Predicting Endosporulation Among Expanding Firmicutes Phylogeny

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Setup Libraries

```
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 3.4.4
```

```
library(stringr)
```

```
## Warning: package 'stringr' was built under R version 3.4.4
```

```
library(reshape2)
library(plyr)
library(gplots)
```

```
## Warning: package 'gplots' was built under R version 3.4.4
```

```
##
## Attaching package: 'gplots'
```

```
## The following object is masked from 'package:stats':
##
##     lowess
```

```
library(openxlsx)
```

```
## Warning: package 'openxlsx' was built under R version 3.4.4
```

```
library(ggpubr)
```

```
## Warning: package 'ggpubr' was built under R version 3.4.4
```

```
## Loading required package: magrittr
```

```
##
## Attaching package: 'ggpubr'
```

```
## The following object is masked from 'package:plyr':
##
##     mutate
```

```
library(ggsignif)
```

```
## Warning: package 'ggsignif' was built under R version 3.4.4
```

```
library(knitr)
```

Download BLAST results and metadata

```
REF_HITS <- read.table("../data/WW_REFERENCE_BLAST_OUT.txt", header = F, sep="\t")
REF_META <- read.xlsx(xlsxFile = "../data/WWRefence_Metadata.xlsx", sheet = 1)
UBA_HITS_DATA <- read.xlsx("../data/tyson_genome_list_1201_firms.xlsx")
UBA_HITS <- read.table("../data/B_subtilis_spo_157_gene_in_PARKS_FIRMICUTES.txt",
                       header = F, sep="\t")
```

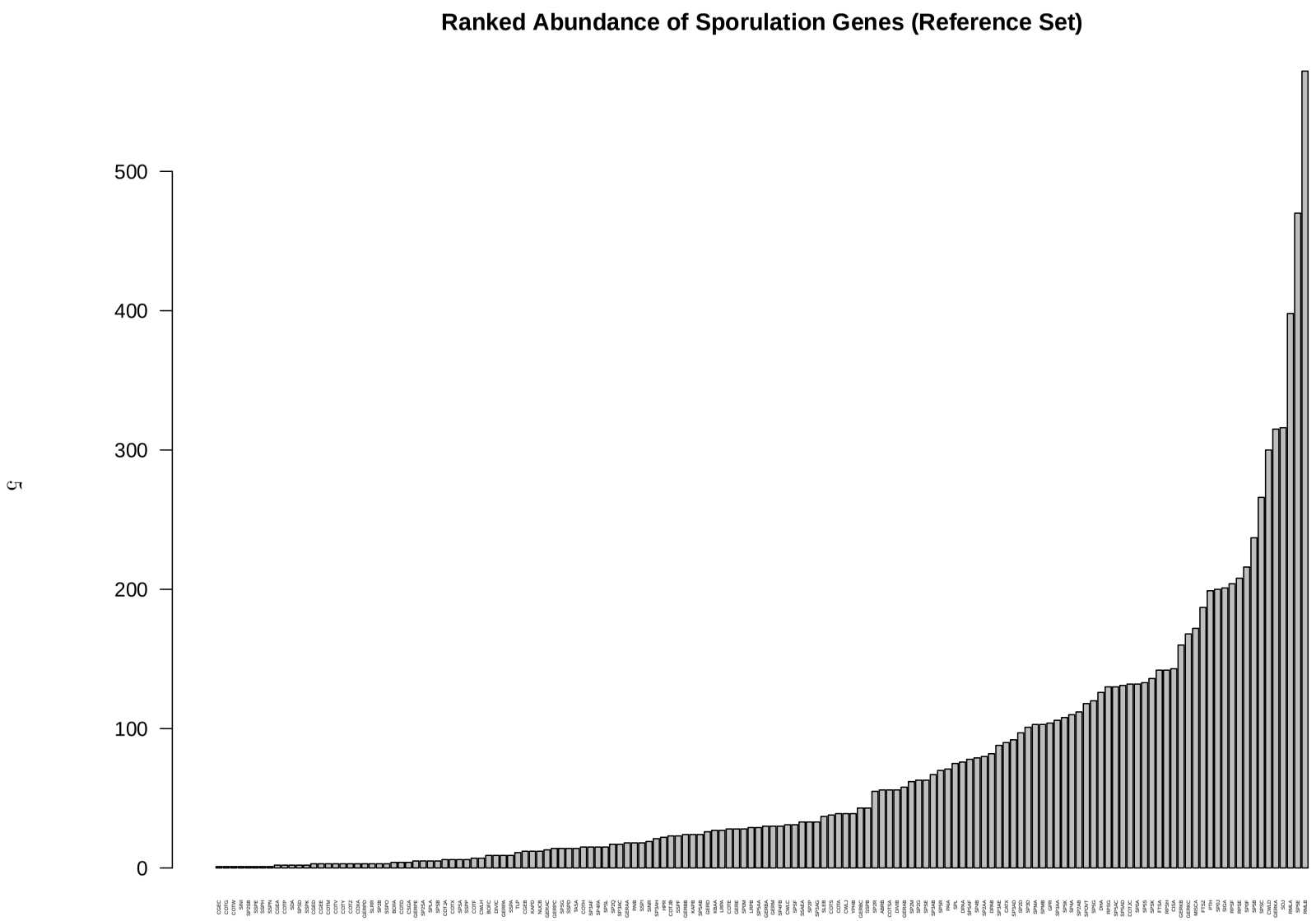
Combine Data

```
colnames(REF_HITS) <- c("qseqid","sseqid","pident","length","qlen","slen","mismatch",
                        "gapopen","qstart","qend","sstart","send","qcov","evalue","bitscore")
REF_HITS <- unique(REF_HITS)
REF_HITS <- REF_HITS[REF_HITS$qcov >= 80,]
REF_HITS <- REF_HITS[REF_HITS$bitscore >= 80,]
REF_HITS <- REF_HITS[order(REF_HITS$bitscore, decreasing = T),]
REF_HITS <- REF_HITS[!duplicated(REF_HITS$sseqid),]

REF_HITS <- REF_HITS[order(REF_HITS$bitscore, decreasing = T),]
REF_GENOMES <- strsplit(as.vector(unlist(strsplit(as.vector(REF_HITS$sseqid),
                                                  "\\|"))[grep("_", unlist(strsplit(as.vector(REF_HITS$
                                                                                    "\\|")))])),"_")
Nuccore_ID = ""
for(i in 1:length(REF_GENOMES)){
  if(REF_GENOMES[[i]][1] == "NC" | REF_GENOMES[[i]][1] == "NZ"){
    p <- str_c(REF_GENOMES[[i]][c(1,2)],"", collapse = "_")
  }
  else(
    p <- str_c(REF_GENOMES[[i]][c(1)],"")
  )
  Nuccore_ID[i] <- p
}
REF_HITS$Nuccore_ID <- Nuccore_ID

feature_type = ""
for(i in 1:length(REF_GENOMES)){
  if(REF_GENOMES[[i]][1] == "NC" | REF_GENOMES[[i]][1] == "NZ"){
    p <- REF_GENOMES[[i]][c(3)]
  }
  else(
    p <- REF_GENOMES[[i]][c(2)]
  )
```

```
  feature_type[i] <- p
}
REF_HITS$feature_type <- feature_type
RefSeq_ID = ""
for(i in 1:length(REF_GENOMES)){
  if(REF_GENOMES[[i]][1] == "NC" | REF_GENOMES[[i]][1] == "NZ"){
    p <- str_c(REF_GENOMES[[i]][c(4,5)],"", collapse = "_")
  }
  else(
    p <- str_c(REF_GENOMES[[i]][c(3)],"", collapse = "_")
  )
  RefSeq_ID[i] <- p
}
REF_HITS$RefSeq_ID <- RefSeq_ID

gene_names <- unlist(strsplit(as.vector(REF_HITS$qseqid),
"\\|"))[grep("_",
unlist(strsplit(as.vector(REF_HITS$qseqid), "\\|")))]
gene_names_spl <- strsplit(gene_names, "_")
unlist(strsplit(gene_names, "_"))
is.odd <- function(x) x %% 2 != 0
gene_names <- unlist(strsplit(gene_names,
"_"))[seq(from=1,
to=length(unlist(strsplit(gene_names, "_"))))[is.odd(seq(from=1,
to=length(unlist(strsplit(gene_names, "_")))))]]
REF_HITS$Gene_Names <- gene_names
REF_HITS <- merge(REF_HITS, REF_META)
#Check to make sure all the genes are in REF_HITS set after filtering
list_genes<-unique(REF_HITS$Gene_Names)
setdiff(list_genes, REF_HITS$Gene_Names)
```

