./assignment_files/GP_sp_assignment_sample_data_df.csv",
           sep = "\t", header =  T,
           row.names = "sampleid")
my_Sample_data = sample_data(GP_sp_assignment_sample_data)
GP_sp_assignment_tax_table = read.table("./assignment_files/GP_sp_assignment_tax_table_df.csv",
           sep = "\t",
           header =  T,
           row.names = "otus")
my_tax_table = tax_table(as.matrix(GP_sp_assignment_tax_table))

asgmt_physeq = phyloseq(my_OTU_table, my_Sample_data, my_tax_table)
```

Print asgmt_physeq

```
print(asgmt_physeq)
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 1413 taxa and 9 samples ]
## sample_data() Sample Data:       [ 9 samples by 8 sample variables ]
## tax_table()   Taxonomy Table:    [ 1413 taxa by 7 taxonomic ranks ]
```

Question 2: How many taxa, samples, and sample vairables are there in asgmt_physeq? (1 point)

```

```

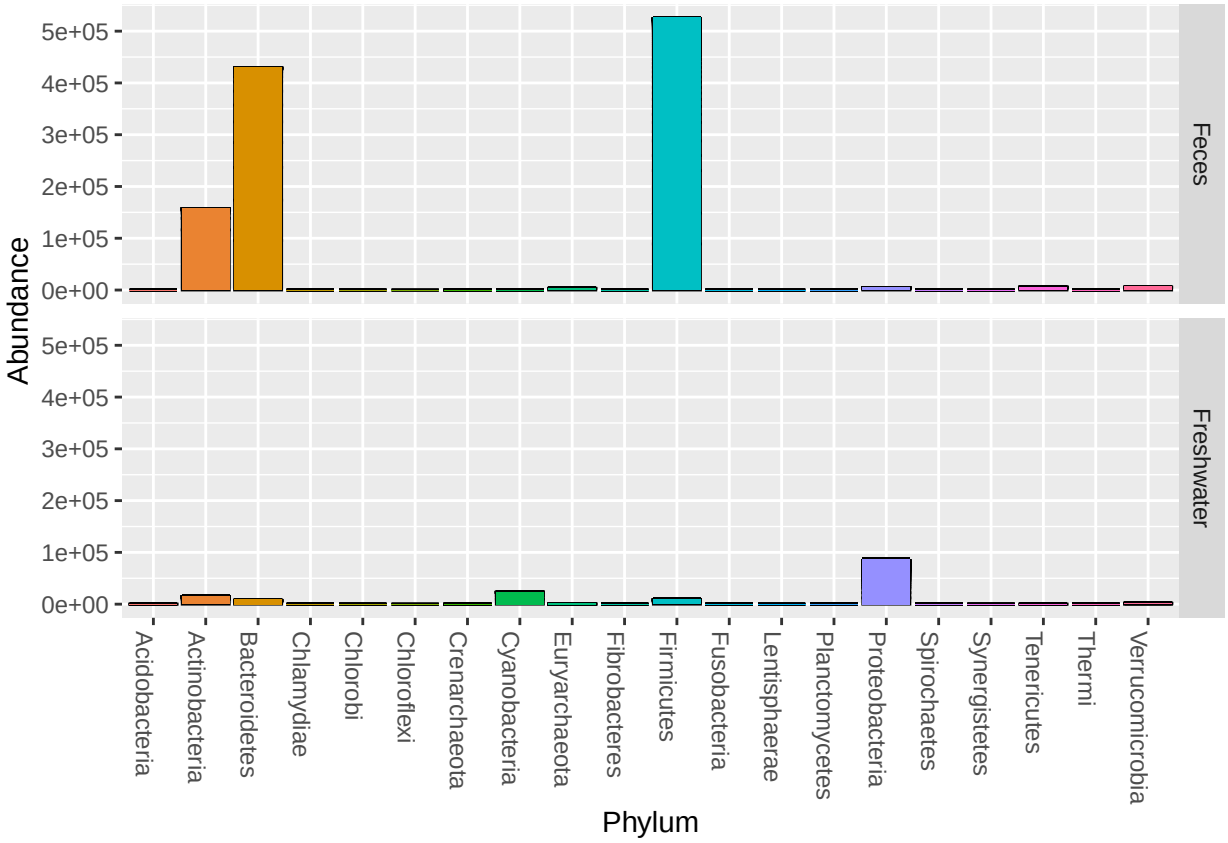
Question 3: List the catagories present in the sample variable "SampleOrigin". (1 point)

