Purcell Project Markdown

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Load phyloseq object

Phyloseq object was generated on the server using serverScript.R, following the running of this script it was downloaded to the local machine and used to make plots

```
# load data
ps0  <- readRDS("data/ps_notree.rds")

# read metadata
meta <- read.csv("data/purcell_meta.csv")

# load metadata into phyloseq object
meta <- sample_data(meta)
meta$Individual <- as.factor(meta$Individual)
row.names(meta) <- meta$Sample_name
ps <- merge_phyloseq(ps0, meta)

# unedited phyloseq object
psOG <- ps

# Assign DNA sequences to refseq slot and replace with simple names to improve readability
dna <- Biostrings::DNAStringSet(taxa_names(ps))
names(dna) <- taxa_names(ps)
ps <- merge_phyloseq(ps, dna)
taxa_names(ps) <- paste0("ASV", seq(ntaxa(ps)))
ps
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 4872 taxa and 60 samples ]
## sample_data() Sample Data:       [ 60 samples by 7 sample variables ]
## tax_table()   Taxonomy Table:    [ 4872 taxa by 6 taxonomic ranks ]
## refseq()      DNAStringSet:      [ 4872 reference sequences ]
```

Filter out Yersinia

```
ps <- subset_taxa(ps, Genus != "Yersinia")
```

Custom Rarefaction Plot

Not run in this Markdown

```
# Data
psdata <- ps

# Loading required library and displaying core configuration
library('doParallel')
detectCores(all.tests = TRUE)

# Setting up and registering the cluster
cl <- makeCluster(detectCores(all.tests = TRUE)-1)
registerDoParallel(cl)

# Calculate alpha diversity
calculate_rarefaction_curves <- function(psdata, measures, depths, parallel = FALSE) {
  require('plyr') # ldply
  require('reshape2') # melt
  require('doParallel')

  # set parallel options if required
  if (parallel) {
    paropts <- list(.packages = c("phyloseq", "reshape2"))
  } else {
    paropts <- NULL
  }

  estimate_rarified_richness <- function(psdata, measures, depth) {
    if(max(sample_sums(psdata)) < depth) return()
    psdata <- prune_samples(sample_sums(psdata) >= depth, psdata)

    rarified_psdata <- rarefy_even_depth(psdata, depth, verbose = FALSE)

    alpha_diversity <- estimate_richness(rarified_psdata, measures = measures)

    # as.matrix forces the use of melt.array, which includes the Sample names (rownames)
    molten_alpha_diversity <- melt(as.matrix(alpha_diversity),
                                   varnames = c('Sample', 'Measure'),
                                   value.name = 'Alpha_diversity')

    molten_alpha_diversity
  }

  names(depths) <- depths # this enables automatic addition of the Depth to the output by ldply
  rarefaction_curve_data <- ldply(depths,
                                  estimate_rarified_richness,
                                  psdata = psdata,
                                  measures = measures,
                                  .id = 'Depth',
                                  .progress = ifelse(interactive() && ! parallel, 'text', 'none'),
                                  .parallel = parallel,
                                  .paropts = paropts)
```

```
  # convert Depth from factor to numeric
  rarefaction_curve_data$Depth <- as.numeric(levels(rarefaction_curve_data$Depth))[rarefaction_curve_da

  rarefaction_curve_data
}

rarefaction_curve_data <- calculate_rarefaction_curves(psdata, c('Observed'),
                                                       rep(c(1, 100, 1:150 * 1000),
                                                           each = 10))
summary(rarefaction_curve_data)
saveRDS(rarefaction_curve_data, file = "Purcell Final/Final_results/rare_object.rds")
```

```
# Data
psdata <- ps

# Load Rarefaction Curve Data Object
rarefaction_curve_data <- readRDS(file = "Purcell Final/Final_results/rare_object.rds")
summary(rarefaction_curve_data)
```

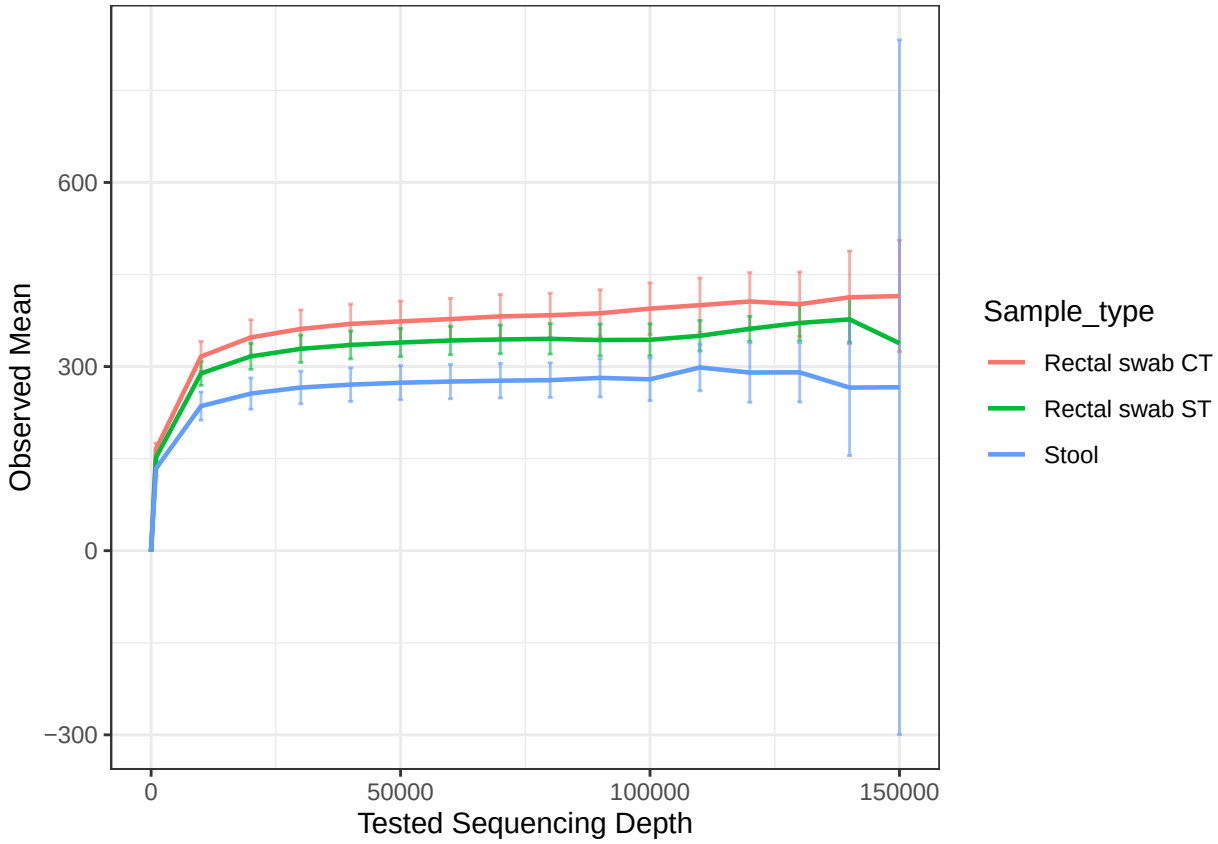
```
##      Depth            Sample          Measure      Alpha_diversity
##  Min.   :     1   X13A   : 1520   Observed:74070   Min.   :  1.0
##  1st Qu.: 29000   X14A   : 1520                    1st Qu.:263.0
##  Median : 60000   X14B   : 1520                    Median :331.0
##  Mean   : 62475   X15A   : 1520                    Mean   :320.4
##  3rd Qu.: 92000   X15C   : 1520                    3rd Qu.:378.0
##  Max.   :150000   X18A   : 1520                    Max.   :546.0
##                   (Other):64950
```

```
# Summarise alpha diversity
rarefaction_curve_data_summary <- ddply(rarefaction_curve_data,
                                        c('Depth', 'Sample', 'Measure'),
                                        summarise,
                                        Alpha_diversity_mean = mean(Alpha_diversity),
                                        Alpha_diversity_sd = sd(Alpha_diversity))

colnames(rarefaction_curve_data_summary) <- gsub("X","",
                                                 colnames(rarefaction_curve_data_summary))
rarefaction_curve_data_summary$Sample <- gsub("X","", rarefaction_curve_data_summary$Sample)

# Add sample data
rarefaction_curve_data_summary_verbose <- merge(rarefaction_curve_data_summary,
                                                data.frame(sample_data(psdata)),
                                                by.x = 'Sample',
                                                by.y = 'row.names')

# Produce summary df of rarefaction data
df_mod <- summarySE(rarefaction_curve_data_summary_verbose,
                    measurevar = "Alpha_diversity_mean",
                    groupvars = c("Depth", "Sample_type"))
```

```
## Warning in qt(conf.interval/2 + 0.5, datac$N - 1): NaNs produced
```

```
df_mod <- df_mod %>%
  subset(Depth == 1 | Depth == 1000 | Depth == 10000 | Depth == 20000 | Depth == 30000 | Depth == 40000
```

```
ggplot(df_mod, aes(x = Depth, y = Alpha_diversity_mean,
                   ymin = Alpha_diversity_mean - ci, ymax = Alpha_diversity_mean + ci,
                   colour = Sample_type)) +
  geom_errorbar(size = 0.5, width = 1000, alpha = 0.6) +
  geom_line(size = 0.8) +
  ylab("Observed Mean") +
  xlab("Tested Sequencing Depth")
```




```
ggsave("Purcell Final/Final_results/1)Rarefaction_Curve.pdf", width = 11, height = 8)
```

Rarefy

```
# Rarefy to even sequencing depth, 90% of minimum sample depth
ps_rare <- rarefy_even_depth(ps, rngseed = 1,
                             sample.size = 0.9 * min(sample_sums(ps)),
                             replace = FALSE)
```

```
## `set.seed(1)` was used to initialize repeatable random subsampling.

## Please record this for your records so others can reproduce.

## Try `set.seed(1); .Random.seed` for the full vector

## ...

## 1300TUs were removed because they are no longer
## present in any sample after random subsampling
```

```
## ...
```

```
sample_sums(ps)
```

```
##    10A    10B    10C    11A    11B    11C    12A    12B    12C    13A    13B
##  96045 149391 133488 101852  87122 135639  60955 141908 134481 164730 149672
##    13C    14A    14B    14C    15A    15B    15C    16A    16B    16C    17A
## 141290 154794 167659 131939 167015 106082 161420 111021 121601 134735 149622
##    17B    17C    18A    18B    18C    19A    19B    19C     1A     1B     1C
## 123675  91485 154502 123487 153504 152567 146504 115610 136572  75035  89197
##    20A    20B    20C     2A     2B     2C     3A     3B     3C     4A     4B
## 187470 110105 106713 107518 114008 100238 136162 126355 100973  98108 118848
##     4C     5A     5B     5C     6A     6B     6C     7A     7B     7C     8A
## 113301 153840 113210  84418 127131 147421  88442 134264 136792 114586 127880
##     8B     8C     9A     9B     9C
##  88579  99543  82179 139662 105460
```

```
sample_sums(ps_rare)
```

```
##   10A   10B   10C   11A   11B   11C   12A   12B   12C   13A   13B   13C   14A
## 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859
##   14B   14C   15A   15B   15C   16A   16B   16C   17A   17B   17C   18A   18B
## 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859
##   18C   19A   19B   19C    1A    1B    1C   20A   20B   20C    2A    2B    2C
## 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859
##    3A    3B    3C    4A    4B    4C    5A    5B    5C    6A    6B    6C    7A
## 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859 54859
##    7B    7C    8A    8B    8C    9A    9B    9C
## 54859 54859 54859 54859 54859 54859 54859 54859
```

Alpha Diversity

```
# Calculate alpha diversity, using Richness and Shannon
alpha_summary <- estimate_richness(ps_rare, measures = c("Observed", "Shannon"))
shapiro.test(alpha_summary$Observed)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha_summary$Observed
## W = 0.98663, p-value = 0.7541
```

```
shapiro.test(alpha_summary$Shannon)
```

```
##
##  Shapiro-Wilk normality test
##
## data:  alpha_summary$Shannon
## W = 0.97173, p-value = 0.1773
```

```
# Blocking Test
rO <- alpha_summary$Observed
rS <- alpha_summary$Shannon

f <- c("Clinician", "Self", "Stool") # treatment levels
```

```r
k <- 3 # number of treatment levels
n <- 20 # number of control blocks

tm <- gl(k, 1, n*k, factor(f)) # matching treatment
blk <- gl(n, k, k*n) # blocking factor

avO <- aov(rO ~ tm + blk)
summary(avO)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## tm           2 108335   54168  21.996 4.51e-07 ***
## blk         19 120686    6352   2.579  0.00635 **
## Residuals   38  93578    2463
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
avS <- aov(rS ~ tm + blk)
summary(avS)
```

```
##             Df Sum Sq Mean Sq F value   Pr(>F)
## tm           2  0.994  0.4970   8.835 0.000706 ***
## blk         19  4.005  0.2108   3.747 0.000260 ***
## Residuals   38  2.137  0.0562
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Test whether the observed number of OTUs differs significantly between samples
# p adjustment using Benjamini and Hochberg
pairwise.t.test(alpha_summary$Observed, sample_data(ps_rare)$Sample_type, p.adjust = "BH")
```

```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  alpha_summary$Observed and sample_data(ps_rare)$Sample_type
##
##                Rectal swab CT Rectal swab ST
## Rectal swab ST 0.0759         -
## Stool          6.3e-06        0.0015
##
## P value adjustment method: BH
```

```r
pairwise.t.test(alpha_summary$Shannon, sample_data(ps_rare)$Sample_type, p.adjust = "BH")
```