plot abundances of genes in reference dataset

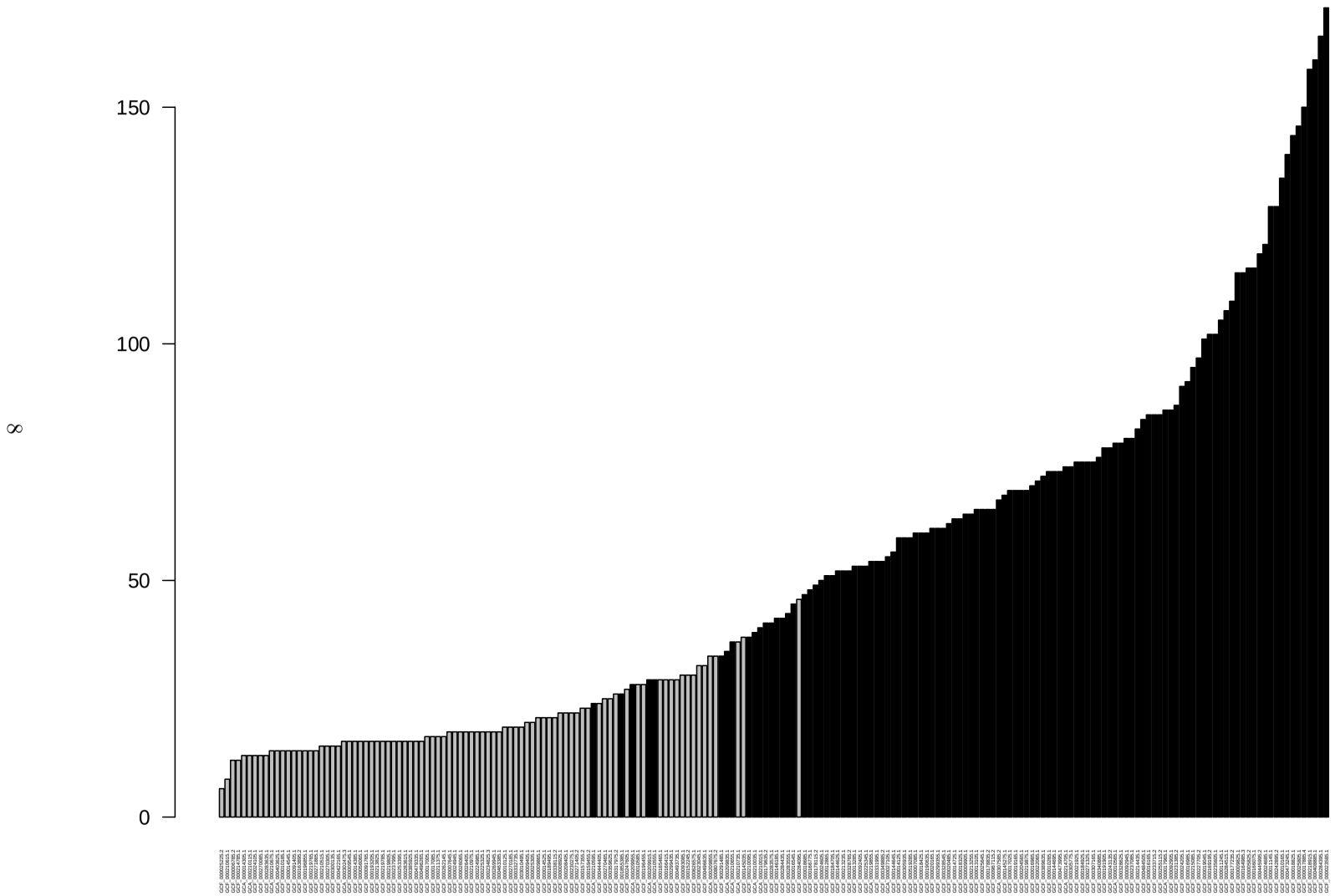
```r
barplot(table(REF_HITS$Gene_Names)[order(table(REF_HITS$Gene_Names))], cex.names = 0.25,
        las= 2, main = "Ranked Abundance of Sporulation Genes (Reference Set)")
```


Ranked Abundance of Sporulation Genes (Reference Set)
500
400
300
200
100
0
g

Label spore formers and non spore formers in reference set

```
spore_cols <- REF_HITS[,c(23,27)]
genome_gene_count <- as.data.frame(table(spore_cols$Assembly))
colnames(genome_gene_count) <- c("Assembly", "Freq")
spore_cols <- unique(spore_cols)
spore_cols<- merge(spore_cols, genome_gene_count)
spore_cols <- spore_cols[order(spore_cols$Freq),]
spore_cols$cols <- spore_cols$`Spore.Forming.(Weller.and.Wu)`
spore_cols[spore_cols$cols == "Y",]$cols <- "black"
spore_cols[spore_cols$cols == "N",]$cols <- "grey"
```

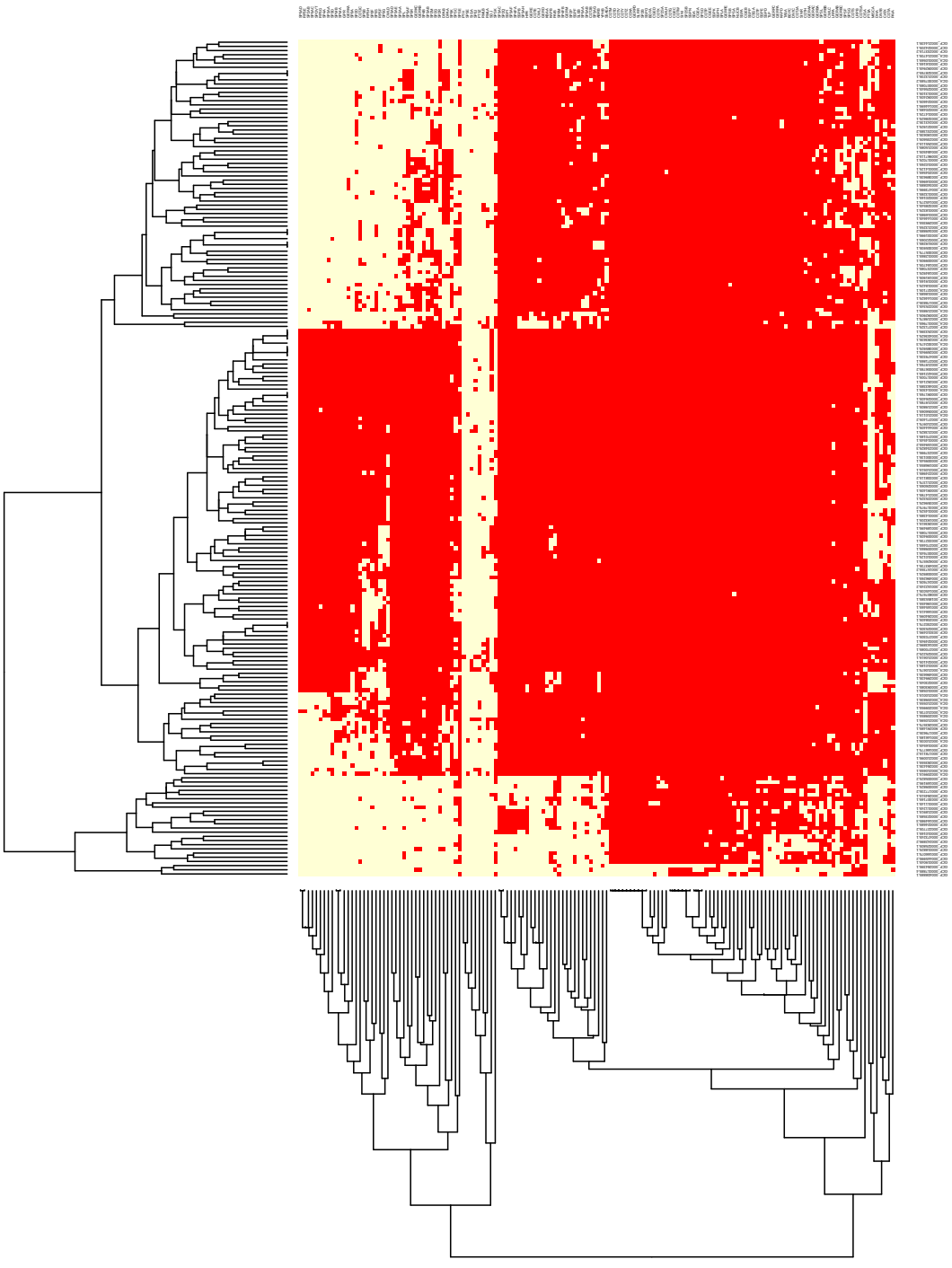
```r
barplot(spore_cols$Freq, las= 2, cex.axis = 1, cex.names = 0.25,
        names.arg = spore_cols$Assembly, col=spore_cols$cols,
        main="Ranked Abundance of Sporulation Genes in Genomes (Reference Set)")
```