```
sample_data(asgmt_physeq)$SampleOrigin %>% as.factor %>% levels()
```

```
## [1] "Feces"      "Freshwater"
```

Question 4: Generate a bar plot for sample vs abudance and facet it for the catagories in SampleOrigin (1 point)

```
p = plot_bar(asgmt_physeq, x= "Phylum", fill = "Phylum", facet_grid=SampleOrigin~.)
p + theme(legend.position="none") + geom_bar(stat = "identity")
```



Question 5: Based on the plot generated in question 4, name all the phylum which big difference in abundance between "Feces" and "Freshwater" samples? (1 point)

Transform the absolute abundance into relative abundance and filter the taxa which have mean relative abundance less than 0.0001

```
# To convert to relative abundance
asgmt_physeq_r  = transform_sample_counts(asgmt_physeq, function(x) x / sum(x) )
# Keep the taxa which have a mean values at least 0.0001
asgmt_physeq_rf = filter_taxa(asgmt_physeq_r, function(x) mean(x) > 0.0001, TRUE)
asgmt_physeq_rf
```

```
## phyloseq-class experiment-level object
```

```
## otu_table()   OTU Table:         [ 238 taxa and 9 samples ]
## sample_data() Sample Data:       [ 9 samples by 8 sample variables ]
## tax_table()   Taxonomy Table:    [ 238 taxa by 7 taxonomic ranks ]
```

Question 6: How many taxa are left after the above filtering? (1 point)

For question 7 to 12, use dataset 'asgmt_physeq'.

Question 7: Generate a Hierarchical clustering plot using the distacne "ward.D2". (2 points)

It will be a four step process

```
asgmt_physeq_otu_df = phyloseq::otu_table(asgmt_physeq) %>%  data.frame()
asgmt_physeq_otu_df[1:5, 1:5]
```

Step1: Extract OTU table as data frame

```
##        M31Fcsw M11Fcsw LMEpi24M SLEpi20M AQC1cm
## 951          0       0        0        0      0
## 155495       0       0        0        0      0
## 1029         0       0        0        0      0
## 341551       0       0        0        0      0
## 108964       0       0        1        0      1
```

```
# transpose the table (required by vegdist)
asgmt_physeq_otu_df_t = t(asgmt_physeq_otu_df)
asgmt_physeq_otu_df_t[1:5, 1:5]
```

Step2: Transpose the table (required by vegdist package)

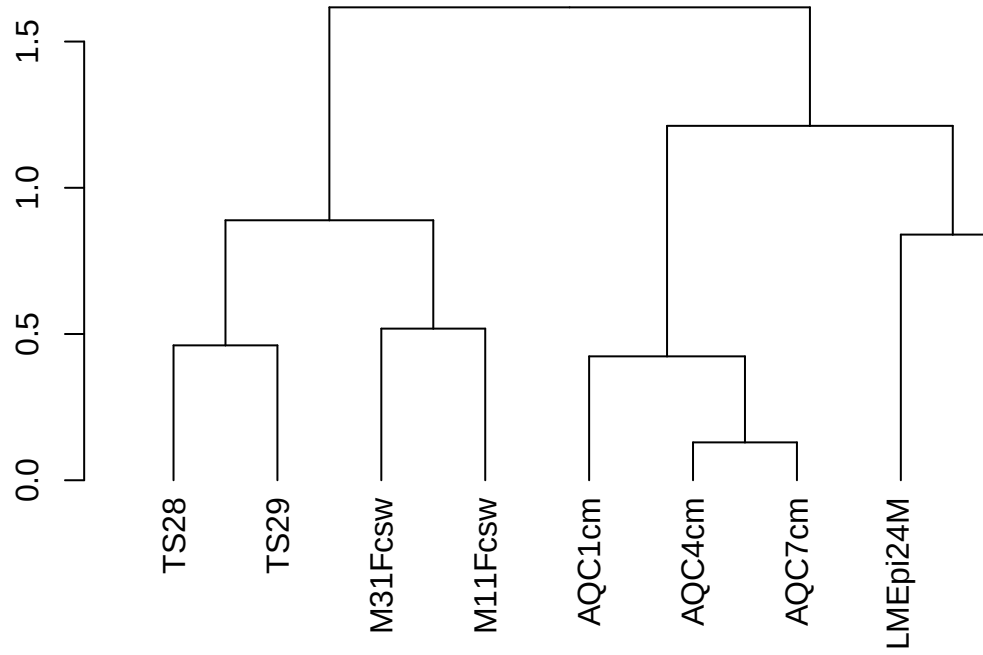
```
##          951 155495 1029 341551 108964
## M31Fcsw    0      0    0      0      0
## M11Fcsw    0      0    0      0      0
## LMEpi24M   0      0    0      0      1
## SLEpi20M   0      0    0      0      0
## AQC1cm     0      0    0      0      1
```