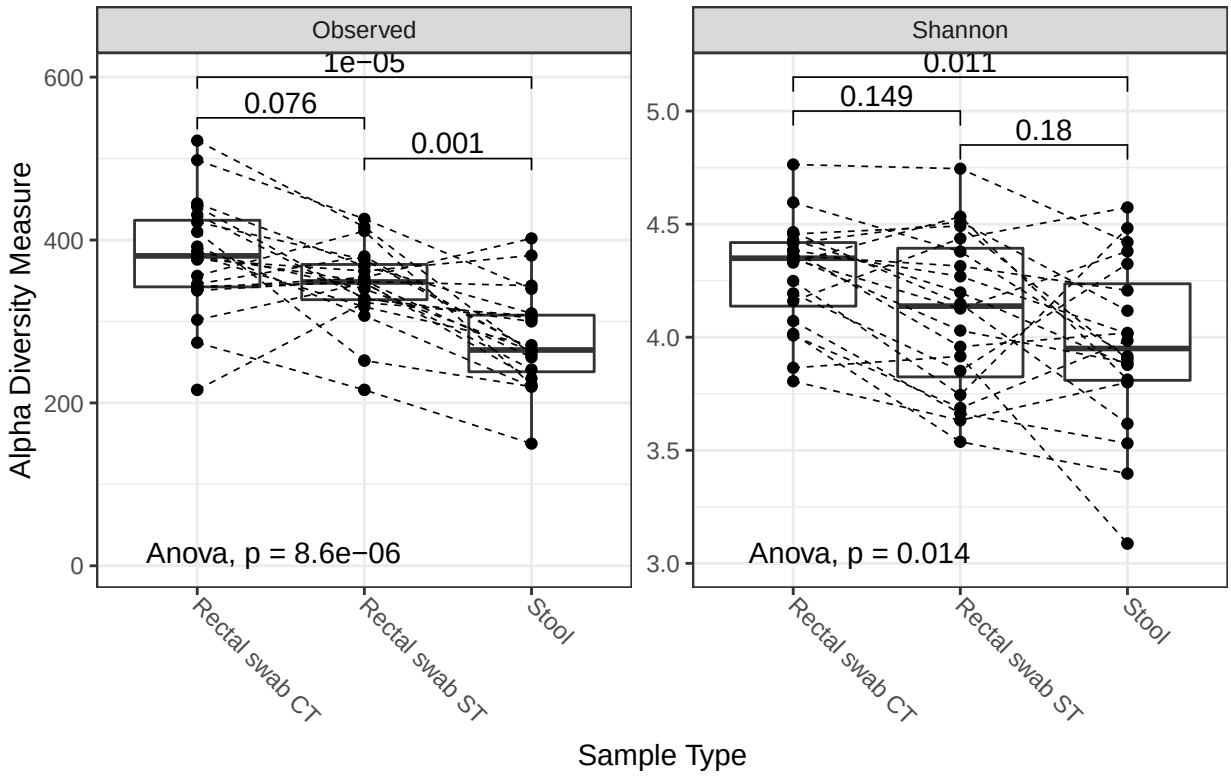
```
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  alpha_summary$Shannon and sample_data(ps_rare)$Sample_type
##
##                Rectal swab CT Rectal swab ST
## Rectal swab ST 0.149          -
## Stool          0.011          0.180
##
## P value adjustment method: BH
```

```r
# Make adjusted p value dataframe
pObs <- pairwise.t.test(alpha_summary$Observed, sample_data(ps_rare)$Sample_type, p.adjust = "BH")
```

```r
pSha <- pairwise.t.test(alpha_summary$Shannon, sample_data(ps_rare)$Sample_type, p.adjust = "BH")

variable <- c("Observed", "Observed", "Observed", "Shannon", "Shannon", "Shannon")
group1 <- c("Rectal swab CT", "Rectal swab ST", "Rectal swab CT",
            "Rectal swab CT", "Rectal swab ST", "Rectal swab CT")
group2 <- c("Stool", "Stool", "Rectal swab ST", "Stool", "Stool", "Rectal swab ST")
pVal <- c(round(pObs$p.value[2,1], 5), round(pObs$p.value[2,2], 3), round(pObs$p.value[1,1], 3),
       round(pSha$p.value[2,1], 3), round(pSha$p.value[2,2], 3), round(pSha$p.value[1,1], 3))
y.position <- c(600, 500, 550, 5.15, 4.85, 5.0)

pAdjusted <- bind_cols(variable, group1, group2, pVal, y.position)
```

```
## New names:
## * NA -> ...1
## * NA -> ...2
## * NA -> ...3
## * NA -> ...4
## * NA -> ...5
```

```r
colnames(pAdjusted) <- c("variable", "group1", "group2", "p", "y.position")

# Plot Observed richness, Shannon, and Simpson diversity values
p <- plot_richness(ps_rare, x = "Sample_type",
                   measures = c("Observed", "Shannon"))

# Add boxplot, individual data points, and linked lines using geom layers
p$layers <- p$layers[-1]
p + geom_boxplot() + geom_point() + xlab("Sample Type") +
  geom_line(aes(group = Individual), size = 0.3, linetype = "dashed") +
  theme(axis.text.x = element_text(angle = 315, hjust = 0),
        aspect.ratio = 1, legend.position = "none") +
  stat_pvalue_manual(pAdjusted) +
  stat_compare_means(method = "anova", label.y = 3)
```




```
ggsave("Purcell Final/Final_results/2)Alpha_Diversity.pdf", width = 7, height = 4.5)
```

Beta Diversity - Bray-Curtis

```
# Ordinate data using Non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity (distance
bray_dist <- phyloseq::distance(ps_rare, method = "bray")
ord.nmds.bray <- ordinate(ps_rare, "NMDS", "bray")
```

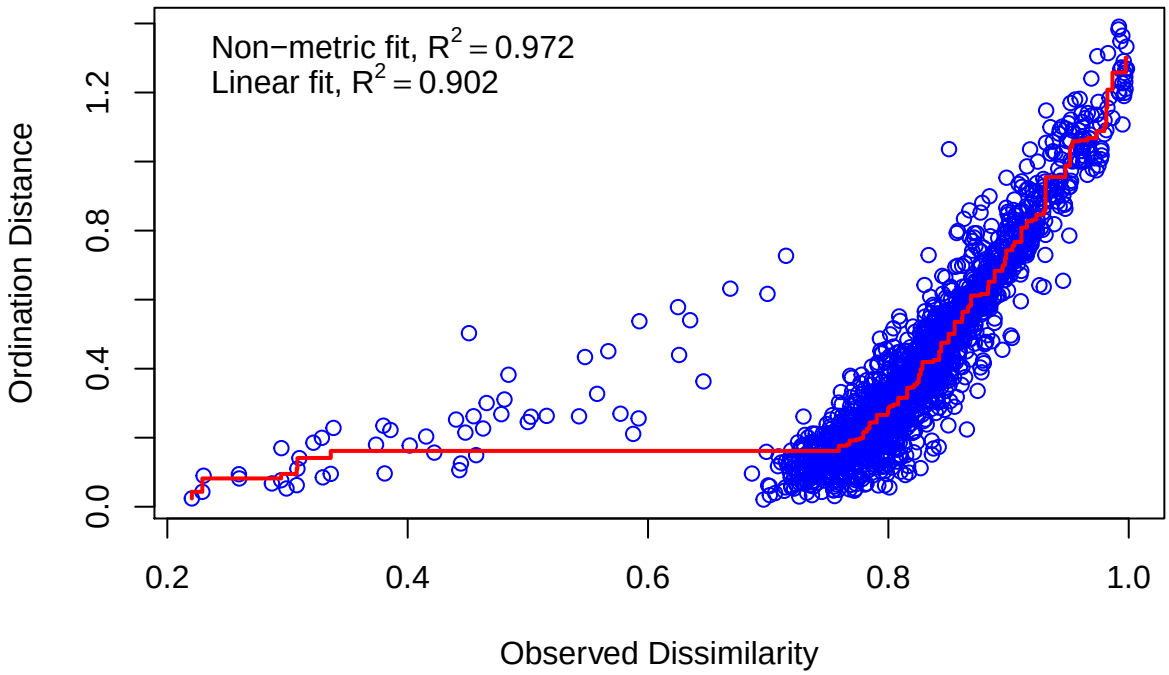
```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.1660649
## Run 1 stress 0.1742839
## Run 2 stress 0.1690271
## Run 3 stress 0.1963773
## Run 4 stress 0.1689969
## Run 5 stress 0.1660679
## ... Procrustes: rmse 0.0007082043  max resid 0.00380969
## ... Similar to previous best
## Run 6 stress 0.179229
## Run 7 stress 0.1805837
## Run 8 stress 0.1690662
## Run 9 stress 0.1909203
## Run 10 stress 0.1688625
## Run 11 stress 0.1660649
## ... New best solution
## ... Procrustes: rmse 0.0001955691  max resid 0.001334372
## ... Similar to previous best
```

```
## Run 12 stress 0.1660682
## ... Procrustes: rmse 0.0007703815  max resid 0.004055105
## ... Similar to previous best
## Run 13 stress 0.1660648
## ... New best solution
## ... Procrustes: rmse 0.0001232208  max resid 0.000492369
## ... Similar to previous best
## Run 14 stress 0.1660648
## ... New best solution
## ... Procrustes: rmse 5.110814e-05  max resid 0.0002284743
## ... Similar to previous best
## Run 15 stress 0.1689939
## Run 16 stress 0.1689967
## Run 17 stress 0.1744556
## Run 18 stress 0.1744547
## Run 19 stress 0.1976383
## Run 20 stress 0.1688245
## *** Solution reached
```

```
# Call newly created file to get the stress value of the plot
ord.nmds.bray
```

```
##
## Call:
## metaMDS(comm = veganifyOTU(physeq), distance = distance)
##
## global Multidimensional Scaling using monoMDS
##
## Data:     wisconsin(sqrt(veganifyOTU(physeq)))
## Distance: bray
##
## Dimensions: 2
## Stress:     0.1660648
## Stress type 1, weak ties
## Two convergent solutions found after 20 tries
## Scaling: centring, PC rotation, halfchange scaling
## Species: expanded scores based on 'wisconsin(sqrt(veganifyOTU(physeq)))'
```

```
# Stress plot
stressplot(ord.nmds.bray)
```




```
# Stats
# Test whether the sample types differ significantly from each other using PERMANOVA
adonis(bray_dist ~ sample_data(ps_rare)$Sample_type)
```

```
##
## Call:
## adonis(formula = bray_dist ~ sample_data(ps_rare)$Sample_type)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                 Df SumsOfSqs MeanSqs F.Model     R2 Pr(>F)
## sample_data(ps_rare)$Sample_type  2    1.4193 0.70965  2.1981 0.0716  0.002 **
## Residuals                        57   18.4025 0.32285         0.9284
## Total                            59   19.8218                 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis(formula = bray_dist ~ sample_data(ps_rare)$Individual)
```

```
##
## Call:
## adonis(formula = bray_dist ~ sample_data(ps_rare)$Individual)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## sample_data(ps_rare)$Individual 19   14.6257 0.76977  5.9258 0.73786  0.001 ***
```

```
## Residuals                       40    5.1961 0.12990         0.26214
## Total                           59   19.8218                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis(bray_dist ~ sample_data(ps_rare)$Sample_type*sample_data(ps_rare)$Individual)
```

```
##
## Call:
## adonis(formula = bray_dist ~ sample_data(ps_rare)$Sample_type *      sample_data(ps_rare)$Individual
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                                                                  Df SumsOfSqs
## sample_data(ps_rare)$Sample_type                                  2    1.4193
## sample_data(ps_rare)$Individual                                  19   14.6257
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual 38    3.7768
## Residuals                                                         0    0.0000
## Total                                                            59   19.8218
##                                                                  MeanSqs
## sample_data(ps_rare)$Sample_type                                       1
## sample_data(ps_rare)$Individual                                        1
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual       0
## Residuals                                                            Inf
## Total
##                                                                  F.Model
## sample_data(ps_rare)$Sample_type                                       0
## sample_data(ps_rare)$Individual                                        0
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual       0
## Residuals
## Total
##                                                                       R2 Pr(>F)
## sample_data(ps_rare)$Sample_type                                 0.07160      1
## sample_data(ps_rare)$Individual                                  0.73786      1
## sample_data(ps_rare)$Sample_type:sample_data(ps_rare)$Individual 0.19054      1
## Residuals                                                        0.00000
## Total                                                            1.00000
```

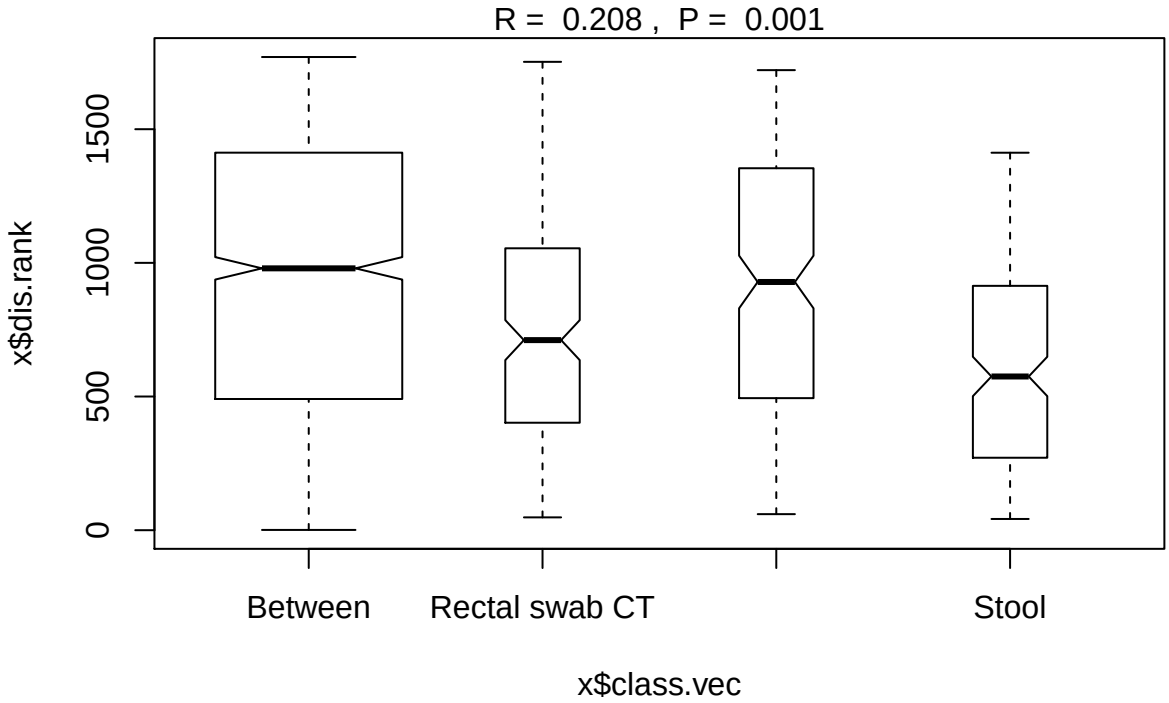
```
anosim(bray_dist, sample_data(ps_rare)$Sample_type)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Sample_type)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.2083
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
```

```
anoSamp <- (anosim(bray_dist, sample_data(ps_rare)$Sample_type))
summary(anoSamp)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Sample_type)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.2083
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0316 0.0487 0.0642 0.0846
##
## Dissimilarity ranks between and within classes:
##                0%     25%   50%      75% 100%    N
## Between         1 491.375 979.5 1411.625 1770 1200
## Rectal swab CT 48 402.500 711.0 1053.125 1752  190
## Rectal swab ST 60 495.250 928.5 1352.125 1721  190
## Stool          42 271.500 575.0  913.000 1412  190
```

```
plot(anoSamp)
```




```
anosim(bray_dist, sample_data(ps_rare)$Individual)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Individual)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.7739
##       Significance: 0.001
```