Ranked Abundance of Sporulation Genes in Genomes (Reference Set)
150
100
50
0
∞

Convert abundance to presence/abscene

```
gene_by_genome <- table(REF_HITS$Gene_Names, REF_HITS$Assembly)
gene_by_genome[gene_by_genome > 0] <- 1
gene_by_genome_df <- as.data.frame(gene_by_genome)
colnames(gene_by_genome_df) <- c("Gene_Names", "Assembly", "Presence")
gene_by_genome_df <- merge(gene_by_genome_df, spore_cols)
gene_by_genome_df[order(gene_by_genome_df$Freq),]
```

```
heatmap.2(gene_by_genome, trace="none", margins = c(10,10), cexRow=0.25,
          cexCol=0.25,
          key = FALSE, sepwidth=c(0.5,0.5), sepcolor="black")
```



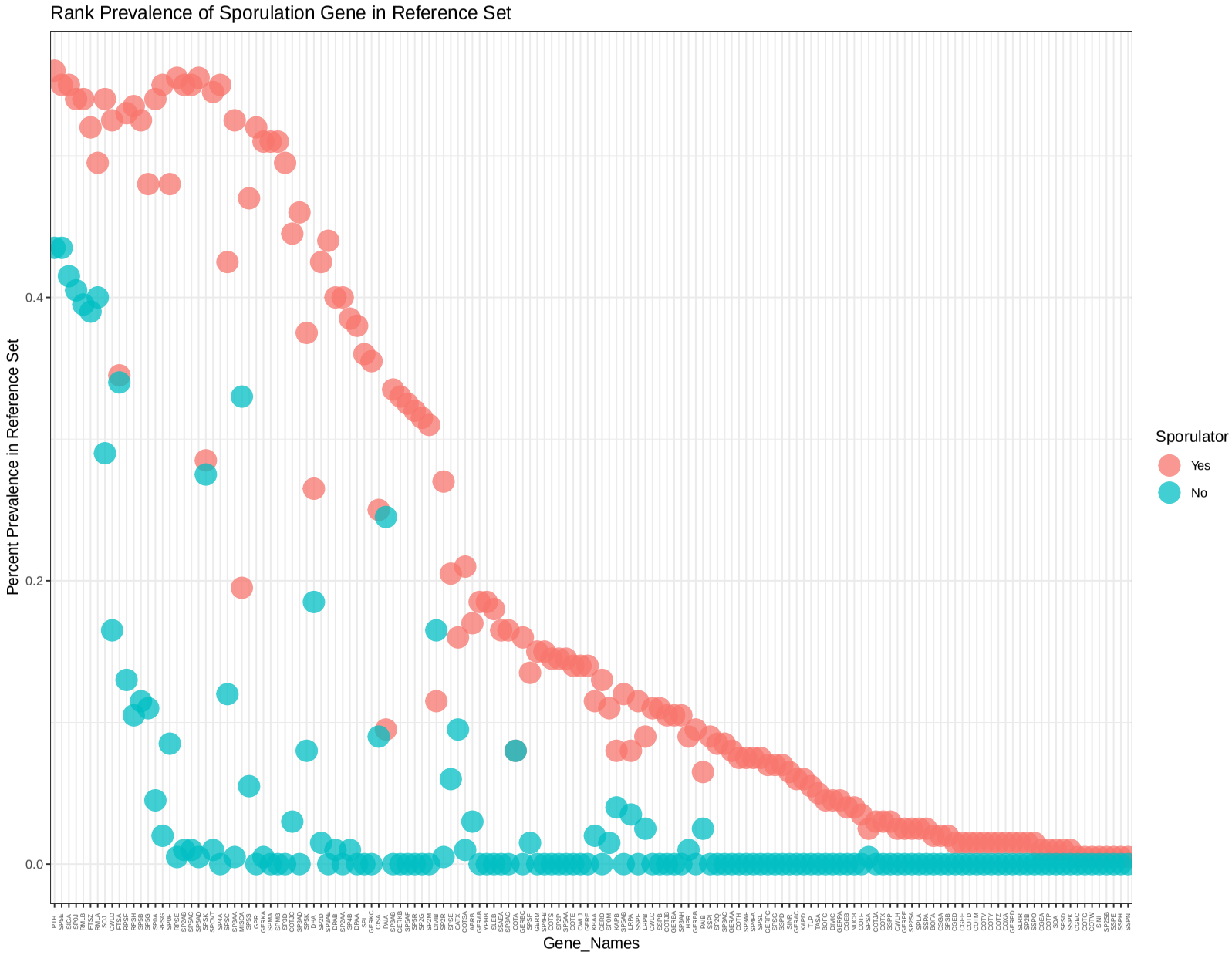
```
gene_presence <- ddply(gene_by_genome_df, .(Gene_Names), summarise, sum_gene_presence = sum(Presence))
gene_by_genome_df <- merge(gene_by_genome_df, gene_presence)
gene_by_genome_df <- gene_by_genome_df[order(gene_by_genome_df$sum_gene_presence),]
```

Convert presence of Gene to percent of presence in among the 200 Genomes

```
gene_by_genome_df_hits <- gene_by_genome_df[gene_by_genome_df$Presence > 0,]
sporu_vs_non <- table(gene_by_genome_df_hits$Gene_Names ,gene_by_genome_df_hits$cols)
sporu_vs_non <- melt(sporu_vs_non)

sporu_vs_non$Percent_Core_Set = sporu_vs_non$value/200
colnames(sporu_vs_non) <- c("Gene_Names", "cols", "hits", "Percent_Core_Set")
```

Plot prevalence of genes in reference dataset in ranked order

```
ggplot(sporu_vs_non,
       aes(y=Percent_Core_Set,
                        x=reorder(Gene_Names,
                                  -rep(sporu_vs_non[sporu_vs_non$cols == "black",]$Percent_Core_Set +
                                                                                  sporu_vs_non[sporu_vs_non$cols == "grey",]$Percent_Core_Set, 2))
           color=cols)) +
  geom_point(stat="identity", size=6, alpha=0.75) +
  ggtitle("Rank Prevalence of Sporulation Gene in Reference Set") + xlab("Gene_Names") +
  ylab("Percent Prevalence in Reference Set") +
  theme_bw(base_size = 10) + scale_color_discrete("Sporulator", labels=c("Yes", "No")) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.4, size = 4))
```


Rank Prevalence of Sporulation Gene in Reference Set
Percent Prevalence in Reference Set
0.0
0.2
0.4
Gene_Names
Sporulator
Yes
No

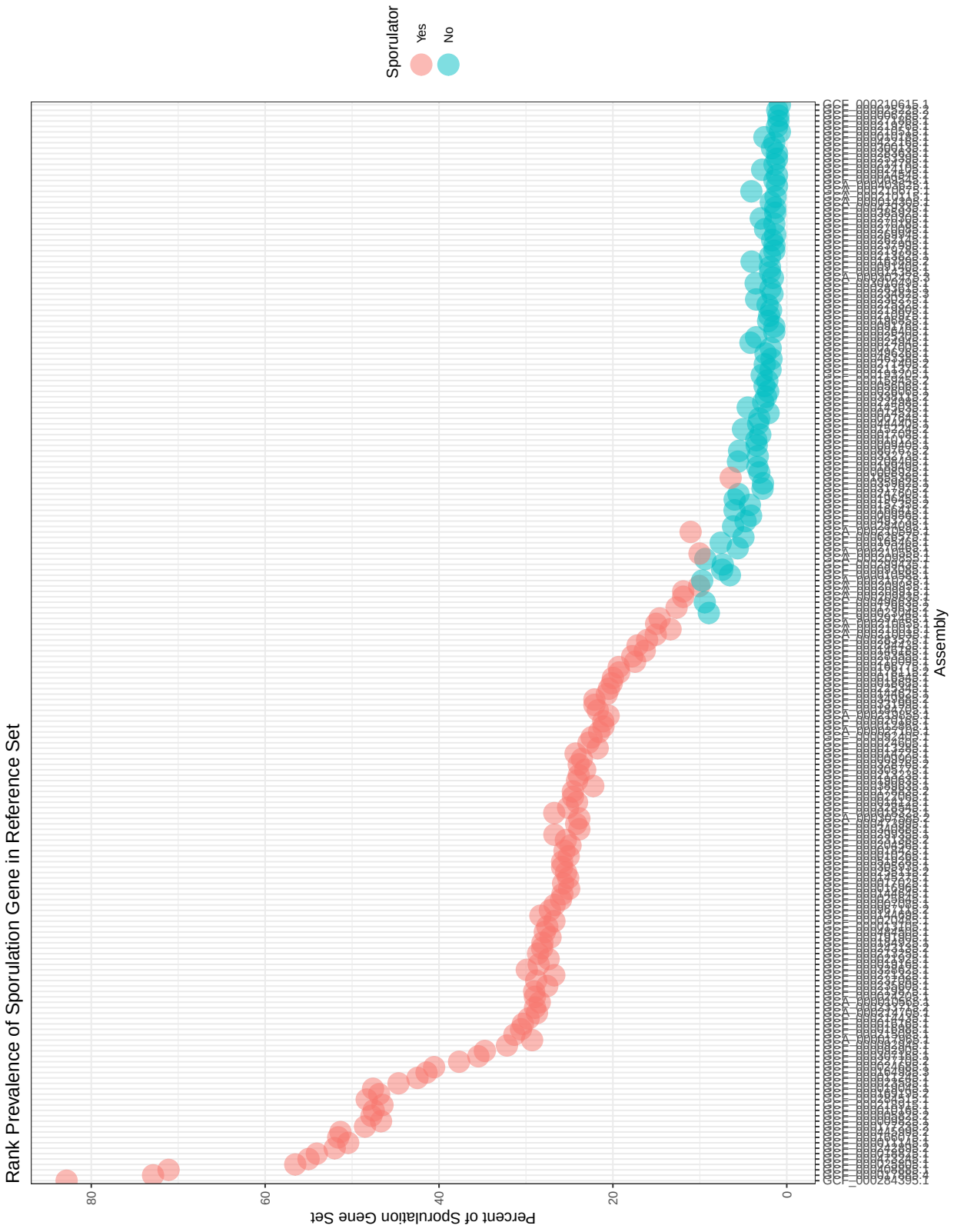
Weight the prevalence of genes in the reference dataset