```
#compute Bray-Curtis dissimilarity
bc_dist = vegan::vegdist(asgmt_physeq_otu_df_t, method = "bray")
bc_dist
```

Step3: Compute Bray-Curtis dissimilarity

```
##            M31Fcsw   M11Fcsw  LMEpi24M  SLEpi20M    AQC1cm    AQC4cm    AQC7cm
## M11Fcsw  0.5184034
## LMEpi24M 0.9904053 0.9938943
## SLEpi20M 0.9970146 0.9972846 0.8399396
## AQC1cm   0.9805934 0.9828535 0.9318942 0.8449668
## AQC4cm   0.9957373 0.9961299 0.9451736 0.8869454 0.3939152
## AQC7cm   0.9960703 0.9965983 0.9485904 0.8737116 0.3498352 0.1294176
## TS28     0.6222115 0.7872989 0.9934247 0.9967061 0.9720616 0.9954971 0.9960830
## TS29     0.6348875 0.8073339 0.9918941 0.9974866 0.9784274 0.9965166 0.9969603
##               TS28
## M11Fcsw
## LMEpi24M
## SLEpi20M
## AQC1cm
## AQC4cm
## AQC7cm
## TS28
## TS29     0.4612095
```

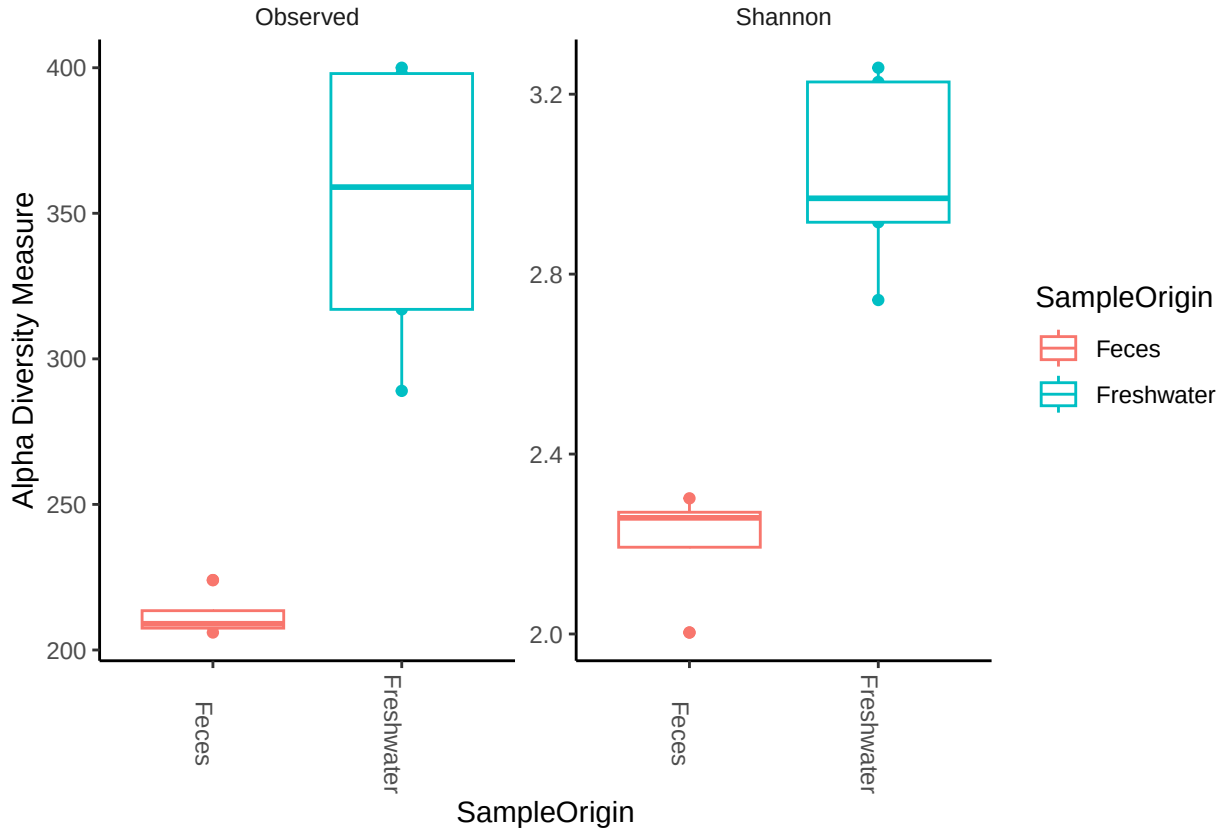
```
#Save as dendrogram
ward = as.dendrogram(hclust(bc_dist, method = "ward.D2"))