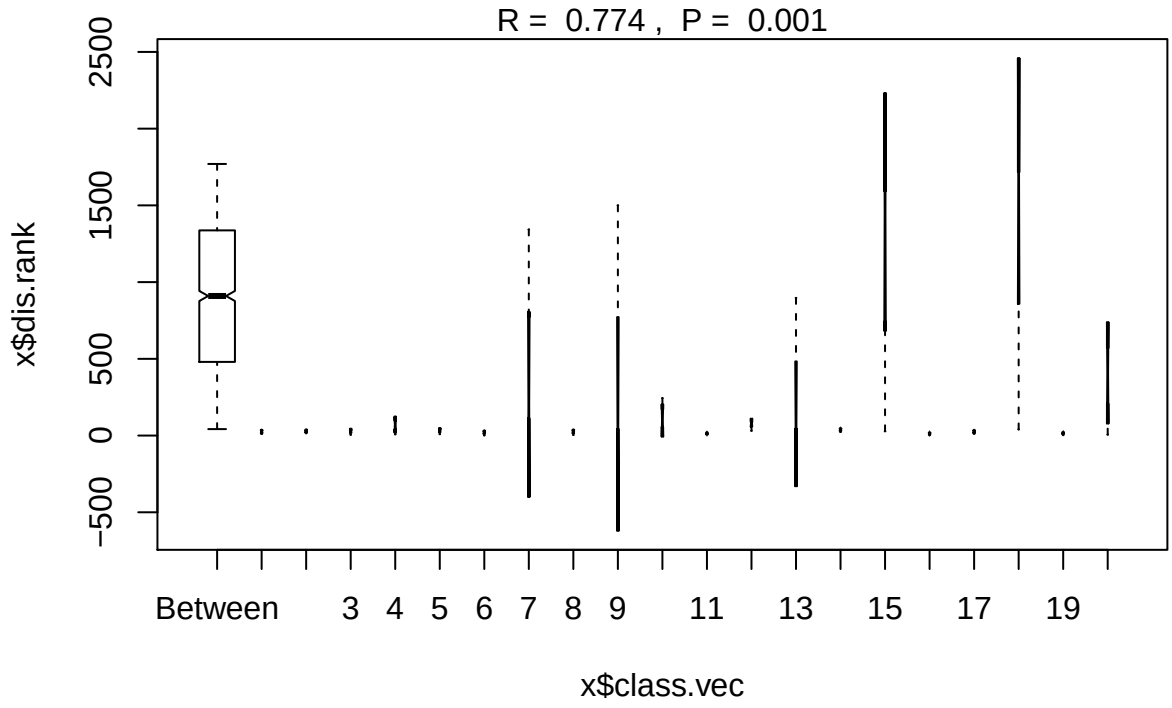
```
##
## Permutation: free
## Number of permutations: 999
```

```
anoInd <- anosim(bray_dist, sample_data(ps_rare)$Individual)
summary(anoInd)
```

```
##
## Call:
## anosim(x = bray_dist, grouping = sample_data(ps_rare)$Individual)
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.7739
##       Significance: 0.001
##
## Permutation: free
## Number of permutations: 999
##
## Upper quantiles of permutations (null model):
##    90%    95%  97.5%    99%
## 0.0855 0.1118 0.1381 0.1564
##
## Dissimilarity ranks between and within classes:
##         0%    25%    50%     75%   100%    N
## Between 42 480.25  909.5 1336.75 1770.0 1710
## 1       10  18.00   26.0   30.50   35.0    3
## 2       15  22.00   29.0   33.00   37.0    3
## 3        5  18.50   32.0   32.50   33.0    3
## 4        8  39.50   71.0   97.00  123.0    3
## 5        9  22.50   36.0   37.00   38.0    3
## 6        1  11.50   22.0   23.00   24.0    3
## 7       19 111.00  203.0  773.00 1343.0    3
## 8        4  15.50   27.0   28.50   30.0    3
## 9       41  42.50   44.0  772.00 1500.0    3
## 10      11  54.50   98.0  171.00  244.0    3
## 11       7  10.00   13.0   17.00   21.0    3
## 12      31  58.00   85.0   86.50   88.0    3
## 13      20  44.50   69.0  483.00  897.0    3
## 14      23  31.00   39.0   41.00   43.0    3
## 15      28 742.50 1457.0 1592.75 1728.5    3
## 16       2   8.00   14.0   15.50   17.0    3
## 17      12  18.50   25.0   29.50   34.0    3
## 18      40 858.50 1677.0 1717.25 1757.5    3
## 19       3   9.50   16.0   17.00   18.0    3
## 20       6 207.25  408.5  570.25  732.0    3
```

```
plot(anoInd)
```

```
## Warning in bxp(list(stats = structure(c(42, 480, 909.5, 1337, 1770, 10, : some
## notches went outside hinges ('box'): maybe set notch=FALSE
```




```
ps.disper <- betadisper(bray_dist, sample_data(ps_rare)$Sample_type)
anova(ps.disper)
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df  Sum Sq   Mean Sq F value  Pr(>F)
## Groups     2 0.01114 0.0055698  2.7521 0.07228 .
## Residuals 57 0.11536 0.0020238
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(ps.disper)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq   Mean Sq      F N.Perm Pr(>F)
## Groups     2 0.01114 0.0055698 2.7521    999  0.072 .
## Residuals 57 0.11536 0.0020238
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
permutest(ps.disper, pairwise = TRUE)
```

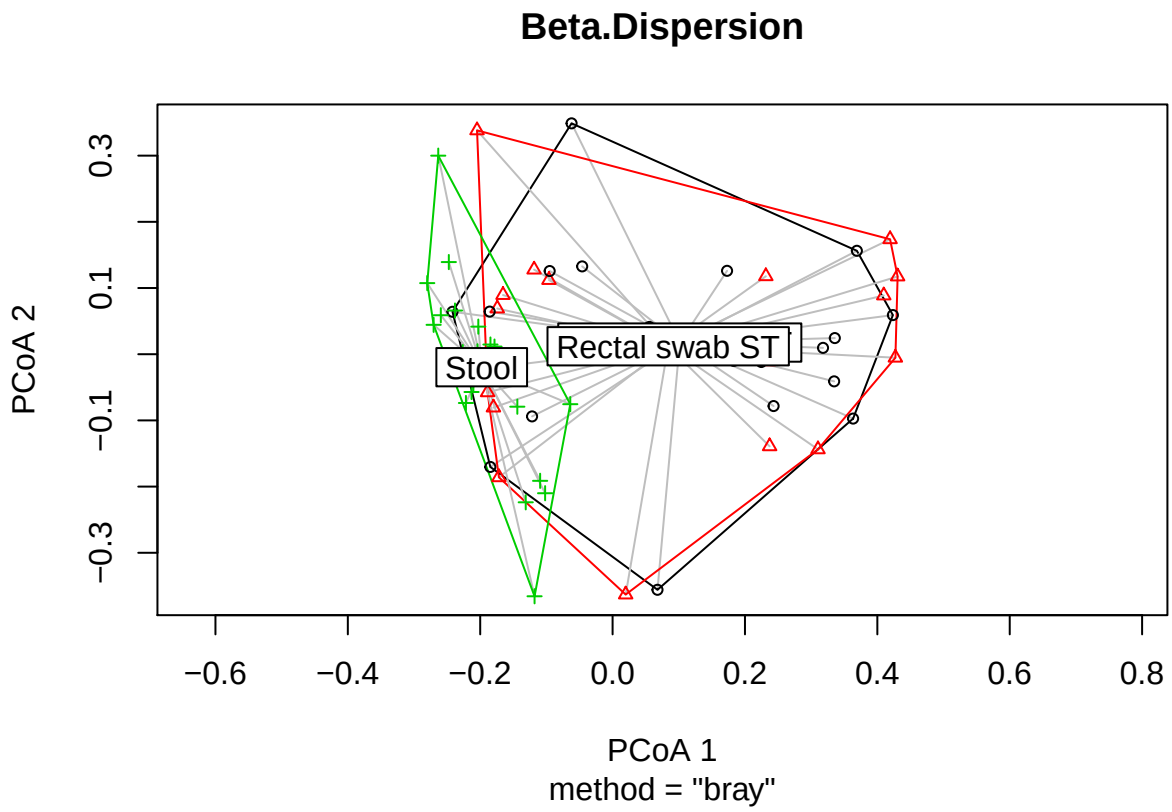
```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
```

```
## Response: Distances
##           Df  Sum Sq   Mean Sq      F N.Perm Pr(>F)
## Groups     2 0.01114 0.0055698 2.7521    999  0.078 .
## Residuals 57 0.11536 0.0020238
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##                Rectal swab CT Rectal swab ST Stool
## Rectal swab CT                       0.161000 0.352
## Rectal swab ST       0.163939                0.030
## Stool                0.352525        0.027001
```

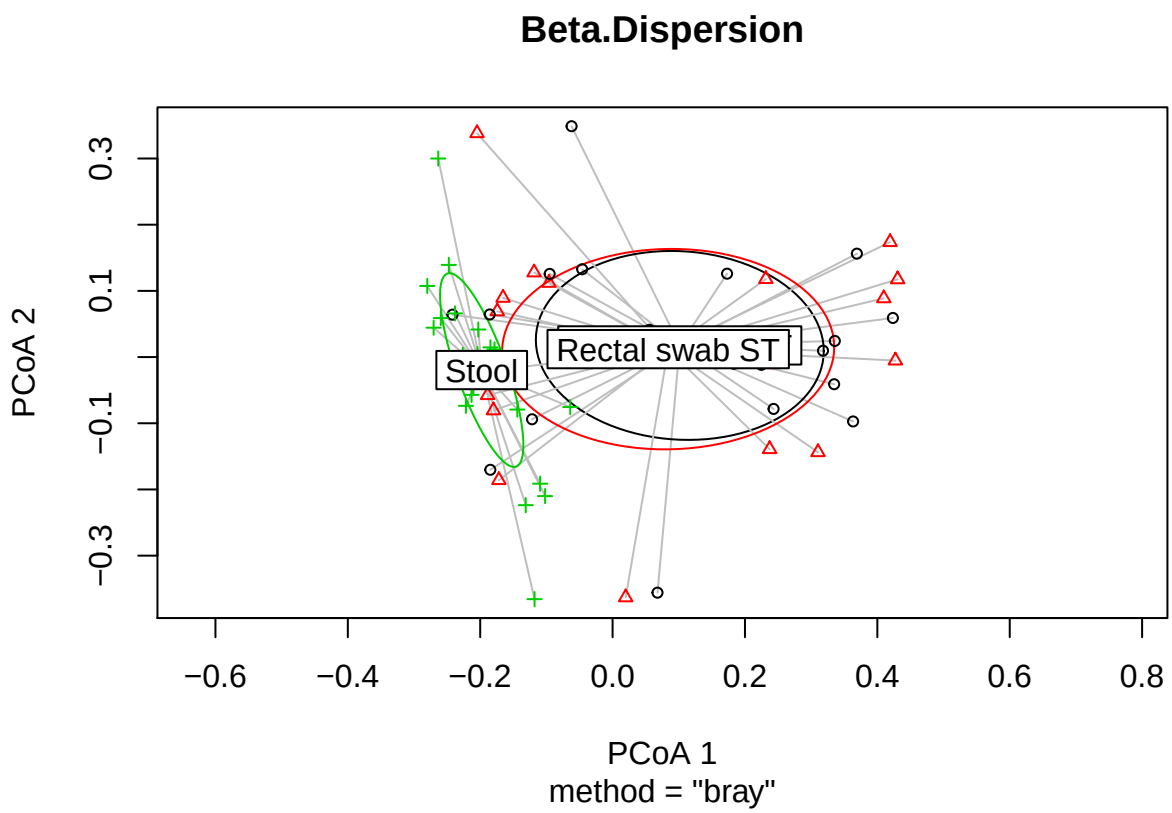
```
TukeyHSD(ps.disper)
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = distances ~ group, data = df)
##
## $group
##                                     diff         lwr         upr     p adj
## Rectal swab ST-Rectal swab CT  0.01962352 -0.01461033 0.053857382 0.3583522
## Stool-Rectal swab CT          -0.01356902 -0.04780288 0.020664837 0.6088017
## Stool-Rectal swab ST          -0.03319255 -0.06742640 0.001041313 0.0592313
```

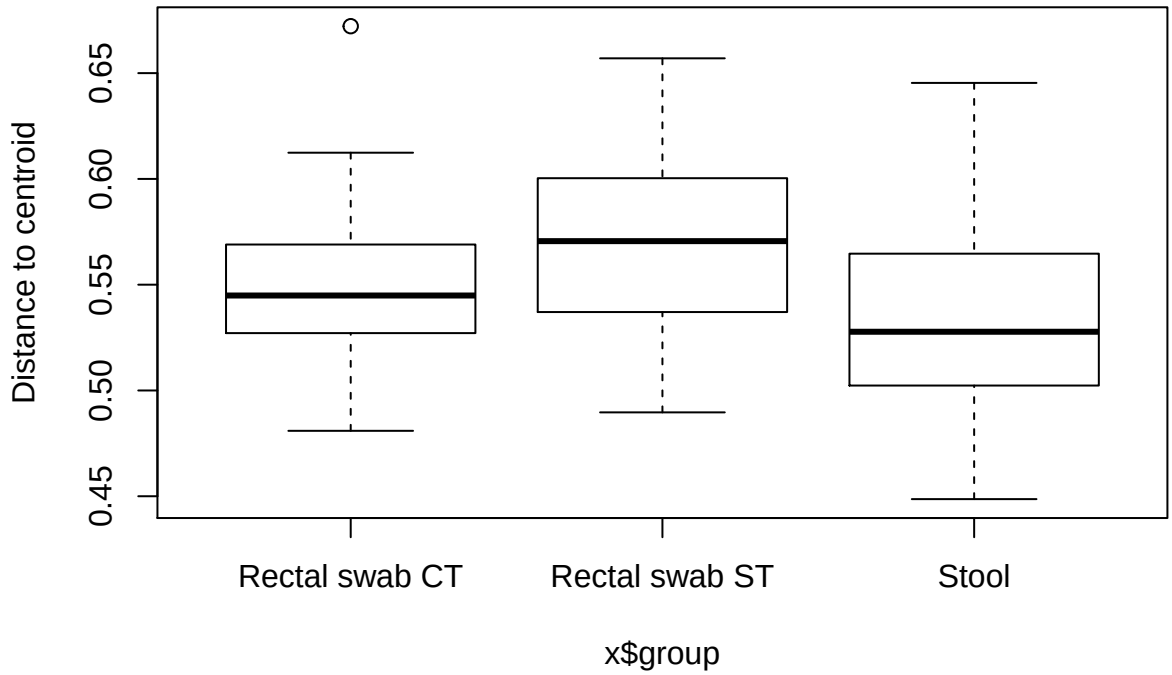
```
# Beta Dispersion Plots
Beta.Dispersion <- ps.disper
plot(Beta.Dispersion)
```



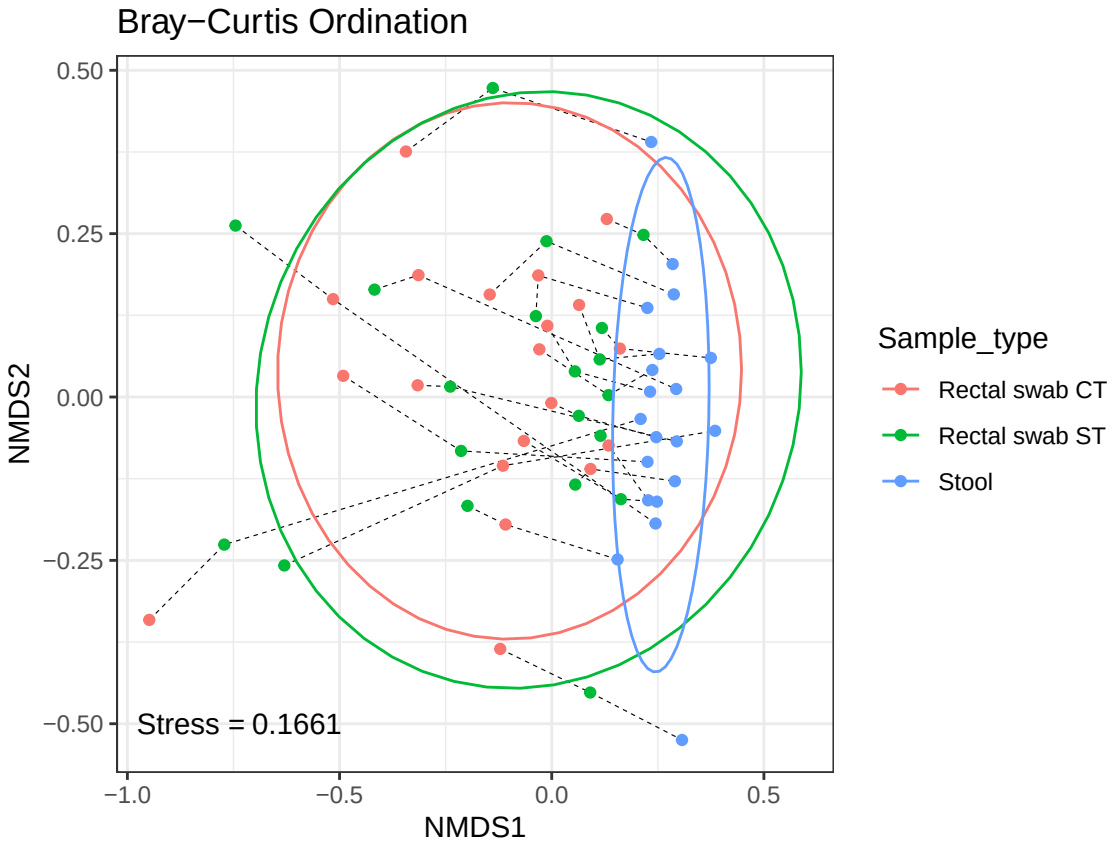

```
plot(Beta.Dispersion, hull = FALSE, ellipse = TRUE)
```



```r
boxplot(Beta.Dispersion)
```




```r
# NMDS plot
cust <- plot_ordination(ps_rare, ord.nmds.bray, justDF = TRUE)

ggplot(cust, aes(x = NMDS1, y = NMDS2)) +
  geom_line(aes(group = Individual), size = 0.2, linetype = "dashed") +
  geom_point(aes(color = Sample_type)) +
  annotate("text", x = -0.85, y = -0.5, label = "Stress =") +
  annotate("text", x = -0.6, y = -0.5, label = round(ord.nmds.bray$stress, 4)) +
  stat_ellipse(aes(color = Sample_type)) +
  ggtitle("Bray-Curtis Ordination") +
  theme(aspect.ratio = 1)
```