The weight is equal to the prevalence of a gene in the reference dataset among sporulators minus the prevalence of a gene in the reference dataset among nonsporulators all divided by the prevalence of a gene in the reference dataset among sporulators. This allows for a gene that is a present in exclusively sporulator to count as one and a gene with some representation among non-sporulators to be discounted by that prevalence. In some cases the genes more prevalent in nonsporulator than sporulators and that negative value was returned to a value of zero.

```
gene_weights <- as.data.frame(cbind(as.vector(sporu_vs_non[sporu_vs_non$cols == "black",]$Gene_Names),
                                    as.vector(as.numeric((sporu_vs_non[sporu_vs_non$cols ==
                                                                       "black",]$Percent_Core_Set-spo
colnames(gene_weights) <- c("Gene_Names", "weight")
gene_weights$weight <- as.numeric(as.character(gene_weights$weight))
gene_weights[gene_weights$weight < 0,]$weight <- 0
gene_by_genome_df_hits <- merge(gene_by_genome_df_hits, gene_weights)

weighted_percents <- ddply(gene_by_genome_df_hits, .(Assembly), summarise, weighted_percent= sum(weight
sporu_vs_non <- table(gene_by_genome_df_hits$Assembly ,gene_by_genome_df_hits$cols)
sporu_vs_non <- melt(sporu_vs_non)
sporu_vs_non <- sporu_vs_non[sporu_vs_non$value != 0, ]
sporu_vs_non$Percent_Core_Set = sporu_vs_non$value/150
colnames(sporu_vs_non) <- c("Assembly", "cols", "hits", "Percent_Core_Set")

sporu_vs_non <- merge(sporu_vs_non, weighted_percents)
```

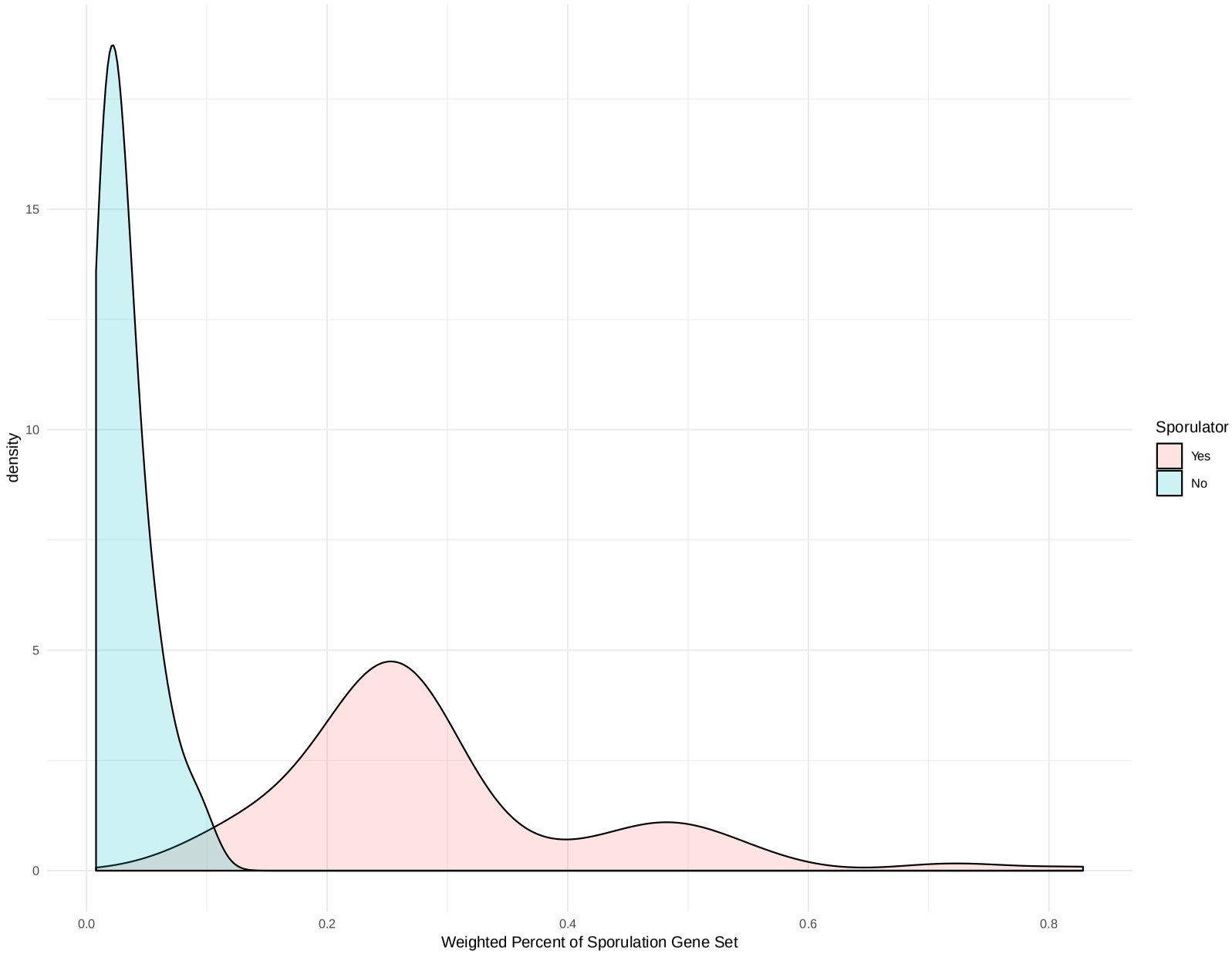
Plot

```
ggplot(sporu_vs_non, aes(y=weighted_percent*100, x=reorder(Assembly, -(hits)), color=cols)) +
  geom_point(stat="identity", size=6, alpha = 0.5) +
  ggtitle("Rank Prevalence of Sporulation Gene in Reference Set") + xlab("Assembly") +
  ylab("Percent of Sporulation Gene Set") +
  theme_bw(base_size = 10) + scale_color_discrete("Sporulator", labels=c("Yes", "No")) +
  theme(axis.text.x = element_text(angle = -90, vjust = 0.4, hjust = 0, size = 8))
```


Rank Prevalence of Sporulation Gene in Reference Set
Percent of Sporulation Gene Set
0
20
40
60
80
Assembly
Sporulator
Yes
No

Plot

```
p <- ggplot(sporu_vs_non) +
  geom_density(aes(x=weighted_percent, fill=cols), alpha=0.2, adjust=2) +
  xlab("Weighted Percent of Sporulation Gene Set") +
  scale_fill_discrete("Sporulator", labels=c("Yes", "No", "?")) +
  theme_minimal(base_size = 10)
p
```


density
15
10
5
0
0.0
0.2
0.4
0.6
0.8
Weighted Percent of Sporulation Gene Set
Sporulator
Yes
No