#Plot
plot(ward)
```



Step4: Save as dendrogram

Question 8: Plot for alpha diversity using two measures, "Observed" and "Shannon". (1 points)

```
plot_richness(asgmt_physeq, x="SampleOrigin", measures=c("Observed", "Shannon"), color = "SampleOrigin"
  geom_boxplot() +
  theme_classic() +
  theme(strip.background = element_blank(), axis.text.x.bottom = element_text(angle = -90))
```



Question 9: Apply wilcox.test to see if the Observed diversity is significantly different for SampleOrigin. (2 points)

```
# Make a dataframe to combine the ouputs of Observed, Shannon and SampleOrigin
my_alph_div = data.frame(
  "Observed" = phyloseq::estimate_richness(asgmt_physeq, measures = "Observed"),
  "Shannon" = phyloseq::estimate_richness(asgmt_physeq, measures = "Shannon"),
  "SampleOrigin" = phyloseq::sample_data(asgmt_physeq)$SampleOrigin)
head(my_alph_div)
```

Step 1: Make a dataframe to combine the outputs of Observed and SampleOrigin.

```
##          Observed  Shannon SampleOrigin
## M31Fcsw       210 2.256019        Feces
## M11Fcsw       206 2.003266        Feces
## LMEpi24M      317 2.742431   Freshwater
## SLEpi20M      289 3.227190   Freshwater
## AQC1cm        400 3.258820   Freshwater
## AQC4cm        398 2.915304   Freshwater
```