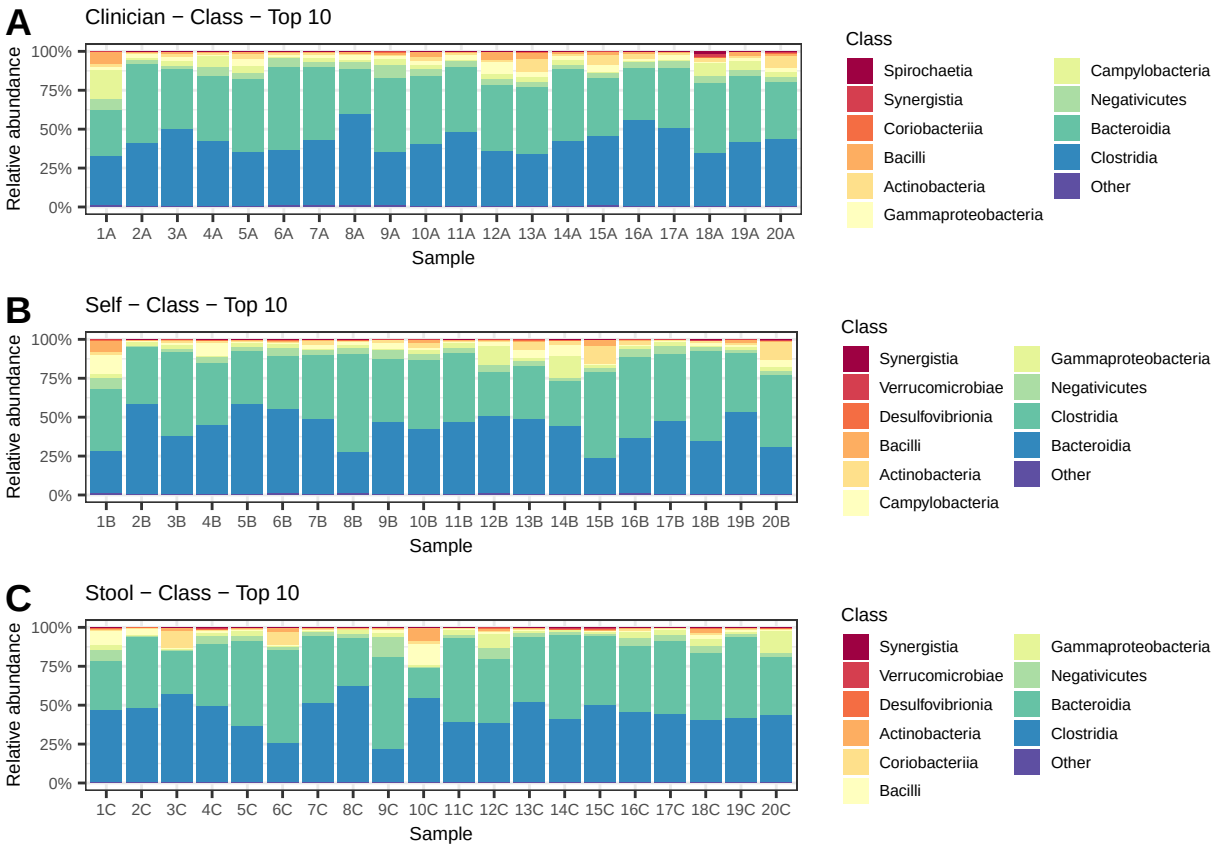

```
ggsave("Purcell Final/Final_results/3)Beta_Diversity.pdf", width = 6, height = 4.5)
```

RELATIVE ABUNDANCE - Using Taxonomic Level Class

```
# Subset Phyloseq Objects
ps_class <- subset_taxa(ps_rare, Class != "NA")

sample_clin <- subset_samples(ps_class, Sample_type == "Rectal swab CT")
sample_self <- subset_samples(ps_class, Sample_type == "Rectal swab ST")
sample_stool <- subset_samples(ps_class, Sample_type == "Stool")

# Relative Abundance - Clinician Taken Swab
clin_class <- tax_glom(sample_clin, taxrank = "Class") # agglomerate taxa
clin_transform <- transform_sample_counts(clin_class, function(x) x/sum(x)) #get abundance in %
clin_melt <- psmelt(clin_transform) # create dataframe from phyloseq object
clin_melt$Class <- as.character(clin_melt$Class) #convert to character
clin_melt <- clin_melt[order(-clin_melt$Abundance),]
clin_melt[!clin_melt$Class %in% c(unique(clin_melt$Class)[1:10]), "Class"] <- "Other"

# Set order of bars
sort.clin <- clin_melt %>%
  plyr::count("Class", wt = "Abundance") %>%
  arrange(desc(freq)) %>%
  pull(Class)
```

```
sort.clin <- sort.clin[!sort.clin %in% "Other"]
sort.clin <- append("Other", sort.clin)

# Plot
t1_class <- clin_melt %>%
  mutate(Sample = factor(Sample, levels = c("1A", "2A", "3A", "4A", "5A",
                                            "6A", "7A", "8A", '9A', "10A",
                                            "11A", "12A", "13A", "14A", "15A",
                                            "16A", "17A", "18A", "19A", "20A"))) %>%
  mutate(Class = factor(Class, levels = rev(sort.clin))) %>%
  ggplot(aes(x = Sample, y = Abundance, fill = Class)) +
  geom_bar(stat = "identity", position = "fill") +
  scale_y_continuous(labels = percent_format()) +
  theme(text = element_text(size = 7)) +
  ggtitle("Clinician - Class - Top 10") +
  ylab("Relative abundance") +
  scale_fill_brewer(palette = "Spectral", guide = guide_legend(ncol = 2)) +
  theme(legend.text = element_text(size = 6), legend.key.size = unit(0.75, "line"))


# Relative Abundance - Self Taken Swab
self_class <- tax_glom(sample_self, taxrank = "Class") # agglomerate taxa
self_class <- transform_sample_counts(self_class, function(x) x/sum(x)) #get abundance in %
self_melt <- psmelt(self_class) # create dataframe from phyloseq object
self_melt$Class <- as.character(self_melt$Class) #convert to character
self_melt <- self_melt[order(-self_melt$Abundance),]
self_melt[!self_melt$Class %in% c(unique(self_melt$Class)[1:10]), "Class"] <- "Other"

# Set order of bars
sort.self <- self_melt %>%
  plyr::count("Class", wt = "Abundance") %>%
  arrange(desc(freq)) %>%
  pull(Class)

sort.self <- sort.self[!sort.self %in% "Other"]
sort.self <- append("Other", sort.self)

# Plot
t2_class <- self_melt %>%
  mutate(Sample = factor(Sample, levels = c("1B", "2B", "3B", "4B", "5B",
                                            "6B", "7B", "8B", "9B", "10B",
                                            "11B", "12B", "13B", "14B", "15B",
                                            "16B", "17B", "18B", "19B", "20B"))) %>%
  mutate(Class = factor(Class, levels = rev(sort.self))) %>%
  ggplot(aes(x = Sample, y = Abundance, fill = Class)) +
  geom_bar(stat = "identity", position = "fill") +
  scale_y_continuous(labels = percent_format()) +
  theme(text = element_text(size = 7)) +
  ggtitle("Self - Class - Top 10") +
  ylab("Relative abundance") +
  scale_fill_brewer(palette = "Spectral", guide = guide_legend(ncol = 2)) +
  theme(legend.text = element_text(size = 6), legend.key.size = unit(0.75, "line"))
```

```r
# Relative Abundance - Stool Sample
stool_class <- tax_glom(sample_stool, taxrank = "Class") # agglomerate taxa
stool_class <- transform_sample_counts(stool_class, function(x) x/sum(x)) #get abundance in %
stool_melt <- psmelt(stool_class) # create dataframe from phyloseq object
stool_melt$Class <- as.character(stool_melt$Class) #convert to character
stool_melt <- stool_melt[order(-stool_melt$Abundance),]
stool_melt[!stool_melt$Class %in% c(unique(stool_melt$Class)[1:10]), "Class"] <- "Other"

# Set order of bars
sort.stool <- stool_melt %>%
  plyr::count("Class", wt = "Abundance") %>%
  arrange(desc(freq)) %>%
  pull(Class)

sort.stool <- sort.stool[!sort.stool %in% "Other"]
sort.stool <- append("Other", sort.stool)

# Plot
t3_class <- stool_melt %>%
  mutate(Sample = factor(Sample, levels = c("1C", "2C", "3C", "4C", "5C",
                                            "6C", "7C", "8C", "9C", "10C",
                                            "11C", "12C", "13C", "14C", "15C",
                                            "16C", "17C", "18C", "19C", "20C"))) %>%
  mutate(Class = factor(Class, levels = rev(sort.stool))) %>%
  ggplot(aes(x = Sample, y = Abundance, fill = Class)) +
  geom_bar(stat = "identity", position = "fill") +
  scale_y_continuous(labels = percent_format()) +
  theme(text = element_text(size = 7)) +
  ggtitle("Stool - Class - Top 10") +
  ylab("Relative abundance") +
  scale_fill_brewer(palette = "Spectral", guide = guide_legend(ncol = 2)) +
  theme(legend.text = element_text(size = 6), legend.key.size = unit(0.75, "line"))

ggarrange(t1_class, t2_class, t3_class, nrow = 3, labels = "AUTO", legend = "right")
```




```
ggsave("Purcell Final/Final_results/4)Relative_Abundance.pdf", width = 7, height = 8)
```

OTU differential abundance testing with DESeq2

```
ps_deseq <- ps %>%
  tax_glom(taxrank = "Genus")

sample_data(ps_deseq)$Sample_type <- gsub(" ", "_", sample_data(ps_deseq)$Sample_type)
sample_data(ps_deseq)$Sample_type <- as.factor(sample_data(ps_deseq)$Sample_type)

# Convert the phyloseq object to a DESeqDataSet
ds <- phyloseq_to_deseq2(ps_deseq, ~ Sample_type)
```

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

```
ds <- DESeq(ds)
```

```
## estimating size factors
```

```
## estimating dispersions
```

```
## gene-wise dispersion estimates
```

```
## mean-dispersion relationship
```

```
## final dispersion estimates
```

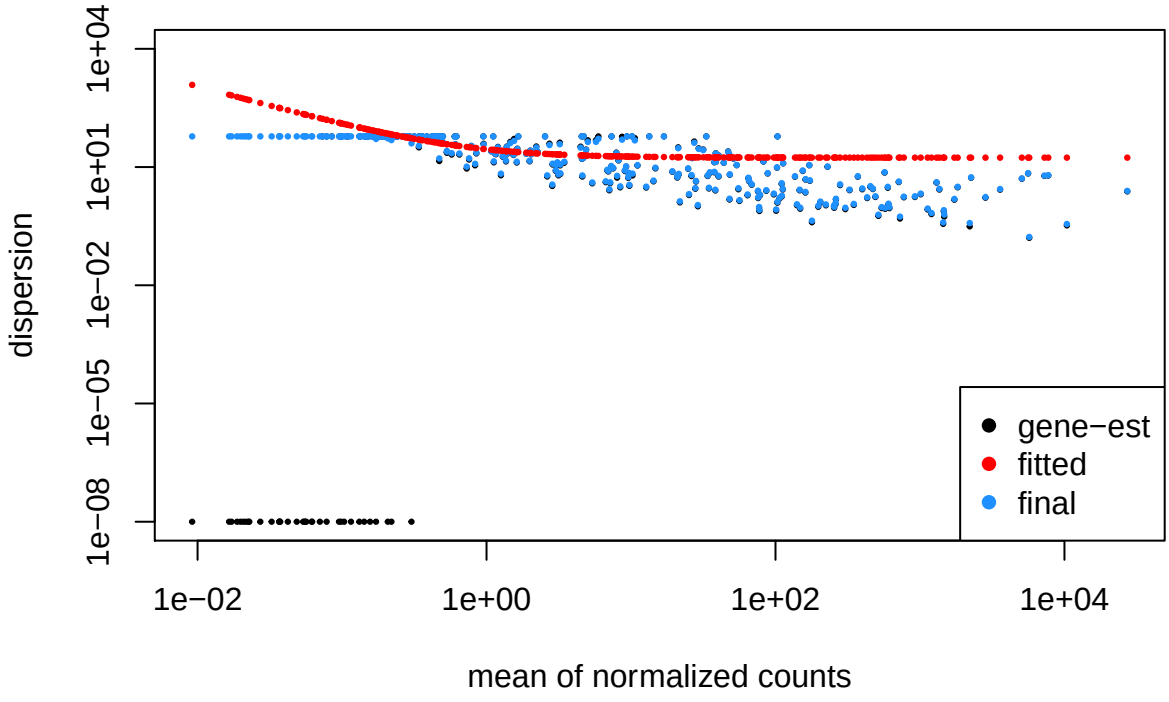
```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 151 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

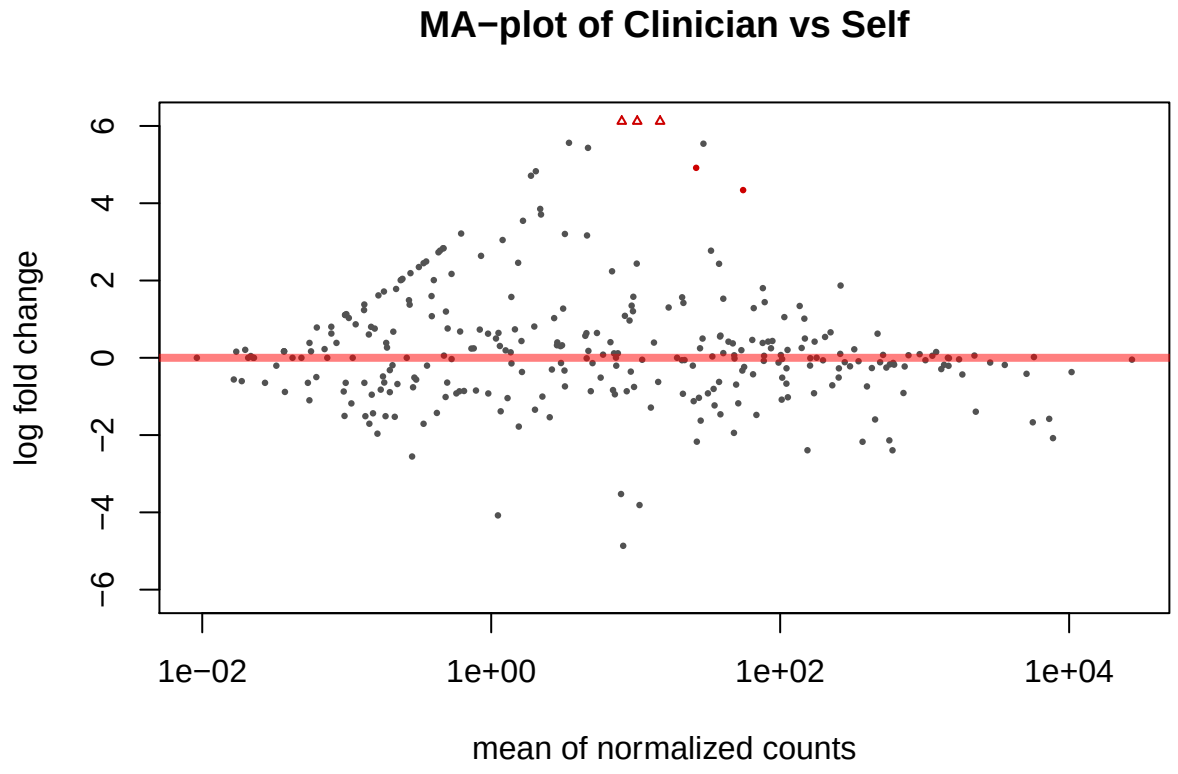
```
## estimating dispersions
```