Bring in Tyson dataset

```
colnames(UBA_HITS) <- c("qseqid","sseqid","pident","length","qlen","slen","mismatch",
                        "gapopen","qstart","qend","sstart","send","qcov","evalue","bitscore")
length(unique(UBA_HITS$Assembly))
UBA_HITS$Genome = str_sub(UBA_HITS$sseqid,1,4)
list_genomes<-unique(UBA_HITS$Genome)


gene_names <- unlist(strsplit(as.vector(UBA_HITS$qseqid),
                              "\\|"))[grep("_", unlist(strsplit(as.vector(UBA_HITS$qseqid),
                                                                "\\|")))]
gene_names_spl <- strsplit(gene_names, "_")

is.odd <- function(x) x %% 2 != 0
gene_names <- unlist(strsplit(gene_names, "_"))[seq(from=1,
to=length(unlist(strsplit(gene_names, "_"))))[is.odd(seq(from=1,
to=length(unlist(strsplit(gene_names, "_"))))]]

UBA_HITS$Gene_Names <- gene_names
list_genes<-unique(UBA_HITS$Gene_Names)

UBA_HITS <- unique(UBA_HITS)
UBA_HITS <- UBA_HITS[UBA_HITS$qcov >= 80,]
UBA_HITS <- UBA_HITS[UBA_HITS$bitscore >= 80,]

UBA_HITS <- UBA_HITS[order(UBA_HITS$bitscore, decreasing = T),]

UBA_HITS <- UBA_HITS[!duplicated(UBA_HITS$sseqid),]

genomes_not_found <- setdiff(list_genomes, UBA_HITS$Genome)
genes_not_found <- setdiff(list_genes, UBA_HITS$Gene_Names)


UBA_HITS$Genome = str_sub(UBA_HITS$sseqid,1,4)

UBA_HITS_DATA$Genome = str_sub(UBA_HITS_DATA$`DDBJ/ENA/GenBank.Accession`,1,4)
UBA_HITS <- merge(UBA_HITS, UBA_HITS_DATA)
UBA_GENES_GENOMES <-UBA_HITS[,c(17,19)]



setarr <- setdiff(UBA_GENES_GENOMES$Gene_Names, REF_HITS$Gene_Names)

UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[1],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[2],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[3],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[4],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[5],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[6],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[7],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[8],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[9],]
```

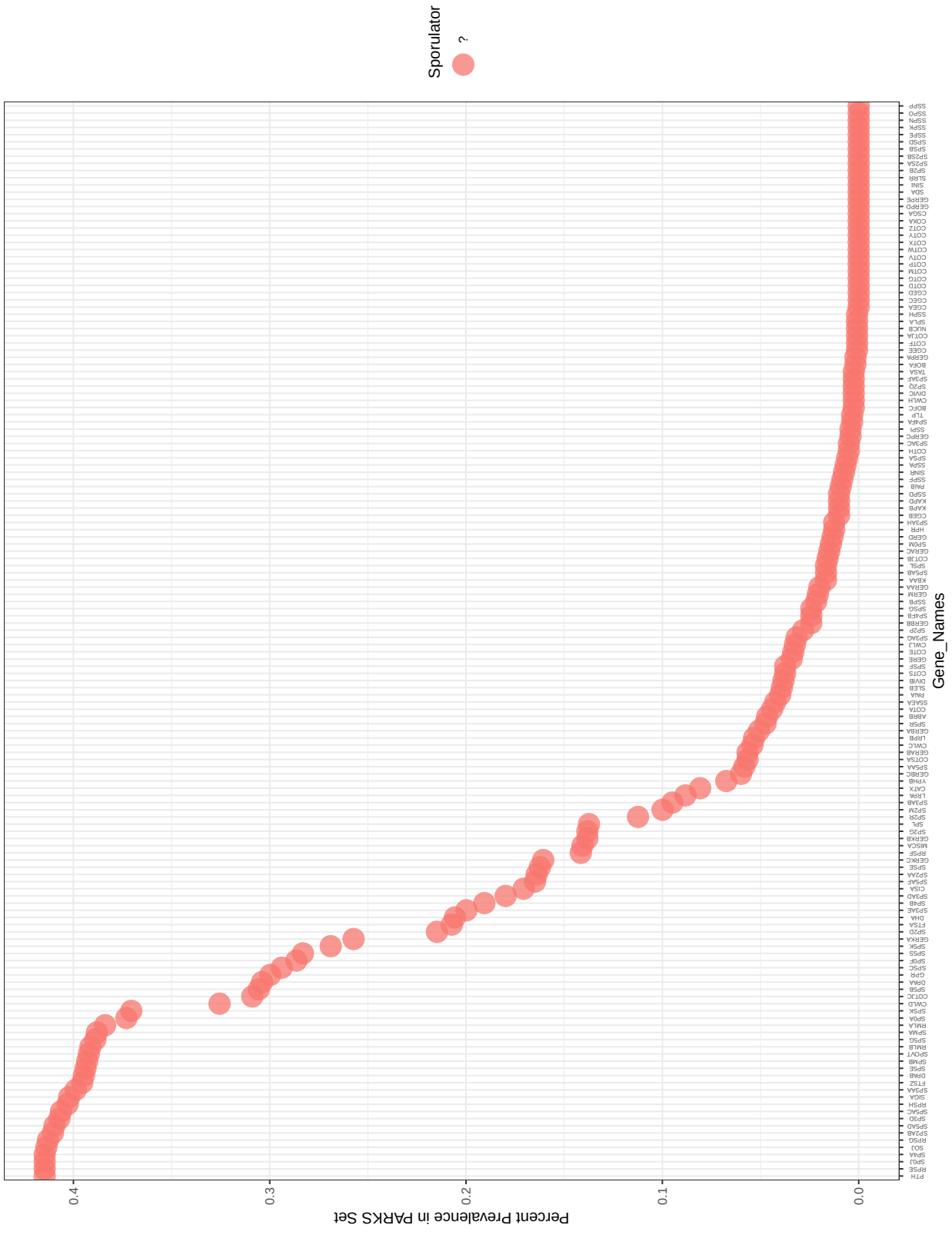
```
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[10],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[11],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[12],]
UBA_GENES_GENOMES <- UBA_GENES_GENOMES[UBA_GENES_GENOMES$Gene_Names != setarr[13],]

genes_not_found <- setdiff(REF_HITS$Gene_Names, UBA_GENES_GENOMES$Gene_Names)

count_by_gene <- as.data.frame(table(UBA_GENES_GENOMES$UBA.Genome.ID,UBA_GENES_GENOMES$Gene_Names))

count_by_gene[count_by_gene$Freq > 0,]$Freq <- 1
colnames(count_by_gene) <- c("Assembly", "Gene_Names", "Presence")

genes_not_found <- as.data.frame(cbind(genes_not_found, rep(0, length(genes_not_found))))
colnames(genes_not_found) <- c("Gene_Names","Presence")


genomes_not_found <-setdiff(UBA_HITS_DATA$UBA.Genome.ID, count_by_gene$Assembly)
genomes_not_found <- as.data.frame(cbind(genomes_not_found,
                                         rep(0, length(genomes_not_found))))
colnames(genomes_not_found) <- c("Assembly","Presence")


not_founds <- merge(c(as.vector(unique(genes_not_found$Gene_Names)),
                      as.vector(unique(count_by_gene$Gene_Names))),
                    c(as.vector(unique(count_by_gene$Assembly)),
                      as.vector(unique(genomes_not_found$Assembly))))



not_founds$Presence <- 0
not_founds <- not_founds[c(2,1,3)]
colnames(not_founds) <- c("Assembly","Gene_Names","Presence")
count_by_gene <- rbind(count_by_gene, not_founds)
```

```
freq_Parks<- ddply(count_by_gene, .(Gene_Names), summarise, Freq=sum(as.numeric(Presence)))
count_by_gene <- merge(count_by_gene, freq_Parks)
count_by_gene$`Spore.Forming.(Weller.and.Wu)` <- "?"
count_by_gene$cols <- "blue"
gene_presence_Parks<- ddply(count_by_gene, .(Assembly),
                            summarise, sum_gene_presence=sum(as.numeric(Presence)))
count_by_gene <- merge(count_by_gene, gene_presence_Parks)

count_by_gene <- count_by_gene[c(2,1,3,5,4,6,7)]

count_by_gene_hits <- count_by_gene[count_by_gene$Presence > 0,]
count_by_gene_hits <- merge(count_by_gene_hits, gene_weights)


weighted_percents <- ddply(count_by_gene_hits, .(Assembly),
                           summarise, weighted_percent= sum(weight)/150)

count_by_gene_hits_count <- table(count_by_gene_hits$Gene_Names ,count_by_gene_hits$cols)
count_by_gene_hits_count <- melt(count_by_gene_hits_count)
```

```
count_by_gene_hits_count$Percent_Core_Set = count_by_gene_hits_count$value/1201
colnames(count_by_gene_hits_count) <- c("Gene_Names", "cols", "hits", "Percent_Core_Set")
```

Plot

```
ggplot(count_by_gene_hits_count,
       aes(y=Percent_Core_Set, x=reorder(Gene_Names, -hits), color=cols)) +
  geom_point(stat="identity", size=6, alpha=0.75) +
  xlab("Gene_Names") +
  ylab("Percent Prevalence in PARKS Set") +
  theme_bw(base_size = 10) +
  scale_color_discrete("Sporulator", labels=c("?")) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.4, size = 4))
```


Sporulator
?
Percent Prevalence in PARKS Set
0.0
0.1
0.2
0.3
0.4
Gene_Names

```
count_by_gene_hits_count <- count_by_gene[count_by_gene$Presence > 0,]
count_by_gene_hits_count <- table(count_by_gene_hits_count$Assembly ,count_by_gene_hits_count$cols)
count_by_gene_hits_count <- melt(count_by_gene_hits_count)
colnames(count_by_gene_hits_count) <- c("Assembly", "cols", "hits")
count_by_gene_hits_count$Percent_Core_Set = count_by_gene_hits_count$hits/150
count_by_gene_hits_count <-merge(count_by_gene_hits_count, weighted_percents)
```

```
PARKS_and_REF <- rbind(sporu_vs_non, count_by_gene_hits_count)
```