```
#Wilcoxon test for Shannon diversity for categories in SampleOrigin
my_alph_div_wt = wilcox.test(Shannon ~ SampleOrigin, data = my_alph_div, exact = FALSE, conf.int = TRUE)
print(my_alph_div_wt$p.value)
```

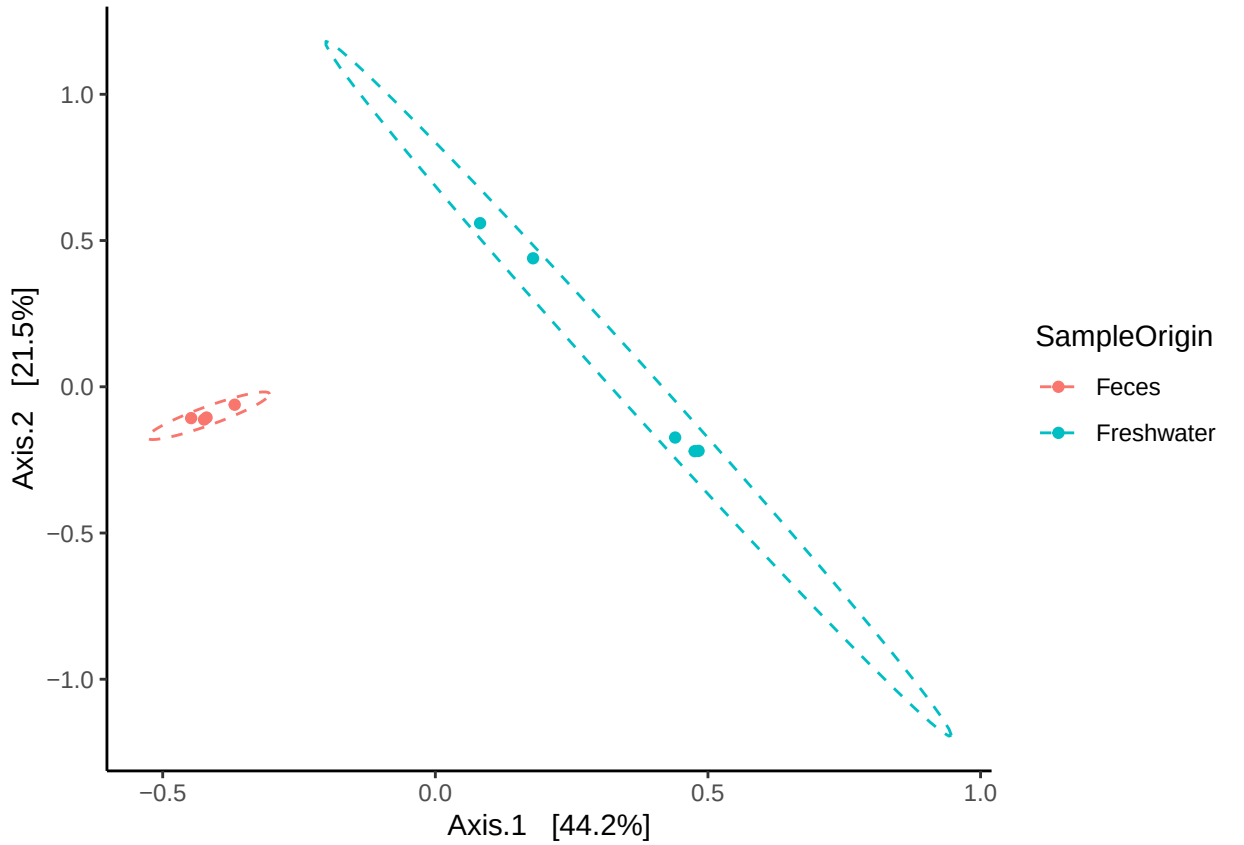
Step 2: Check the significance level for wilcox.test

```
## [1] 0.01996445
```

Note down the p-value? is the difference significant i.e is p-value less than 0.05?

Question 10: Make a PCoA plot using the "bray" method as distance the beta diversity. (1 point)

```
ordination = ordinate(asgmt_physeq, method="PCoA", distance="bray")
plot_ordination(asgmt_physeq, ordination, color="SampleOrigin") +
  theme_classic() +
  theme(strip.background = element_blank()) +
  stat_ellipse(linetype = 2)
```



Question 11: Apply DESeq2 method to identify the differentially abundant taxa based on SampleOrigin column. (1 point)

```
set.seed(2345)
# run_deseq2 command run the program deseq2 to identify DA taxa
# Running this command takes a few seconds
asgmt_physeq_deseq2 = run_deseq2(asgmt_physeq,
                            group = "SampleOrigin",
                            transform = "log10p", # log transformation
                            norm = "rarefy",  # common method for normalization
                            p_adjust = "BH", # adjusted p-value methods
                            )
```

```
## You set ‘rngseed‘ to FALSE. Make sure you’ve set & recorded
##  the random seed of your session for reproducibility.
## See ‘?set.seed‘
```

```
## ...
```

```
## 313OTUs were removed because they are no longer
## present in any sample after random subsampling
```