```
## fitting model and testing
```

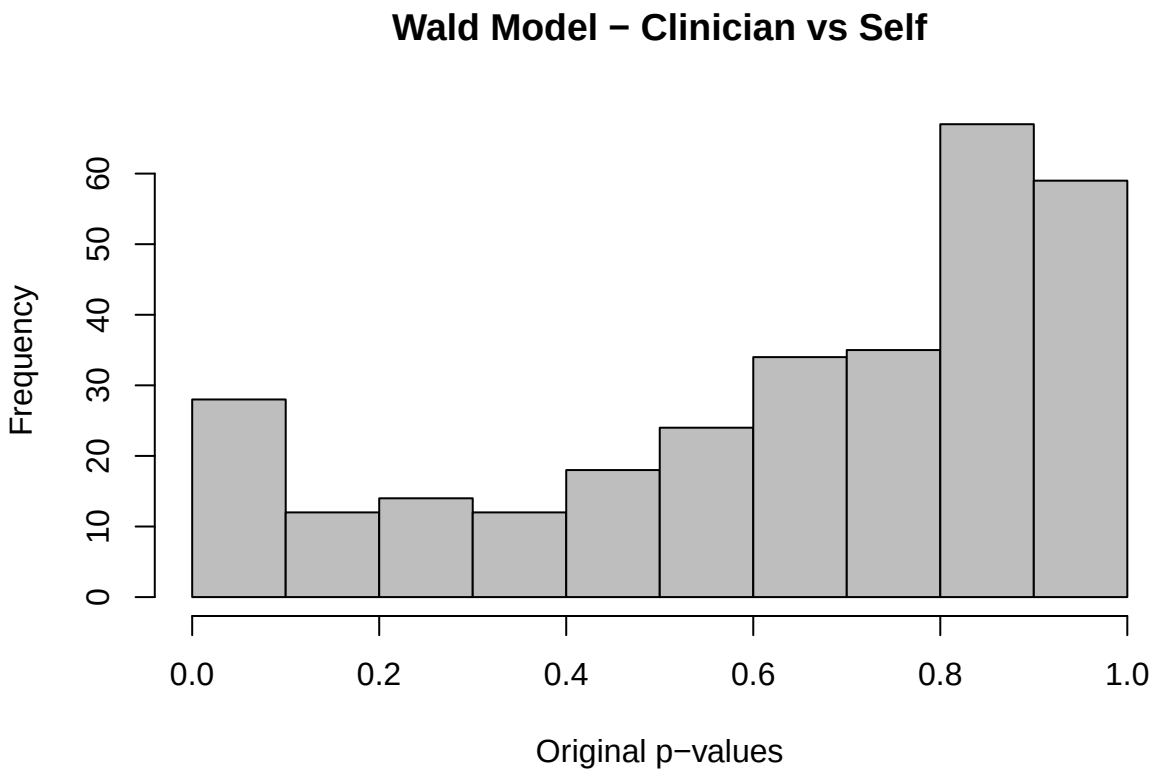
```
# Plot of Dispersion Estimates
plotDispEsts(ds, ylim = c(1e-8, 1e4))
```




```
# Extract the result table from the ds object usind the DESeq2 function results and filter the OTUs usi
alpha <- 0.01

# Swab CT vs Swab ST
resCTST <- results(ds, contrast = c("Sample_type", "Rectal_swab_CT", "Rectal_swab_ST"),
                   alpha = alpha)
resCTST <- resCTST[order(resCTST$padj, na.last = NA), ]
plotMA(resCTST, alpha = 0.01, main = "MA-plot of Clinician vs Self")
```



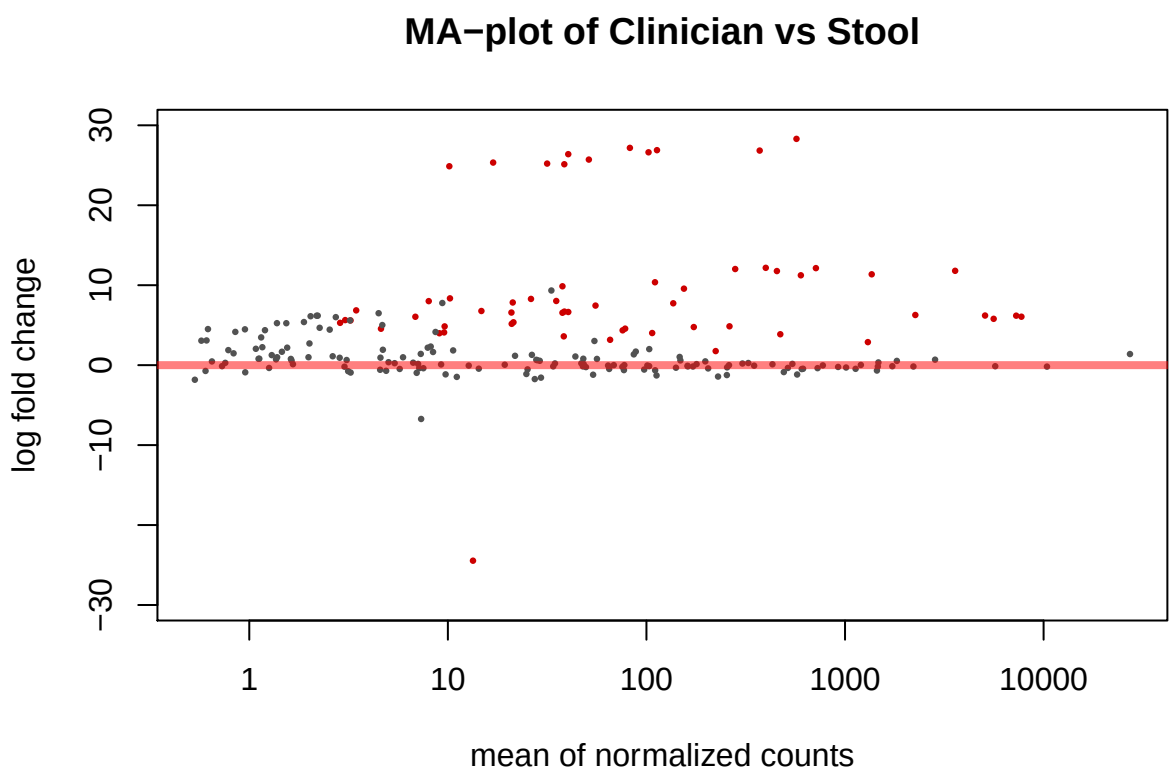

```
hist(resCTST$pvalue, col = "gray", main = "Wald Model - Clinician vs Self", xlab = "Original p-values")
```




```
resCTST_sig <- resCTST[(resCTST$padj < alpha), ]
resCTST_sig <- cbind(as(resCTST_sig, "data.frame"), as(tax_table(ps)[rownames(resCTST_sig), ], "matrix")
head(resCTST_sig)
```

```
##          baseMean log2FoldChange     lfcSE     stat       pvalue         padj
## ASV473  55.398735       4.339790 0.7977869 5.439786 5.334449e-08 8.081691e-06
## ASV930  10.246362       7.072850 1.2770450 5.538450 3.051598e-08 8.081691e-06
## ASV1129  8.010744       6.131688 1.1954151 5.129338 2.907624e-07 2.936700e-05
## ASV658  26.218338       4.915902 1.1727001 4.191952 2.765650e-05 1.764129e-03
## ASV1164 14.753210       6.139523 1.4686762 4.180311 2.911104e-05 1.764129e-03
##          Kingdom          Phylum               Class            Order
## ASV473  Bacteria Proteobacteria Gammaproteobacteria  Pseudomonadales
## ASV930  Bacteria     Firmicutes          Clostridia    Clostridiales
## ASV1129 Bacteria     Firmicutes          Clostridia    Clostridiales
## ASV658  Bacteria Proteobacteria Gammaproteobacteria    Aeromonadales
## ASV1164 Bacteria Proteobacteria Gammaproteobacteria Enterobacterales
##                   Family                      Genus
## ASV473  Pseudomonadaceae                  Pseudomonas
## ASV930    Clostridiaceae  Clostridium_sensu_stricto_5
## ASV1129   Clostridiaceae Clostridium_sensu_stricto_13
## ASV658    Aeromonadaceae                   Aeromonas
## ASV1164       Hafniaceae       Hafnia-Obesumbacterium
```

```r
# Swab CT vs Stool
resCTS <- results(ds, contrast = c("Sample_type", "Rectal_swab_CT", "Stool"),
                  alpha = alpha)
resCTS <- resCTS[order(resCTS$padj, na.last = NA), ]
plotMA(resCTS, alpha = 0.01, main = "MA-plot of Clinician vs Stool")
```



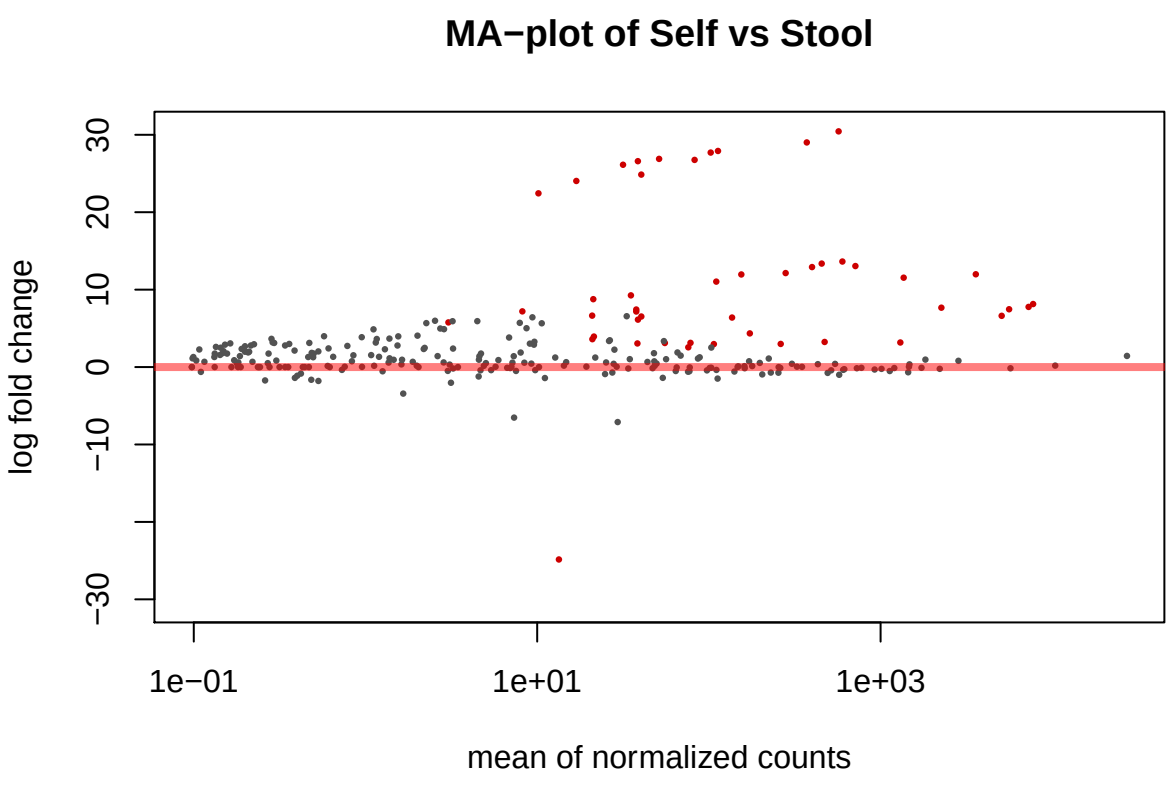

```r
hist(resCTS$pvalue, col = "gray", main = "Wald Model - Clinician vs Stool", xlab = "Original p-values")
```