Plot

```
ggplot(PARKS_and_REF, aes(y=Percent_Core_Set, x=reorder(Assembly, -hits), color=cols)) +
  geom_point(stat="identity", size=6, alpha=0.75) +
  xlab("Gene_Names") +
  ylab("Percent Prevalence\nof Sporulation Set in Genomes") +
  theme_bw(base_size = 10) +
  scale_color_discrete("Sporulator", labels=c("Yes","No","?")) +
  theme(axis.text.x = element_text(angle = 90, vjust = 0.4, size = 4))
```

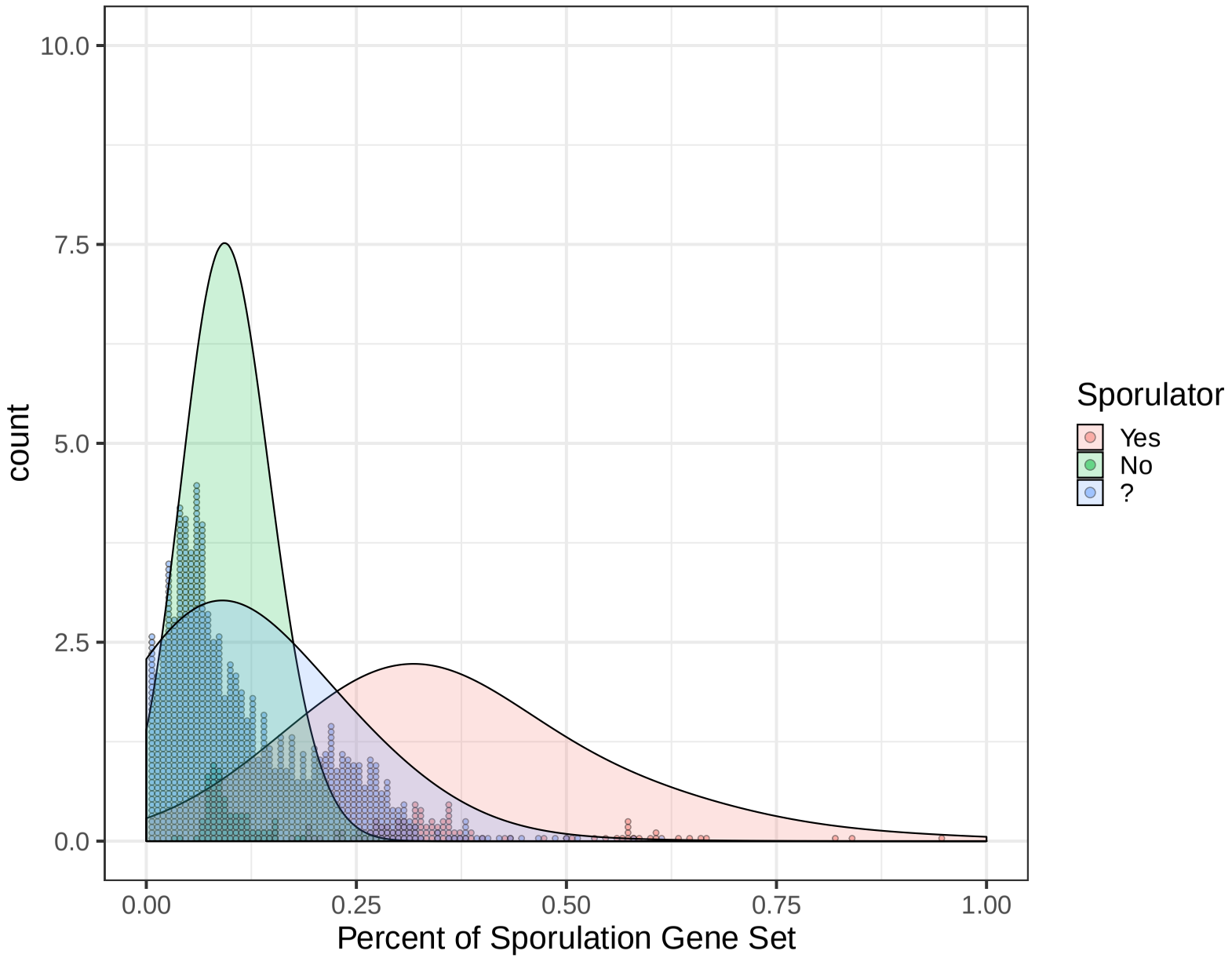
Sporulator
Yes
No
?
Percent Prevalence
of Sporulation Set in Genomes
0.00
0.25
0.50
0.75
Gene_Names

Plot

```
p <- ggplot(PARKS_and_REF) +
  geom_dotplot(data=PARKS_and_REF, aes(x=Percent_Core_Set, fill=cols),
               alpha=0.5, dotsize = 1.2, binwidth = 1/180) +
  scale_fill_discrete("Sporulator", labels=c("Yes", "No", "?")) +
  theme_bw(base_size = 20)

p <- p + geom_density(aes(x=Percent_Core_Set, fill=cols), alpha=0.2, adjust=5) +
  xlab("Percent of Sporulation Gene Set") +
  scale_fill_discrete("Sporulator", labels=c("Yes", "No", "?")) +
  theme_bw(base_size = 20) + scale_x_continuous(limits=c(0,1)) +
  scale_y_continuous(limits=c(0,10))
```

```
## Scale for 'fill' is already present. Adding another scale for 'fill',
## which will replace the existing scale.
```

```
p
```

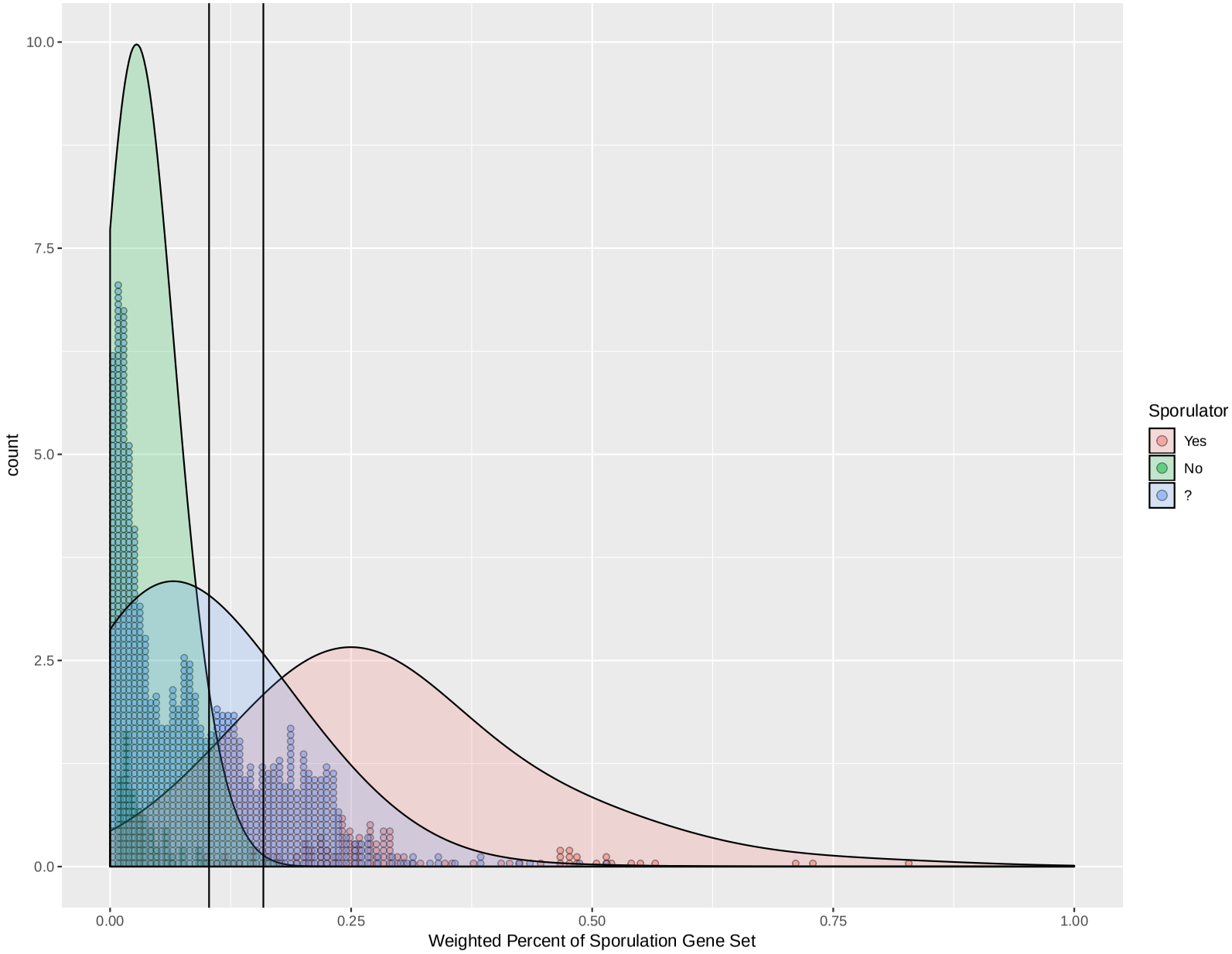

10.0
7.5
5.0
2.5
0.0
count
0.00
0.25
0.50
0.75
1.00
Percent of Sporulation Gene Set
Sporulator
Yes
No
?

