## Read in YAML guide

```
library(yaml)
yamls <- yaml.load_file("de.yml")
sample1 <- yamls$sample2
sample2 <- yamls$sample2

## [1] "wtambr"

sample2

## [1] "wtbmbr"</pre>
```

## Read in Data

Read in raw count data per gene.

```
counts <- read.delim("../sam2countsResults.tsv",row.names=1)

#check the file
head(counts)
summary(counts)
colnames(counts)
#need to convert NA to 0 counts
counts[is.na(counts)] <- 0</pre>
```

## Subset per DE expirement

I am going to start by subsetting the particular treatments I am looking at.

# colnames(counts)

```
##
    [1] "tf2ambr1"
                         "tf2ambr3"
                                         "tf2ambr4"
                                                          "tf2ambr6"
    [5] "tf2aother1"
                         "tf2aother2"
                                         "tf2aother4"
                                                          "tf2aother7"
                                                          "tf2bother1"
  [9] "tf2bmbr2"
                         "tf2bmbr5"
                                         "tf2bmbr6"
## [13] "tf2bother3"
                                                          "tf2cmbr1.4"
                         "tf2bother4"
                                         "tf2bother6"
## [17] "tf2cmbr3"
                         "tf2cmbr6"
                                         "tf2cmbr7"
                                                          "tf2cother2"
                                                          "wtambr2"
## [21] "tf2cother5"
                         "tf2cother6"
                                         "tf2cother7"
## [25] "wtambr4"
                         "wtambr5"
                                         "wtaother1"
                                                          "wtaother5"
## [29] "wtaother6"
                                                          "wtbmbr2"
                         "wtaother7"
                                         "wtaother8"
## [33] "wtbmbr3"
                         "wtbmbr6"
                                         "wtbmbr8"
                                                          "wtbother1.4"
## [37] "wtbother3"
                         "wtbother5"
                                         "wtbother8"
                                                          "wtcmbr10"
## [41] "wtcmbr1.4.6"
                         "wtcmbr2"
                                         "wtcmbr3"
                                                          "wtcmbr7"
## [45] "wtcmbr9"
                         "wtcother1.3.4" "wtcother2"
                                                          "wtcother6"
```

```
counts1 <- counts[,grep(sample1, colnames(counts), value = TRUE)]
count1Len <- length(colnames(counts1)) #used in to specify library group in next step.

counts2 <- counts[,grep(sample2, colnames(counts), value = TRUE)]
count2Len <- length(colnames(counts2)) #used to specify library group in next step.

counts <- cbind(counts1, counts2)

head(counts)</pre>
```

```
##
                 wtambr2 wtambr4 wtambr5 wtbmbr2 wtbmbr3 wtbmbr6 wtbmbr8
## Solyc00g005040.2.1
                   0
                          2
                                  8
                                       2
## Solyc00g005050.2.1
                     0
                          6
                                 6
                                        20
                                               5
                                                    18
                                                            0
## Solyc00g005060.1.1
                    0
                           0
                                 1
                                       1
                                              2
                                                    1
                                                           1
## Solyc00g005070.1.1
                           3
                                 9
                     24
                                        14
                                              6
                                                    12
                                                           14
                    9
## Solyc00g005080.1.1
                           15
                                 19
                                        25
                                              15
                                                    27
                                                           0
## Solyc00g005150.1.1
                           1
                                 2
                                        0
                                             0
                                                     3
                                                            0
```

# Add column specifying library Group

Make a vector called group that will be used to make a new column named group to identify library region type.

```
group <- c(rep(sample1, count1Len), rep(sample2, count2Len))
d <- DGEList(counts=counts,group=group)</pre>
```

#### d\$samples

```
## wtambr2 wtambr 395165 1
## wtambr4 wtambr 792542 1
## wtambr5 wtambr 632686 1
## wtbmbr2 wtbmbr 1355352 1
## wtbmbr3 wtbmbr 1213142 1
## wtbmbr6 wtbmbr 1598917 1
## wtbmbr8 wtbmbr 48352 1
```