```
resCTS_sig <- resCTS[(resCTS$padj < alpha), ]
resCTS_sig <- cbind(as(resCTS_sig, "data.frame"), as(tax_table(ps)[rownames(resCTS_sig), ], "matrix"))
head(resCTS_sig)
```

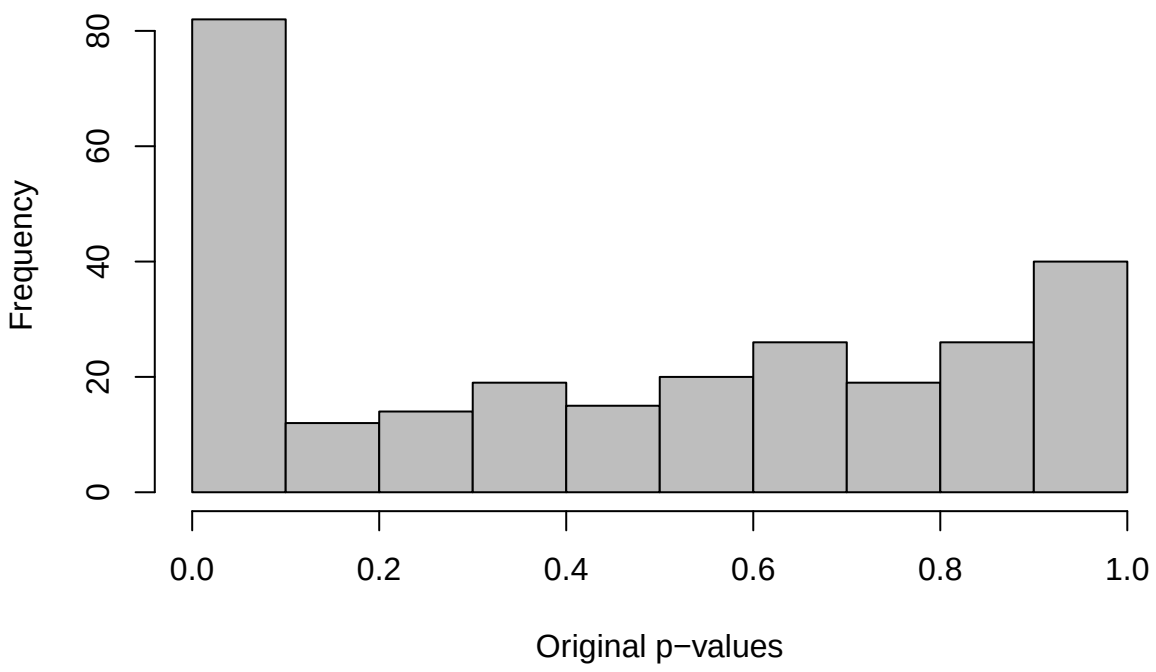
```
##          baseMean log2FoldChange     lfcSE     stat        pvalue          padj
## ASV324  371.68353       26.83942 1.1951058 22.45778 1.074225e-111 2.245130e-109
## ASV262  569.52608       28.30378 1.3237517 21.38149 1.987046e-101  2.076463e-99
## ASV365  102.45995       26.62194 1.5030862 17.71152  3.417104e-70  2.380583e-68
## ASV662  112.98959       26.88915 1.6814757 15.99140  1.467033e-57  7.665245e-56
## ASV283   82.54958       27.17414 1.7632089 15.41175  1.364563e-53  5.703875e-52
## ASV5   3585.31720       11.79910 0.7864744 15.00252  7.068126e-51  2.462064e-49
##         Kingdom           Phylum           Class
## ASV324 Bacteria       Firmicutes   Negativicutes
## ASV262 Bacteria       Firmicutes      Clostridia
## ASV365 Bacteria       Firmicutes      Clostridia
## ASV662 Bacteria       Firmicutes         Bacilli
## ASV283 Bacteria     Synergistota     Synergistia
## ASV5   Bacteria Campilobacterota Campylobacteria
##                                      Order
## ASV324      Veillonellales-Selenomonadales
## ASV262                        Clostridia_or
## ASV365 Peptostreptococcales-Tissierellales
## ASV662                       Lactobacillales
## ASV283                        Synergistales
## ASV5                       Campylobacterales
##                                       Family          Genus
## ASV324                        Veillonellaceae Negativicoccus
## ASV262                  Hungateiclostridiaceae Fastidiosipila
## ASV365 Peptostreptococcales-Tissierellales_fa      Gallicola
## ASV662                           Aerococcaceae      Facklamia
```

```
## ASV283                          Synergistaceae Pyramidobacter
## ASV5                          Campylobacteraceae  Campylobacter
```

```r
# Swab ST vs Stool
resSTS <- results(ds, contrast = c("Sample_type", "Rectal_swab_ST", "Stool"),
                  alpha = alpha)
resSTS <- resSTS[order(resSTS$padj, na.last = NA), ]
plotMA(resSTS, alpha = 0.01, main = "MA-plot of Self vs Stool")
```




```r
hist(resSTS$pvalue, col = "gray", main = "Wald Model - Self vs Stool", xlab = "Original p-values")
```




```
resSTS_sig <- resSTS[(resSTS$padj < alpha), ]
resSTS_sig <- cbind(as(resSTS_sig, "data.frame"), as(tax_table(ps)[rownames(resSTS_sig), ], "matrix"))
head(resSTS_sig)
```

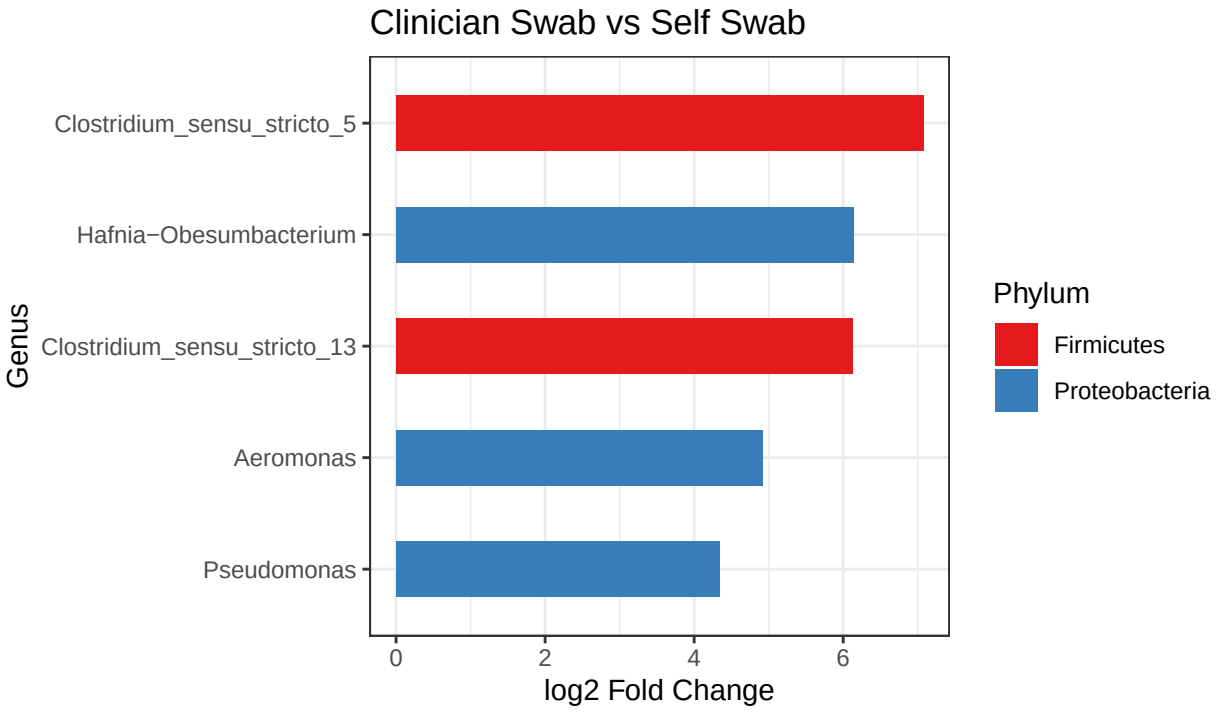
```
##          baseMean log2FoldChange     lfcSE     stat        pvalue          padj
## ASV324  371.68353       29.01285 1.1939499 24.29989 1.965677e-130 5.366298e-128
## ASV262  569.52608       30.44088 1.3230614 23.00791 3.884562e-117 5.302427e-115
## ASV365  102.45995       27.70526 1.5015745 18.45081  5.137379e-76  4.675015e-74
## ASV662  112.98959       27.91069 1.6802657 16.61088  5.813687e-62  3.967841e-60
## ASV5   3585.31720       11.98355 0.7864328 15.23785  1.982790e-52  1.082603e-50
## ASV283   82.54958       26.75502 1.7629576 15.17621  5.082879e-52  2.312710e-50
##         Kingdom           Phylum           Class
## ASV324 Bacteria       Firmicutes   Negativicutes
## ASV262 Bacteria       Firmicutes      Clostridia
## ASV365 Bacteria       Firmicutes      Clostridia
## ASV662 Bacteria       Firmicutes         Bacilli
## ASV5   Bacteria Campilobacterota Campylobacteria
## ASV283 Bacteria     Synergistota     Synergistia
##                                     Order
## ASV324      Veillonellales-Selenomonadales
## ASV262                        Clostridia_or
## ASV365 Peptostreptococcales-Tissierellales
## ASV662                      Lactobacillales
## ASV5                     Campylobacterales
## ASV283                       Synergistales
##                                       Family          Genus
## ASV324                        Veillonellaceae Negativicoccus
## ASV262                 Hungateiclostridiaceae Fastidiosipila
## ASV365 Peptostreptococcales-Tissierellales_fa      Gallicola
## ASV662                           Aerococcaceae      Facklamia
```

```
## ASV5                        Campylobacteraceae  Campylobacter
## ASV283                          Synergistaceae Pyramidobacter
```

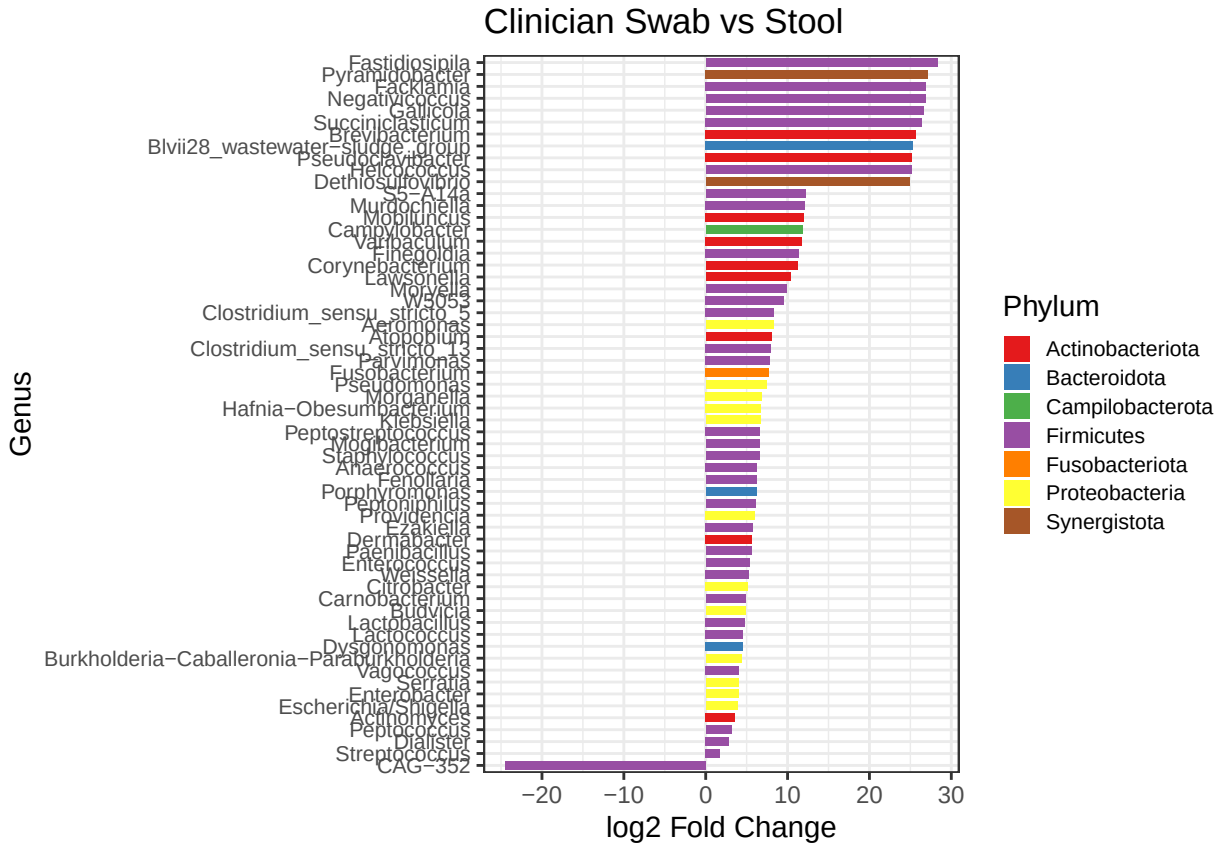
```
# Save .csv of significant fold change results
resCTST_sig$Comparison <- "Clinician Taken Swab vs Self Taken Swab"
resCTS_sig$Comparison <- "Clinician Taken Swab vs Stool"
resSTS_sig$Comparison <- "Self Taken Swab vs Stool"

SignificantResults <- rbind(resCTST_sig, resCTS_sig, resSTS_sig)
write.csv(SignificantResults, file = "Purcell Final/Final_results/SignificantFoldChangeResults.csv")
```

Differential Abundance Figure

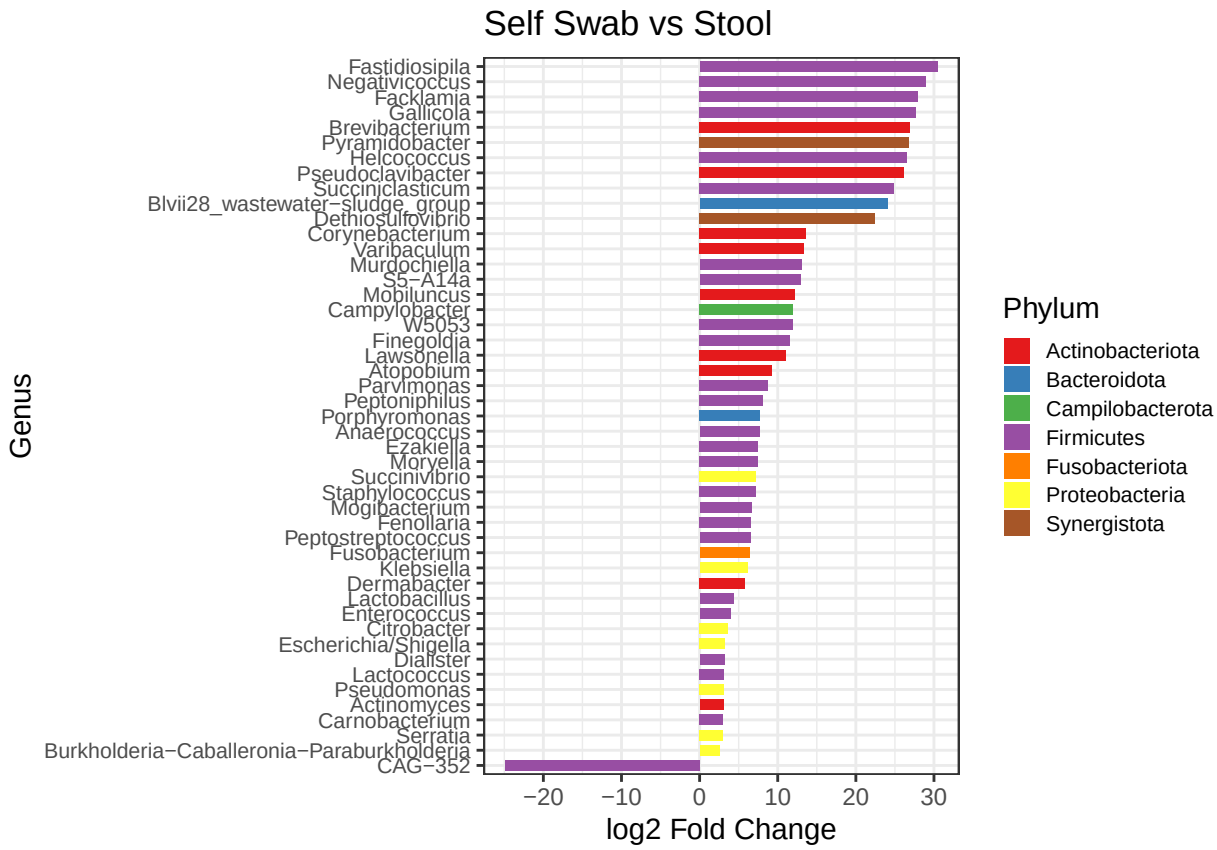
```
ggplot(resCTST_sig, aes(x = log2FoldChange, y = reorder(Genus, log2FoldChange), fill= Phylum)) +
  geom_bar(stat = "identity", position = "identity", width = 0.5) +
  labs(title = "Clinician Swab vs Self Swab", y = "Genus", x = "log2 Fold Change") +
  theme(aspect.ratio = 1) +
  scale_fill_brewer(palette = "Set1")
```