Plot

```
p <- ggplot(PARKS_and_REF) +
  geom_dotplot(data=PARKS_and_REF, aes(x=weighted_percent, fill=cols),
               alpha=0.5,dotsize = 1.2, binwidth = 1/180) +
  scale_fill_discrete("Sporulator", labels=c("Yes", "No"))

p <- p +
  geom_density(aes(x=as.numeric(weighted_percent), fill=cols), alpha=0.2, adjust=5) +
  xlab("Weighted Percent of Sporulation Gene Set") +
  scale_fill_discrete("Sporulator", labels=c("Yes", "No", "?")) +
  scale_x_continuous(limits=c(0,1))
```

```
## Scale for 'fill' is already present. Adding another scale for 'fill',
## which will replace the existing scale.
```

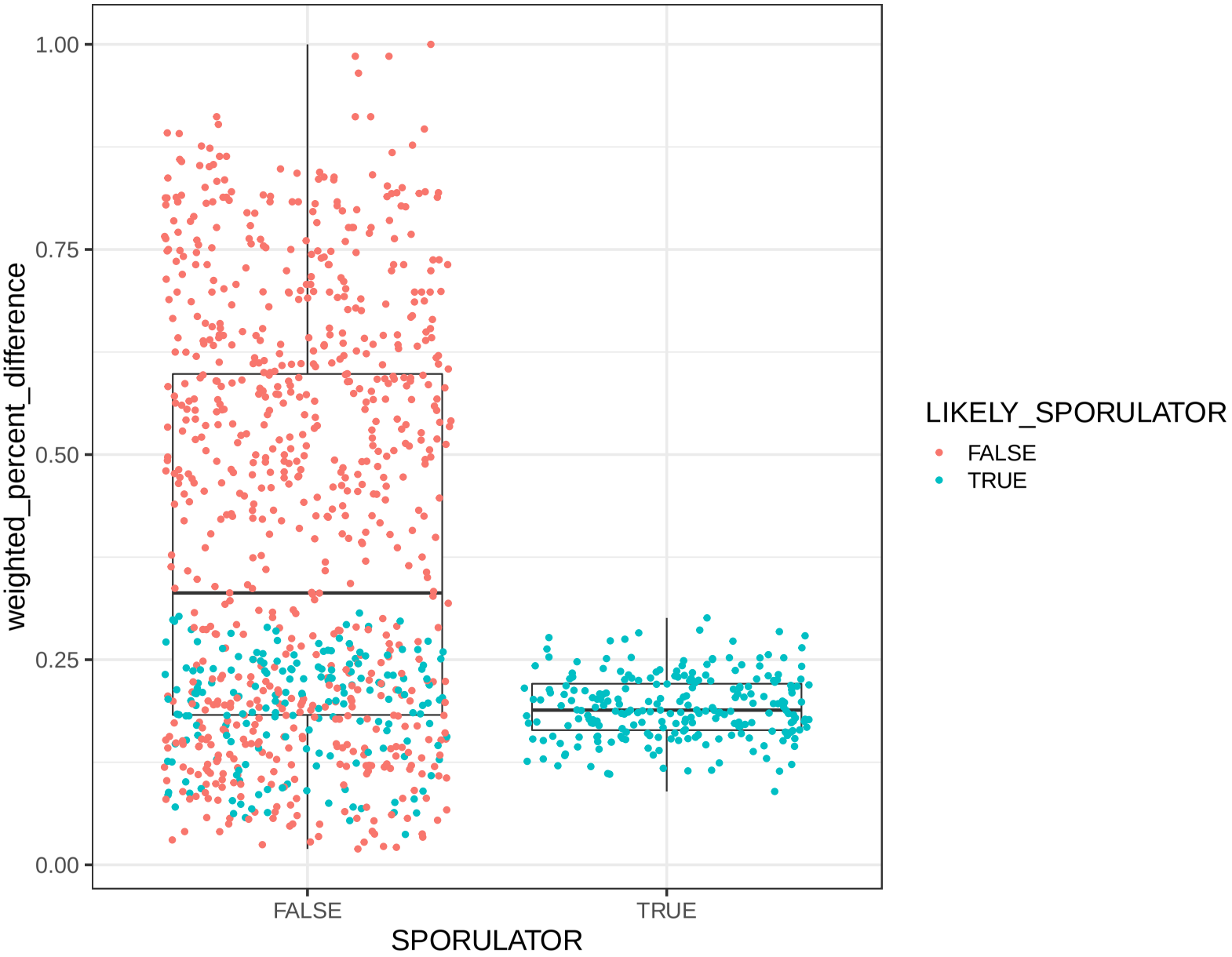
```
mu <- ddply(PARKS_and_REF, "cols", summarise,
            grp.mean=mean(Percent_Core_Set), grp.sd= sd(Percent_Core_Set))

weighted_mu <- ddply(PARKS_and_REF, "cols", summarise,
                     grp.mean=mean(weighted_percent), grp.sd= sd(weighted_percent))
p <- p +
  geom_vline(aes(xintercept=weighted_mu[2,2] +
                   3.290*weighted_mu[2,3]))
p <- p +
  geom_vline(aes(xintercept=weighted_mu[1,2] - 1*weighted_mu[1,3]))
p
```


10.0
7.5
5.0
2.5
0.0
count
0.00
0.25
0.50
0.75
1.00
Weighted Percent of Sporulation Gene Set
Sporulator
Yes
No
?

```
PARKS <- PARKS_and_REF[PARKS_and_REF$cols == "blue", ]
PARKS$LIKELY_SPORULATOR <- PARKS_and_REF[PARKS_and_REF$cols == "blue", ]$weighted_percent > weighted_mu
  3.290*weighted_mu[2,3]
PARKS$SPORULATOR <- PARKS_and_REF[PARKS_and_REF$cols == "blue", ]$weighted_percent > weighted_mu[1,2] -

PARKS <- ddply(PARKS, .(Assembly), mutate,
               weighted_percent_difference = (Percent_Core_Set - weighted_percent)/Percent_Core_Set,
               weighted_difference = (Percent_Core_Set - weighted_percent))
```

Plot

```
ggplot(PARKS) +
  geom_boxplot(aes(x=SPORULATOR, y=weighted_percent_difference), position = "dodge") +
  geom_jitter(aes(x=SPORULATOR, y=weighted_percent_difference, color=LIKELY_SPORULATOR)) +
  theme_bw(base_size = 18)
```


1.00
0.75
0.50
0.25
0.00
weighted_percent_difference
FALSE
TRUE
SPORULATOR
LIKELY_SPORULATOR
FALSE
TRUE