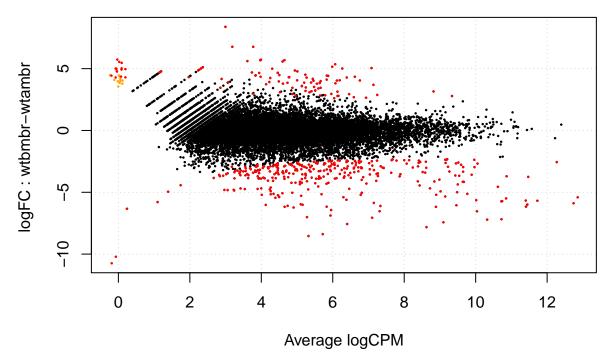
```
cpm.d <- cpm(d)
d <- d[rowSums(cpm.d>5)>=3,] #change to 5
d <- estimateCommonDisp(d,verbose=T)</pre>
```

```
## Disp = 0.4578 , BCV = 0.6766
```

```
d <- calcNormFactors(d)
d <- estimateCommonDisp(d)

DEtest <- exactTest(d,pair=c(sample1,sample2))
head(DEtest$table)</pre>
```

```
logFC logCPM
##
                                        PValue
## Solyc00g005050.2.1 0.3365 3.396 8.895e-01
## Solyc00g005070.1.1 -0.0197 5.707 7.960e-01
## Solyc00g005080.1.1 -1.2336 4.665 5.943e-02
## Solyc00g005440.1.1 0.4710 4.833 5.326e-01
## Solyc00g005840.2.1 -0.9441 4.854 1.210e-01
## Solyc00g006470.1.1 -5.6131 11.650 1.043e-12
results <- topTags(DEtest, n=Inf)
head(results)
## Comparison of groups: wtbmbr-wtambr
                       logFC logCPM
                                       PValue
## Solyc00g011160.1.1 -7.949 11.326 7.094e-19 9.940e-15
## Solyc06g024230.1.1 -7.315 11.605 6.008e-18 4.209e-14
## Solyc11g027710.1.1 -7.243 12.717 1.586e-17 6.061e-14
## Solyc00g068970.2.1 -7.215 13.105 1.730e-17 6.061e-14
## Solyc06g024240.1.1 -7.960 8.720 4.075e-17 1.142e-13
## Solyc06g024350.1.1 -8.110 8.383 1.180e-16 2.755e-13
dim(results$table)
## [1] 14012
sum(results$table$FDR<.05) # How many are DE genes?</pre>
## [1] 436
summary(decideTestsDGE(DEtest,p.value=.05))
      [,1]
##
## -1
       330
## 0 13576
        106
## 1
sig.genes <- rownames(results$table[results$table$FDR<0.05,]) # outputs just significant gene names
plotSmear(d,de.tags=sig.genes)
```



Subset by all the ones with a significant score

```
results.sig <- subset(results$table, results$table$FDR < 0.05)
```

What are the genes that are misexpressed? For this we need to add some annotation

Essentially we are merging two annotations files to 1.) only sig genes 2.) all genes

```
annotation1<- read.delim("../ITAG2.3_all_Arabidopsis_ITAG_annotations.tsv", header=FALSE)
colnames(annotation1) <- c("ITAG", "SGN_annotation")
annotation2<- read.delim ("../ITAG2.3_all_Arabidopsis_annotated.tsv")
annotation <- merge(annotation1, annotation2, by = "ITAG")

#Making the only significant gene table
results.sig$ITAG <- rownames(results.sig) #change row.names to ITAG for merging
results.sig.annotated <- merge(results.sig,annotation,by = "ITAG") #This is merging to only sig genes
#Making all table
results$table$ITAG <- rownames(results$table)
results.all.annotated <- merge(results$table, annotation,by = "ITAG")</pre>
```

Write table with results

```
write.table(results.all.annotated, "DE_all.txt", sep="\t", row.names=F)
write.table(results.sig.annotated, "DE_sig.txt", sep="\t", row.names=F)
```

library(rmarkdown) render("skeletonDE.Rmd", "pdf\_document")