```
ggsave("Purcell Final/Final_results/5)Differential_Abundance_clinVSself.pdf", width = 7, height = 4)

clinVSstool <- ggplot(resCTS_sig, aes(x = log2FoldChange,
                                      y = reorder(Genus, log2FoldChange),
                                      fill= Phylum)) +
  geom_bar(stat = "identity", position = "identity", width = 0.7) +
  labs(title = "Clinician Swab vs Stool", y = "Genus", x = "log2 Fold Change") +
  scale_fill_brewer(palette = "Set1") +
  theme(axis.text.y = element_text(size = 8),
        legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line"))
clinVSstool
```




```
selfVSstool <- ggplot(resSTS_sig, aes(x = log2FoldChange,
                                      y = reorder(Genus, log2FoldChange),
                                      fill= Phylum)) +
  geom_bar(stat = "identity", position = "identity", width = 0.7) +
  labs(title = "Self Swab vs Stool", y = "Genus", x = "log2 Fold Change") +
  scale_fill_brewer(palette = "Set1") +
  theme(axis.text.y = element_text(size = 8),
        legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line"))
selfVSstool
```




```
ggarrange(clinVSstool, selfVSstool, ncol = 2, common.legend = TRUE, legend = "right", labels = "AUTO")
```

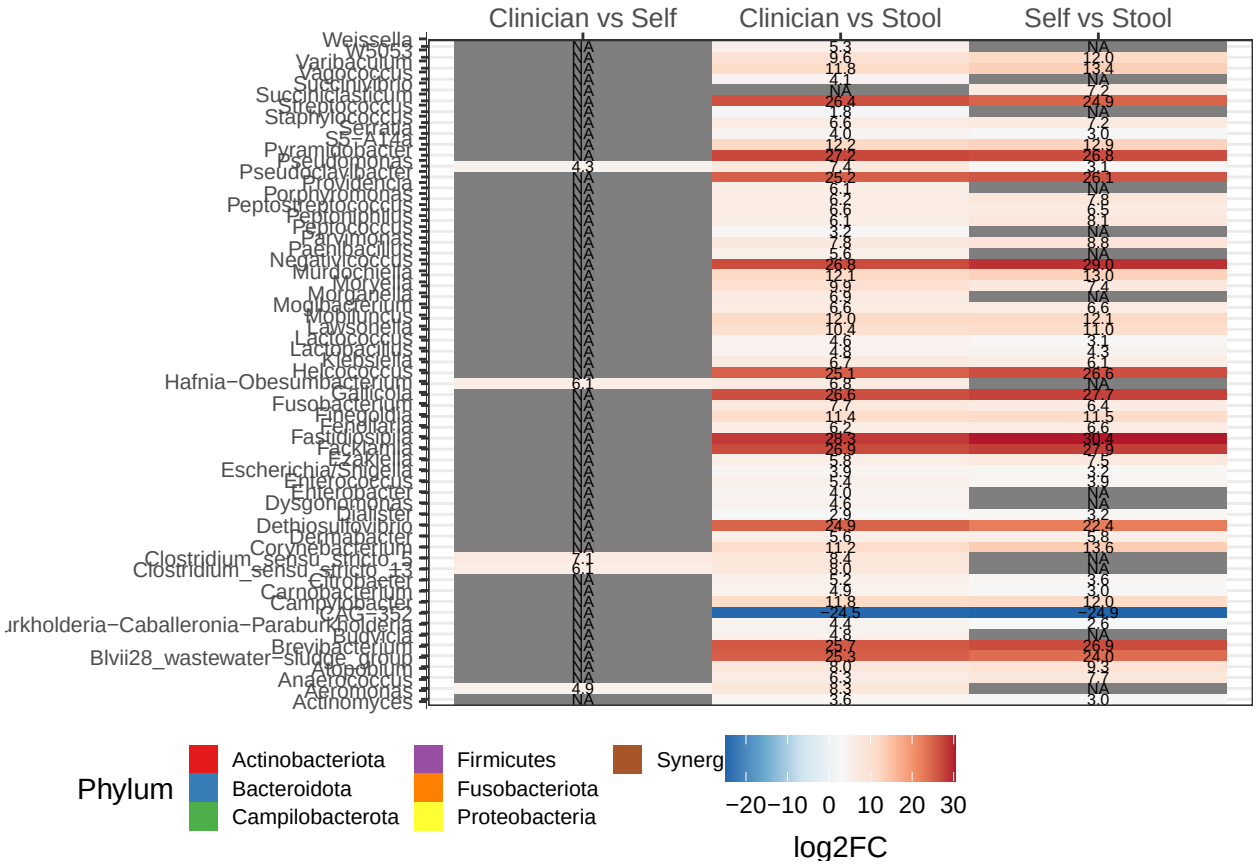

```
ggsave("Purcell Final/Final_results/5)Differential_Abundance_swabsVSstool.pdf", width = 12, height = 8)
```

Differential Abundance - ggplot Heatmap

```
diffCTST <- resCTST_sig %>%
  select(log2FoldChange, Phylum, Genus)
colnames(diffCTST)[1] <- "CTST_log2FoldChange"

diffCTS <- resCTS_sig %>%
  select(log2FoldChange, Phylum, Genus)
colnames(diffCTS)[1] <- "CTS_log2FoldChange"

diffSTS <- resSTS_sig %>%
  select(log2FoldChange, Phylum, Genus)
colnames(diffSTS)[1] <- "STS_log2FoldChange"

heat <- rbind.fill(as.data.frame(t(diffCTS)), as.data.frame(t(diffSTS)))
heat <- rbind.fill(as.data.frame(heat), as.data.frame(t(diffCTST)))
heat <- t(heat)
heat <- as.data.frame(heat)
colnames(heat) <- c("CTS", "CTS_phylum", "CTS_genus",
                    "STS", "STS_phylum", "STS_genus",
                    "CTST", "CTST_phylum", "CTST_genus")
```

```
heat$sigPhylum <- as.character(heat$CTS_phylum)
heat$sigPhylum[nrow(heat)] <- as.character(heat$STS_phylum[nrow(heat)])

heat$sigGenus <- as.character(heat$CTS_genus)
heat$sigGenus[nrow(heat)] <- as.character(heat$STS_genus[nrow(heat)])

heat <- select(heat, -CTS_genus, -STS_genus, -CTST_genus, -CTS_phylum, -STS_phylum, -CTST_phylum)

# file for ggplot based heatmap
SamplingComparison <- c(1:(nrow(heat)*3))
SamplingComparison[1:nrow(heat)] <- "CTS"
SamplingComparison[(nrow(heat)+1):(nrow(heat)*2)] <- "STS"
SamplingComparison[((nrow(heat)*2)+1):(nrow(heat)*3)] <- "CTST"
log2FC <- c(1:(nrow(heat)*3))
log2FC[1:nrow(heat)] <- as.numeric(as.character(heat$CTS))
log2FC[(nrow(heat)+1):(nrow(heat)*2)] <- as.numeric(as.character(heat$STS))
log2FC[((nrow(heat)*2)+1):(nrow(heat)*3)] <- as.numeric(as.character(heat$CTST))
Phylum <- c(1:(nrow(heat)*3))
Phylum[1:nrow(heat)] <- heat$sigPhylum
Phylum[(nrow(heat)+1):(nrow(heat)*2)] <- heat$sigPhylum
Phylum[((nrow(heat)*2)+1):(nrow(heat)*3)] <- heat$sigPhylum
Genus <- c(1:(nrow(heat)*3))
Genus[1:nrow(heat)] <- heat$sigGenus
Genus[(nrow(heat)+1):(nrow(heat)*2)] <- heat$sigGenus
Genus[((nrow(heat)*2)+1):(nrow(heat)*3)] <- heat$sigGenus
ftp <- as.data.frame(cbind(SamplingComparison, log2FC, Phylum, Genus))

ftp$log2FC <- as.numeric(as.character(ftp$log2FC))
ftp$SamplingComparison <- factor(ftp$SamplingComparison, levels = c("CTST", "CTS", "STS"))

heatLog <- ggplot(ftp, aes(SamplingComparison, Genus, fill = log2FC)) + geom_tile() +
  geom_text(aes(label = sprintf("%2.1f", log2FC)), size = 2) +
  theme(axis.title = element_blank(), legend.position = "bottom",
        axis.text.y = element_blank(),
        axis.text.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        plot.background = element_blank(),
        plot.margin = margin(t = 2, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 0, r = 0, b = 0, l = 0, unit = "pt")) +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5)) +
  scale_fill_distiller(palette = "RdBu") +
  scale_x_discrete(position = "top", labels = (c("Clinician vs Self",
                                                 "Clinician vs Stool",
                                                 "Self vs Stool")))

heatPhylum <- ggplot(ftp, aes(SamplingComparison, Genus, fill = Phylum)) + geom_tile() +
  theme(axis.title = element_blank(), legend.position = "bottom",
        axis.text.y = element_text(size = 8),
        axis.text.x = element_blank(), axis.ticks.x = element_blank(),
        plot.margin = margin(t = 13.5, r = 5, b = 11, l = 0, unit = "pt"),
        legend.margin = margin(t = 0, r = 0, b = 0, l = 0, unit = "pt"),
        legend.text = element_text(size = 8), legend.key.size = unit(0.75, "line")) +
  scale_fill_brewer(palette = "Set1", guide = guide_legend(ncol = 3))
```

```
heatChanges <- ggarrange(heatPhylum, heatLog, widths = c(1, 2))
heatChanges
```




```
ggsave("Purcell Final/Final_results/6)Differential_Abundance_heatmap.pdf", width = 8, height = 8,
       device = cairo_pdf)
```

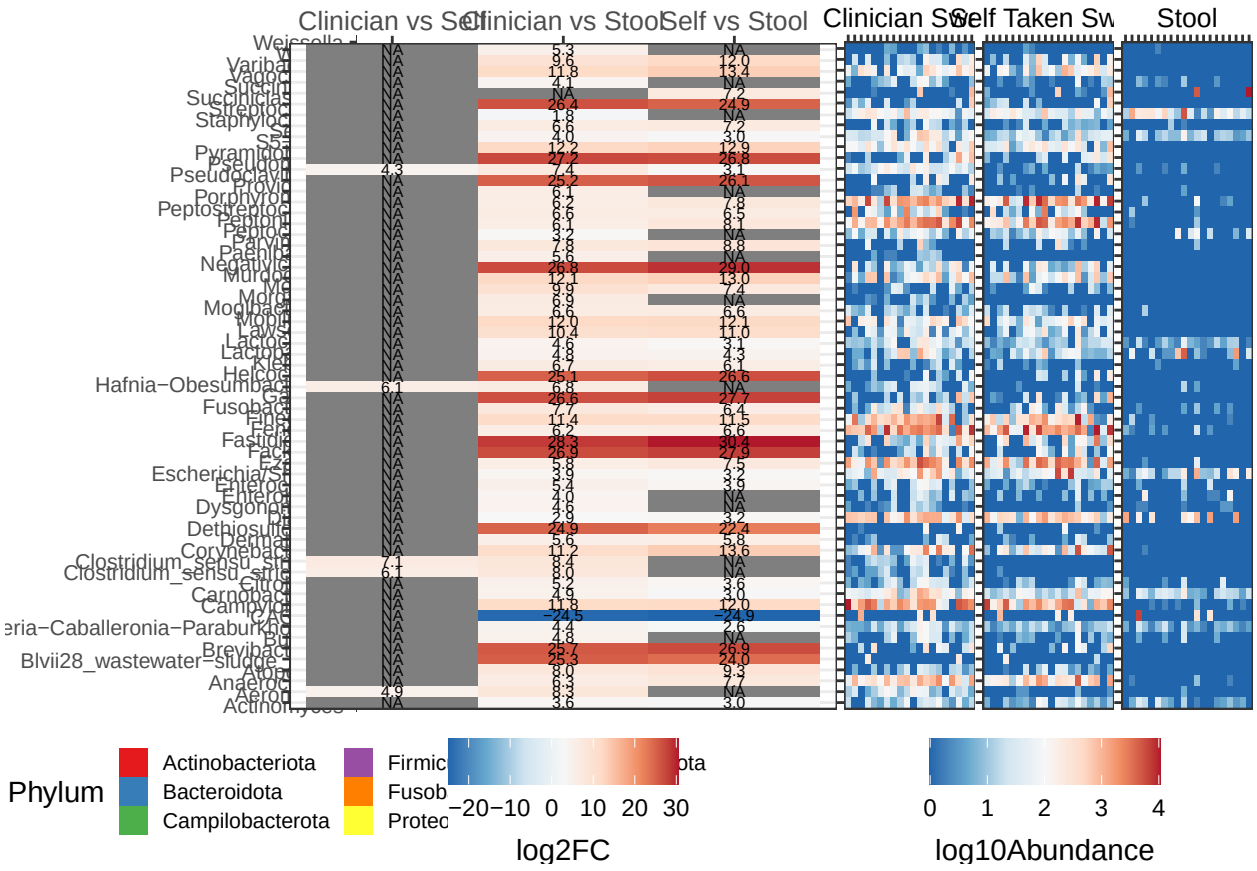
Species Abundance ggplot Heatmap

```
# Make figure with individual abundance to go next to heatmap
heat_ps <- subset_taxa(ps_rare, Genus %in% heat$sigGenus)
heat_ps <- heat_ps %>%
  tax_glom(taxrank = "Genus")

# Clinician Swab
heat_clin <- subset_samples(heat_ps, Sample_type == "Rectal swab CT")
melted_clin <- psmelt(heat_clin)
melted_clin <- select(melted_clin, Individual, Genus, Abundance)
melted_clin$Abundance[melted_clin$Abundance == 0] <- 1
melted_clin$log2Abundance <- log2(melted_clin$Abundance)
melted_clin$log10Abundance <- log10(melted_clin$Abundance)

heatCS <- ggplot(melted_clin, aes(Individual, Genus, fill = log10Abundance)) + geom_tile() +
  scale_x_discrete(position = "top") + xlab("Clinician Swab") +
  theme(axis.title.x = element_text(family = "Helvetica", size = 10, face = "plain"),
```

```
        axis.title.y = element_blank(),
        axis.text = element_blank(), legend.position = "bottom", legend.background = element_blank(),
        plot.margin = margin(t = 1, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 11, r = 0, b = 0, l = 0, unit = "pt")) +
  scale_fill_distiller(palette = "RdBu") +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5))

# Self Swab
heat_self <- subset_samples(heat_ps, Sample_type == "Rectal swab ST")
melted_self <- psmelt(heat_self)
melted_self <- select(melted_self, Individual, Genus, Abundance)
melted_self$Abundance[melted_self$Abundance == 0] <- 1
melted_self$log2Abundance <- log2(melted_self$Abundance)
melted_self$log10Abundance <- log10(melted_self$Abundance)

heatSS <- ggplot(melted_self, aes(Individual, Genus, fill = log10Abundance)) + geom_tile() +
  scale_x_discrete(position = "top") + xlab("Self Taken Swab") +
  theme(axis.title.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        axis.title.y = element_blank(),
        axis.text = element_blank(), legend.position = "bottom", legend.background = element_blank(),
        plot.margin = margin(t = 1, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 11, r = 0, b = 0, l = 0, unit = "pt")) +
  scale_fill_distiller(palette = "RdBu") +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5))