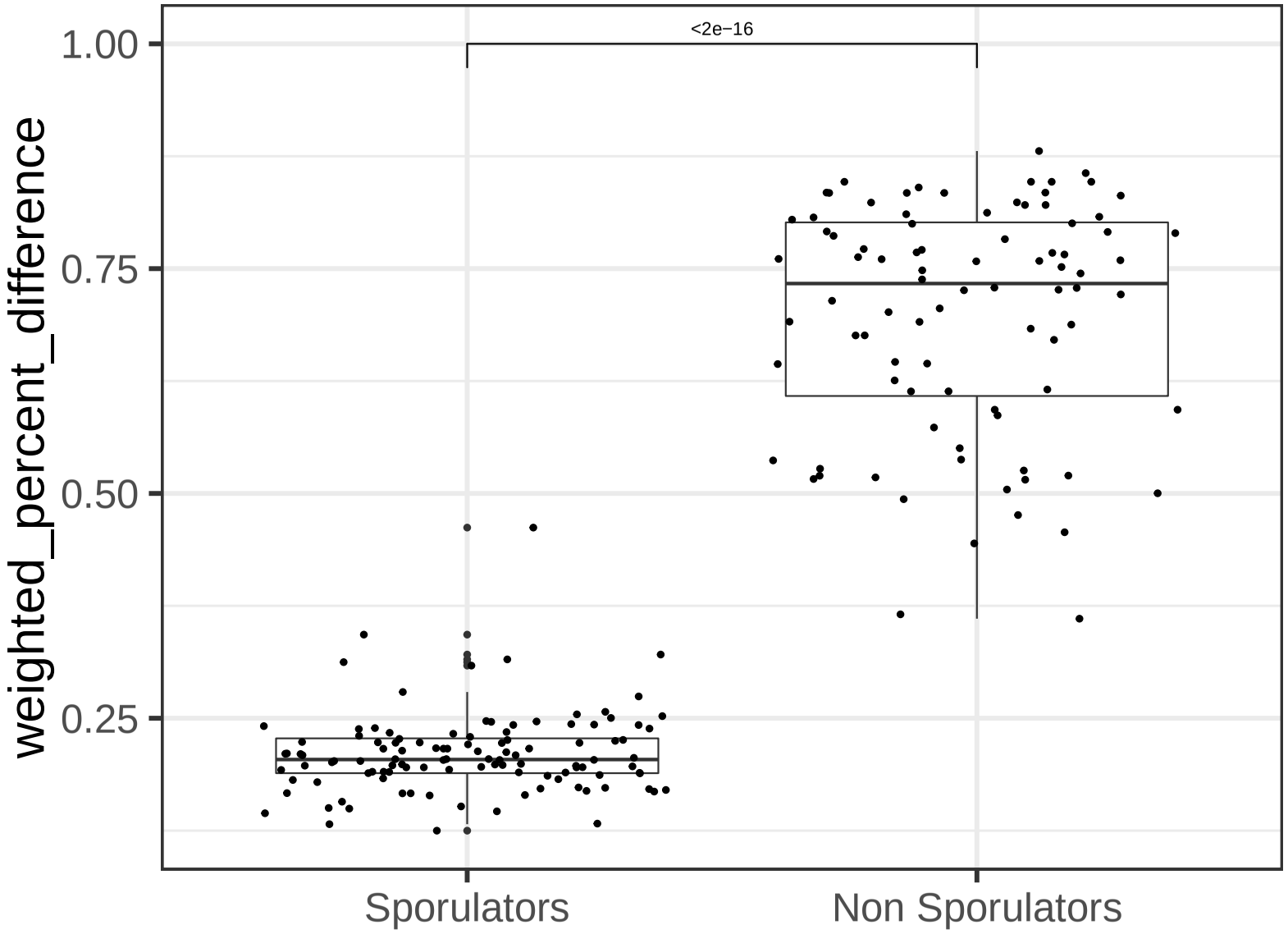
```
REFS <- PARKS_and_REF[PARKS_and_REF$cols == "black" | PARKS_and_REF$cols == "grey", ]

REFS <- ddply(REFS, .(Assembly, cols), mutate,
              weighted_percent_difference = (Percent_Core_Set - weighted_percent)/Percent_Core_Set,
              weighted_difference = (Percent_Core_Set - weighted_percent))
```

Plot

```
anno_df1 <- compare_means(weighted_percent_difference ~ cols, data = REFS, method = "t.test", p.adjust.method = "holm") %>%
 mutate(y_pos = 1, p.adj = format.pval(p.adj, digits = 2))

anno_df1 <- compare_means(weighted_percent ~ cols, data = REFS, method = "t.test", p.adjust.method = "holm") %>%
 mutate(y_pos = 1, p.adj = format.pval(p.adj, digits = 2))
```

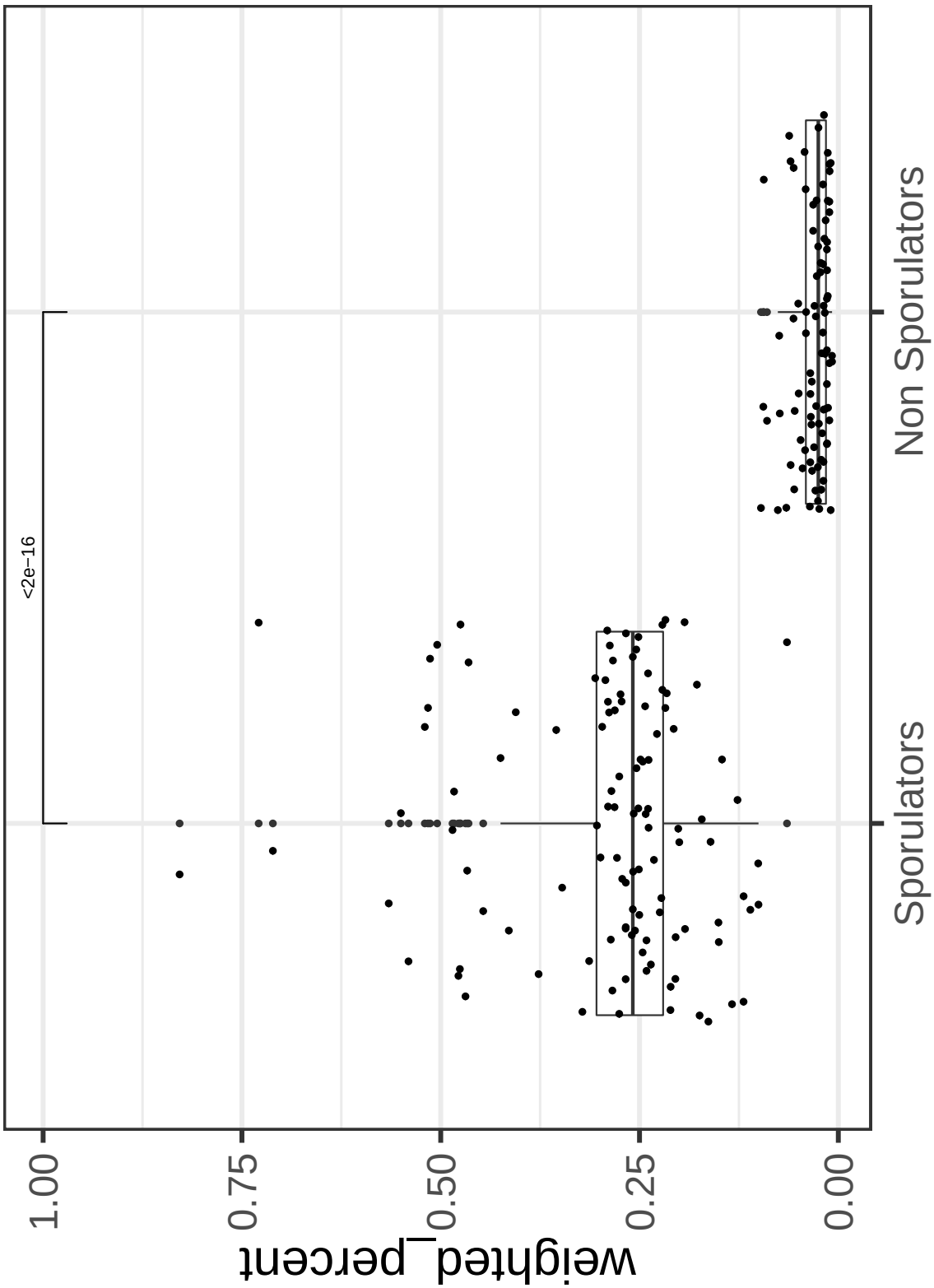
```
ggplot(REFS) +
  geom_boxplot(aes(x=cols, y=weighted_percent_difference), position = "dodge") +
  geom_jitter(aes(x=cols, y=weighted_percent_difference)) + theme_bw(base_size = 30) +
  scale_x_discrete(labels=(c("Sporulators","Non Sporulators"))) + xlab("") +
    geom_signif(
    data=anno_df1,
    aes(xmin = group1, xmax = group2, annotations = p.adj, y_position = y_pos),
    manual= TRUE
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```


weighted_percent_difference
1.00
0.75
0.50
0.25
<2e−16
Sporulators
Non Sporulators

```
ggplot(REFS) +
  geom_boxplot(aes(x=cols, y=weighted_percent), position = "dodge") +
  geom_jitter(aes(x=cols, y=weighted_percent)) + theme_bw(base_size = 30) +
  scale_x_discrete(labels=(c("Sporulators","Non Sporulators"))) + xlab("") +
    geom_signif(
    data=anno_df1,
    aes(xmin = group1, xmax = group2, annotations = p.adj, y_position = y_pos),
    manual= TRUE
  )
```

```
## Warning: Ignoring unknown aesthetics: xmin, xmax, annotations, y_position
```


weighted_percent
1.00
0.75
0.50
0.25
0.00
<2e−16
Sporulators
Non Sporulators

With the expanding phylogeny of Firmicutes that has arose from metagenomic binning efforts. We can look back and see if there are new major branches at which we now predict a loss of endosporulation to occur.

As a first pass at this I used the web-based AnnoTree software. The software allows you to visualize the prevalance of genes or sets of genes at varied phylogenetic depth. I selected the 9 genes that formed the most inclusize cluster of "genes for sporulators by sporulators" and visualized their prevalence at the phylum level.

```
include_graphics("../data/heatmap9.png")
```