```
## ...
```

```
## converting counts to integer mode
```

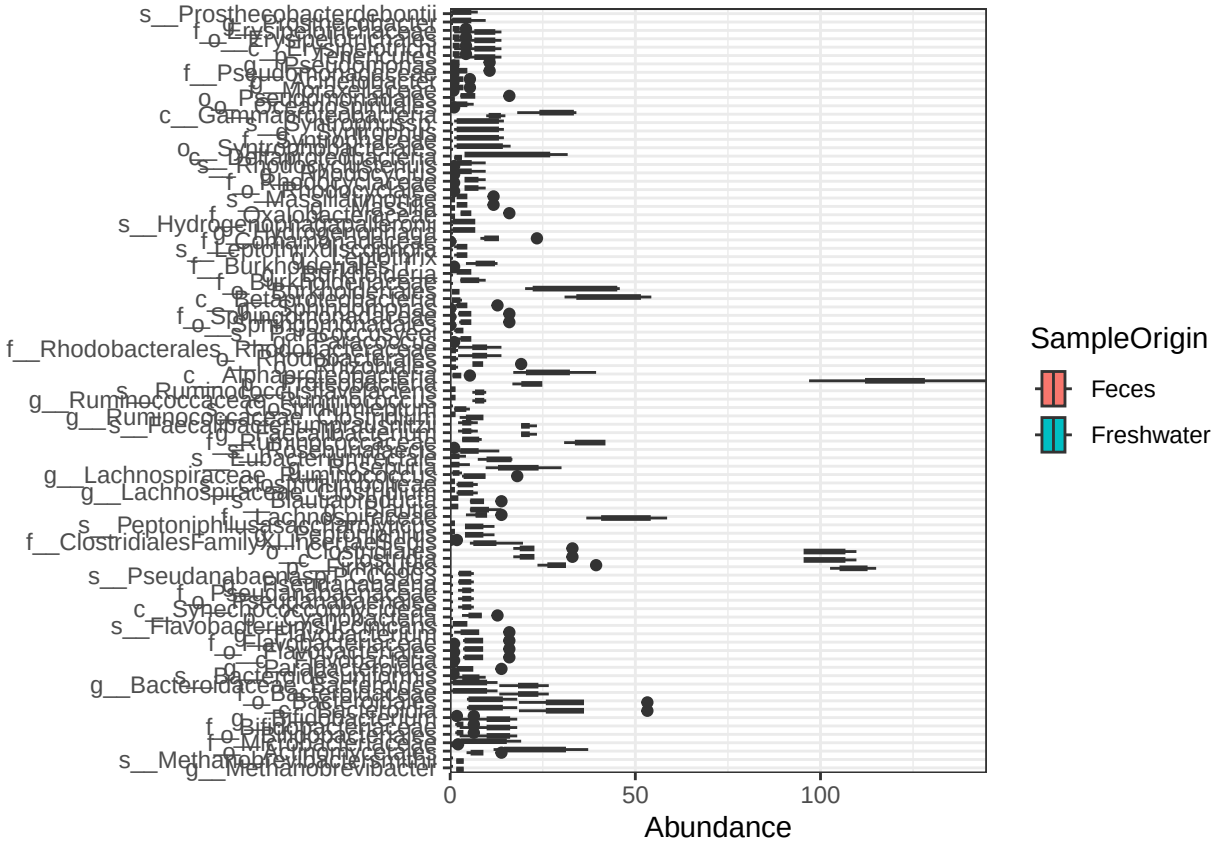
```
## -- note: fitType=’parametric’, but the dispersion trend was not well captured by the
##    function: y = a/x + b, and a local regression fit was automatically substituted.
##    specify fitType=’local’ or ’mean’ to avoid this message next time.
```