# Stool
heat_stool <- subset_samples(heat_ps, Sample_type == "Stool")
melted_stool <- psmelt(heat_stool)
melted_stool <- select(melted_stool, Individual, Genus, Abundance)
melted_stool$Abundance[melted_stool$Abundance == 0] <- 1
melted_stool$log2Abundance <- log2(melted_stool$Abundance)
melted_stool$log10Abundance <- log10(melted_stool$Abundance)

heatSt <- ggplot(melted_stool, aes(Individual, Genus, fill = log10Abundance)) + geom_tile() +
  scale_x_discrete(position = "top") + xlab("Stool") +
  theme(axis.title.x = element_text(family = "Helvetica", size = 10, face = "plain"),
        axis.title.y = element_blank(),
        axis.text = element_blank(), legend.position = "bottom", legend.background = element_blank(),
        plot.margin = margin(t = 1, r = 0, b = 0, l = 0, unit = "pt"),
        legend.margin = margin(t = 11, r = 0, b = 0, l = 0, unit = "pt")) +
  scale_fill_distiller(palette = "RdBu") +
  guides(fill = guide_colourbar(title.position = "bottom", title.hjust = 0.5))


heatAbundance <- ggarrange(heatCS, heatSS, heatSt, ncol = 3, common.legend = TRUE, legend = c("bottom"))
```

Combined Heatmaps

```
ggarrange(heatChanges, heatAbundance, widths = c(2, 1), legend = c("bottom"))
```




```
ggsave("Purcell Final/Final_results/7)Differential_Abundance_heatmap_extra.pdf",
       width = 11, height = 8, device = cairo_pdf)
```

Boxplot Sanity Checks

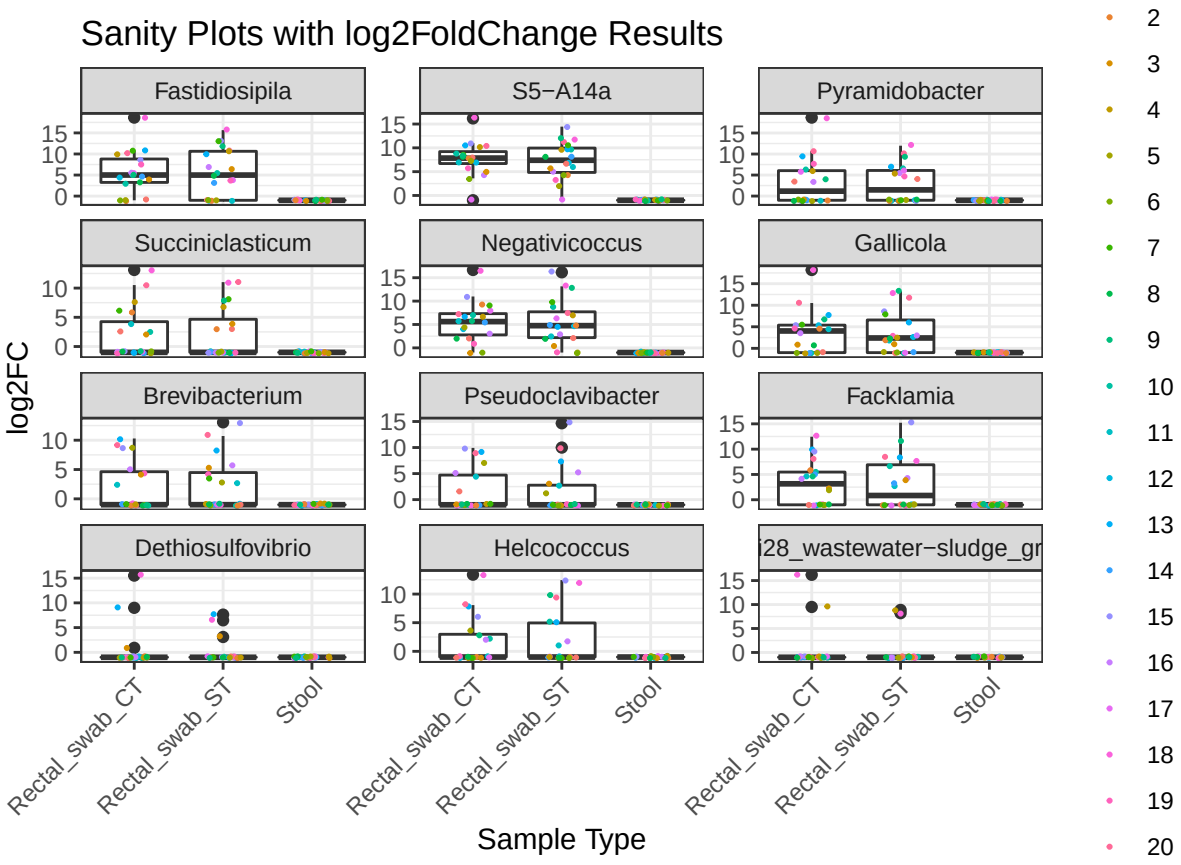
```
resCTS_sig <- resCTS_sig[order(-resCTS_sig$log2FoldChange),]

int <- row.names(resCTS_sig)[1:12]
ASVlabs <- tax_table(ps)[int, 6]
names(ASVlabs) <- int
ASVlabs <- as.list(ASVlabs)

ASV_labeller <- function(variable,value){
  return(ASVlabs[value])
}

# Sanity Plots with Fold Change
tcounts <- t(log2((counts(ds[int, ], normalized = TRUE, replaced = FALSE) + .5))) %>%
  merge(colData(ds), ., by = "row.names") %>%
  gather(ASV, log2FC, (ncol(.)-length(int) + 1):ncol(.))

tcounts %>%
  select(Row.names, Sample_type, Individual, ASV, log2FC) %>%
  head %>%
```

```
  knitr::kable()
```

Row.names	Sample_type	Individual	ASV	log2FC
10A	Rectal_swab_CT	10	ASV262	3.017179
10B	Rectal_swab_ST	10	ASV262	5.359164
10C	Stool	10	ASV262	-1.000000
11A	Rectal_swab_CT	11	ASV262	4.888552
11B	Rectal_swab_ST	11	ASV262	-1.000000
11C	Stool	11	ASV262	-1.000000

```
ggplot(tcounts, aes(Sample_type, log2FC)) +
  geom_boxplot() + geom_jitter(width = 0.2, height = 0.2, size = 0.4, aes(color = Individual)) +
  facet_wrap(~ASV, scales = "free_y", labeller = ASV_labeller, nrow = 4) +
  labs(x = "Sample Type",
       y = "log2FC",
       title = "Sanity Plots with log2FoldChange Results") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

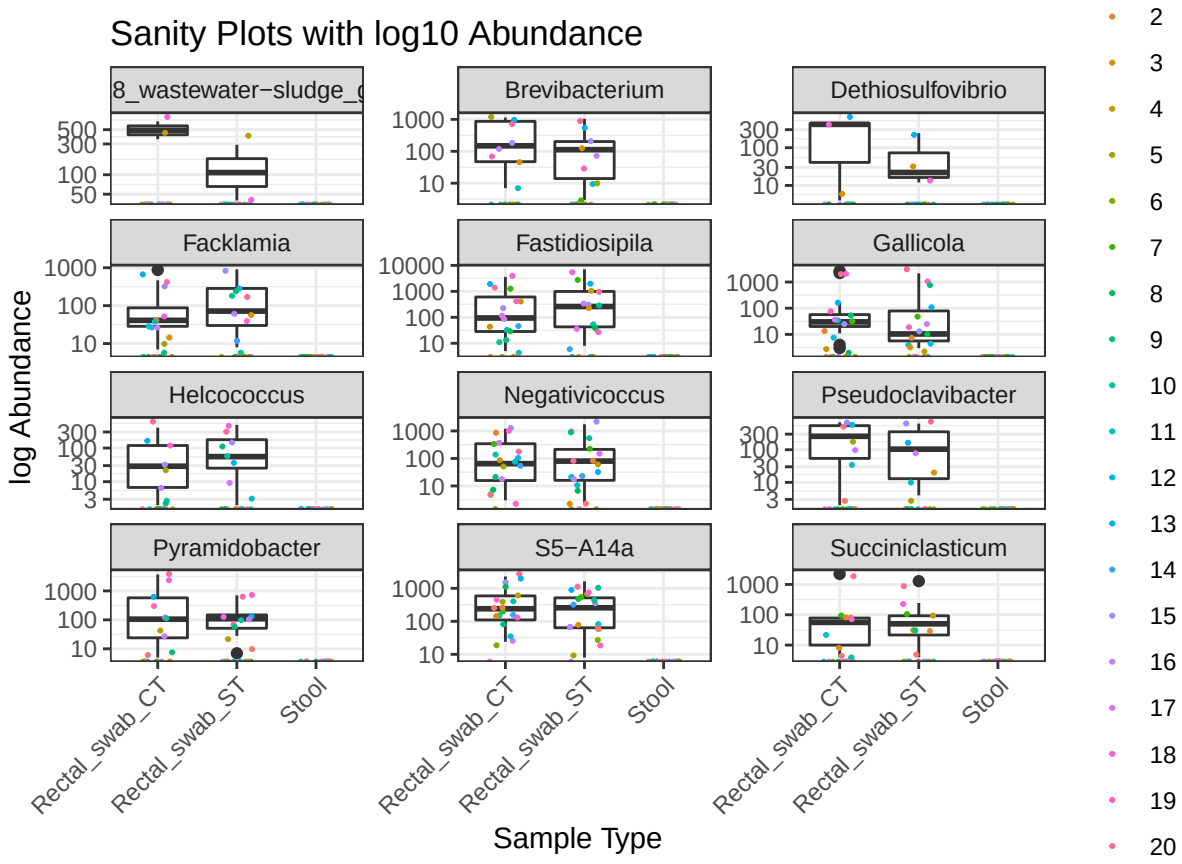
```
## Warning: The labeller API has been updated. Labellers taking `variable` and
## `value` arguments are now deprecated. See labellers documentation.
```




```
ggsave("Purcell Final/Final_results/8)Sanity_FoldChange_plots.pdf", width = 7, height = 8)

# Sanity Plots with Abundance
sanity_ps <- subset_taxa(ps_deseq, taxa_names(ps_deseq) %in% int)
```

```
sanity <- psmelt(sanity_ps)

ggplot(sanity, aes(Sample_type, Abundance)) +
  geom_boxplot() + geom_jitter(width = 0.2, height = 0.2, size = 0.4, aes(color = Individual)) +
  facet_wrap(~Genus, scales = "free_y", nrow = 4) +
  scale_y_log10() +
  labs(x = "Sample Type",
       y = "log Abundance",
       title = "Sanity Plots with log10 Abundance") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
## Warning: Transformation introduced infinite values in continuous y-axis
## Warning: Removed 474 rows containing non-finite values (stat_boxplot).
```




```
ggsave("Purcell Final/Final_results/8)Sanity_logAbundance_plots.pdf", width = 7, height = 8)
```

```
## Warning: Transformation introduced infinite values in continuous y-axis
## Warning: Transformation introduced infinite values in continuous y-axis
## Warning: Removed 474 rows containing non-finite values (stat_boxplot).
```

Session Info

```
sessionInfo()
```

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Sierra 10.12.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_NZ.UTF-8/en_NZ.UTF-8/en_NZ.UTF-8/C/en_NZ.UTF-8/en_NZ.UTF-8
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] DESeq2_1.26.0               SummarizedExperiment_1.16.1
##  [3] DelayedArray_0.12.3         BiocParallel_1.20.1
##  [5] matrixStats_0.56.0          Biobase_2.46.0
##  [7] GenomicRanges_1.38.0        GenomeInfoDb_1.22.1
##  [9] IRanges_2.20.2              S4Vectors_0.24.4
## [11] BiocGenerics_0.32.0         scales_1.1.1
## [13] tidyr_1.1.0                 ggpubr_0.4.0
## [15] extrafont_0.17              ggplot2_3.3.2
## [17] phyloseq_1.30.0             dplyr_1.0.0
## [19] vegan_2.5-6                 permute_0.9-5
## [21] Rmisc_1.5                   plyr_1.8.6
## [23] lattice_0.20-41             RColorBrewer_1.1-2
##
## loaded via a namespace (and not attached):
##   [1] colorspace_1.4-1       ggsignif_0.6.0         ellipsis_0.3.1
##   [4] rio_0.5.16             htmlTable_2.0.1        XVector_0.26.0
##   [7] base64enc_0.1-3        rstudioapi_0.11        farver_2.0.3
##  [10] bit64_0.9-7.1          AnnotationDbi_1.48.0   codetools_0.2-16
##  [13] splines_3.6.3          geneplotter_1.64.0     knitr_1.29
##  [16] ade4_1.7-15            Formula_1.2-3          jsonlite_1.7.0
##  [19] annotate_1.64.0        broom_0.7.0            Rttf2pt1_1.3.8
##  [22] cluster_2.1.0          png_0.1-7              compiler_3.6.3
##  [25] backports_1.1.8        Matrix_1.2-18          acepack_1.4.1
##  [28] htmltools_0.5.0        tools_3.6.3            igraph_1.2.5
##  [31] gtable_0.3.0           glue_1.4.1             GenomeInfoDbData_1.2.2
##  [34] reshape2_1.4.4         Rcpp_1.0.5             carData_3.0-4
##  [37] cellranger_1.1.0       vctrs_0.3.2            Biostrings_2.54.0
##  [40] multtest_2.42.0        ape_5.4                nlme_3.1-148
##  [43] extrafontdb_1.0        iterators_1.0.12       xfun_0.15
##  [46] stringr_1.4.0          openxlsx_4.1.5         lifecycle_0.2.0
##  [49] XML_3.99-0.3           rstatix_0.6.0          zlibbioc_1.32.0
##  [52] MASS_7.3-51.6          hms_0.5.3              biomformat_1.14.0
##  [55] rhdf5_2.30.1           yaml_2.2.1             curl_4.3
```

```
##  [58] memoise_1.1.0          gridExtra_2.3          rpart_4.1-15
##  [61] RSQLite_2.2.0          latticeExtra_0.6-29    stringi_1.4.6
##  [64] highr_0.8              genefilter_1.68.0      foreach_1.5.0
##  [67] checkmate_2.0.0        zip_2.0.4              rlang_0.4.7
##  [70] pkgconfig_2.0.3        bitops_1.0-6           evaluate_0.14
##  [73] purrr_0.3.4            Rhdf5lib_1.8.0         labeling_0.3
##  [76] htmlwidgets_1.5.1      cowplot_1.0.0          bit_1.1-15.2
##  [79] tidyselect_1.1.0       magrittr_1.5           R6_2.4.1
##  [82] generics_0.0.2         Hmisc_4.4-0            DBI_1.1.0
##  [85] pillar_1.4.6           haven_2.3.1            foreign_0.8-76
##  [88] withr_2.2.0            mgcv_1.8-31            survival_3.2-3
##  [91] abind_1.4-5            RCurl_1.98-1.2         nnet_7.3-14
##  [94] tibble_3.0.3           crayon_1.3.4           car_3.0-8
##  [97] rmarkdown_2.3          jpeg_0.1-8.1           locfit_1.5-9.4
## [100] grid_3.6.3             readxl_1.3.1           data.table_1.12.8
## [103] blob_1.2.1             forcats_0.5.0          digest_0.6.25
## [106] xtable_1.8-4           munsell_0.5.0
```