```
asgmt_physeq_deseq2
```

```
## microbiomeMarker-class inherited from phyloseq-class
## normalization method:              [ RLE ]
## microbiome marker identity method: [ DESeq2: Wald ]
## marker_table() Marker Table:       [ 91 microbiome markers with 5 variables ]
## otu_table()    OTU Table:          [ 837 taxa and  9 samples ]
## sample_data()  Sample Data:        [ 9 samples by  8 sample variables ]
## tax_table()    Taxonomy Table:     [ 837 taxa by 1 taxonomic ranks ]
```

Question 12: Plot the differentially abundant taxa identified by deseq2 method . (1 point)

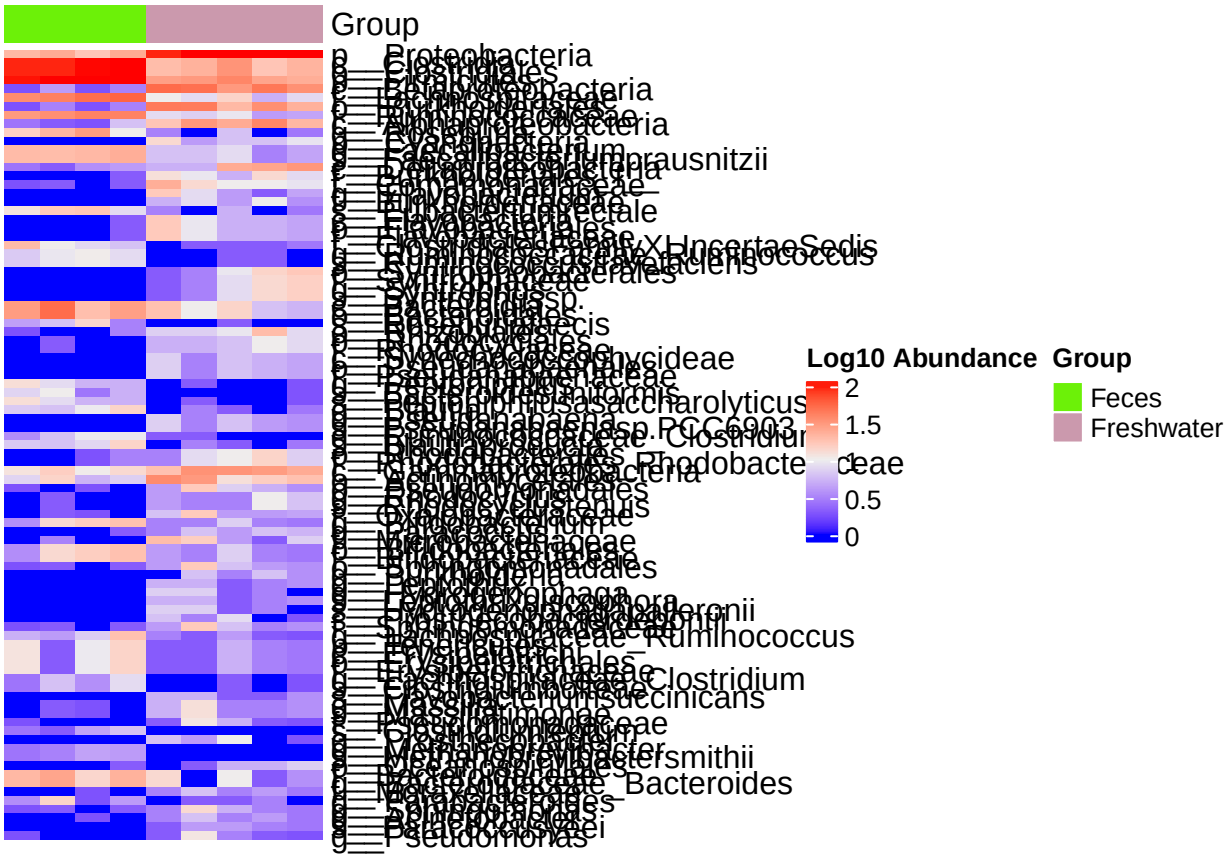
```
plot_DA =  microbiomeMarker::plot_abundance(asgmt_physeq_deseq2, group = "SampleOrigin")
plot_DA
```




```
plot_DA_hmap = microbiomeMarker::plot_heatmap(asgmt_physeq_deseq2, group = "SampleOrigin")
```

```
## Warning in transform_log10(otu): OTU table contains zeroes. Using log10(1 + x)
## instead.
```

```
plot_DA_hmap
```


Group
Log10 Abundance
2
1.5
1
0.5
0
Group
Feces
Freshwater