Skeleton Key for RNAseq analysis

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See README.md for more detailed instructions of how to use script

Analysis

libraries

```
library(edgeR)
library(yaml)
```

Read in YAML guide

```
yamls <- yaml.load_file("de.yml")
```

This part assigns your YMAL to a object in R. This will be used throughout the script to specify which sample types you are comparing.

```
sample1 <- yamls$sample1
sample2 <- yamls$sample2

sample1
```

```
## [1] "wtambr"
```

```
sample2
```

```
## [1] "wtbmbr"
```

Read in Data

Read in raw count data per gene.

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

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

Subset DE expirement

Start by subsetting the particular treatments which are being compared.

```
colnames(counts)
```

```
##  [1] "tf2ambr1"      "tf2ambr3"      "tf2ambr4"      "tf2ambr6"
##  [5] "tf2aother1"    "tf2aother2"    "tf2aother4"    "tf2aother7"
##  [9] "tf2bmbr2"      "tf2bmbr5"      "tf2bmbr6"      "tf2bother1"
## [13] "tf2bother3"    "tf2bother4"    "tf2bother6"    "tf2cmbr1.4"
## [17] "tf2cmbr3"      "tf2cmbr6"      "tf2cmbr7"      "tf2cother2"
## [21] "tf2cother5"    "tf2cother6"    "tf2cother7"    "wtambr2"
## [25] "wtambr4"       "wtambr5"       "wtaother1"     "wtaother5"
## [29] "wtaother6"     "wtaother7"     "wtaother8"     "wtbmbr2"
## [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)
```

```
##                    wtambr2 wtambr4 wtambr5 wtbmbr2 wtbmbr3 wtbmbr6 wtbmbr8
## Solyc00g005040.2.1       0       2       8       2       4       3       0
## Solyc00g005050.2.1       0       6       6      20       5      18       0
## Solyc00g005060.1.1       0       0       1       1       2       1       1
## Solyc00g005070.1.1      24       3       9      14       6      12      14
## Solyc00g005080.1.1       9      15      19      25      15      27       0
## Solyc00g005150.1.1       0       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)
```

Check to see if the group column matches your sample name and they are appropriate.

```
d$samples
```

```
##          group lib.size norm.factors
## 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
```

Differential expression using edgeR

Make sure there is full understanding on each edgeR command being used. The manual is amazing so read it *before* running the DE analysis below edgeR manual.

```
cpm.d <- cpm(d) #counts per mutant
d <- d[rowSums(cpm.d>5)>=3,] #This might be a line to adjust. It is removing genes with low counts.
d <- estimateCommonDisp(d,verbose=T)
```

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

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

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

```
##                       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       FDR
## 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     4
```

```
sum(results$table$FDR<.05) # How many are DE genes?
```

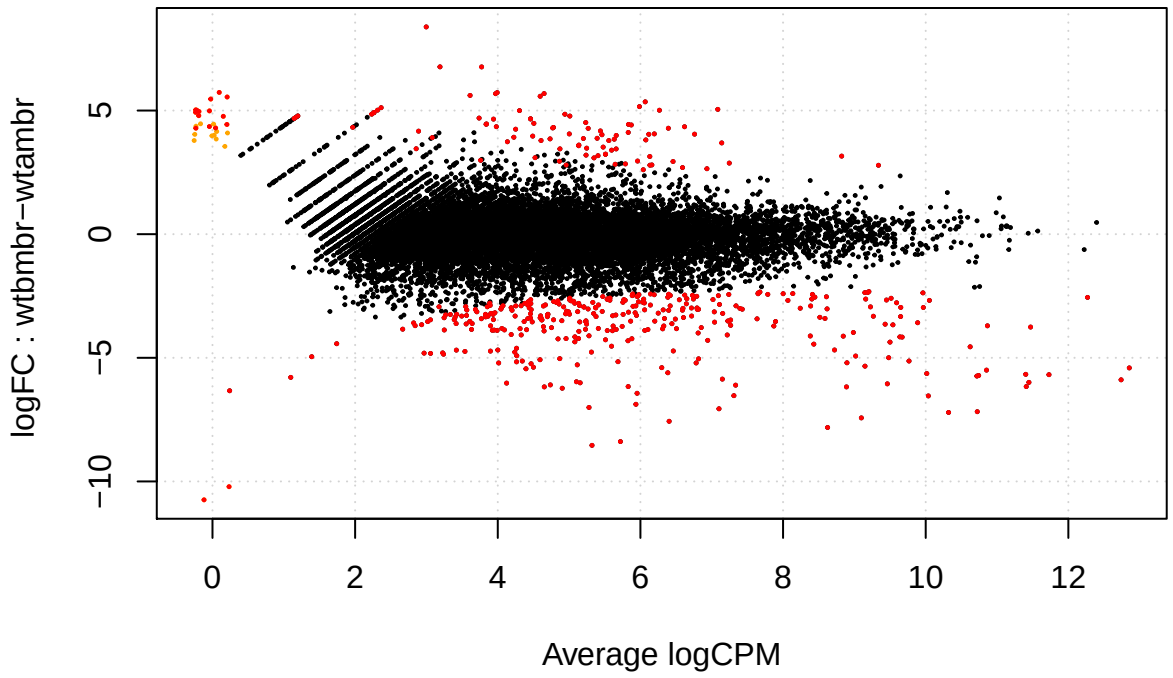
```
## [1] 436
```

```
summary(decideTestsDGE(DEtest,p.value=.05))
```

```
##    [,1]
## -1   330
## 0  13576
## 1    106
```

```
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 genes with a significant FDR score.

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

dim(results.sig)

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

```
##                 ITAG
## 1 Solyc00g005000.2.1
## 2 Solyc00g005040.2.1
## 3 Solyc00g005050.2.1
```

```
## 4 Solyc00g005080.1.1
## 5 Solyc00g005900.1.1
## 6 Solyc00g006490.2.1
##
## 1                                                    Aspartic proteinase nepenthesin I (AHRD V1 **--
## 2                                                      Potassium channel (AHRD V1 ***- D0EM91_9ROSI)
## 3
## 4
## 5 Oxygen-evolving enhancer protein 1, chloroplastic (AHRD V1 ***- PSBO_SOLTU); contains Interpro doma
## 6    Serine/threonine-protein phosphatase 6 regulatory subunit 3 (AHRD V1 **-- SAPS3_HUMAN); contains
##         AGI symbol
## 1 AT3G20015   <NA>
## 2 AT5G46240   KAT1
## 3 AT5G11680   <NA>
## 4 ATCG01280 YCF2.2
## 5 AT5G66570  MSP-1
## 6 AT1G07990   <NA>
##
## 1
## 2
## 3
## 4
## 5
## 6 SIT4 phosphatase-associated family protein; similar to SIT4 phosphatase-associated family protein
##   X..identity alignment.length e.value bit.score percent.query.align
## 1       63.76              447  7e-148       520               89.94
## 2       66.02              103   2e-37       150               85.71
## 3       76.96              204   1e-88       322               98.98
## 4       91.25               80   2e-38       153               79.80
## 5       69.62               79   4e-26       112               78.79
## 6       61.92              856   0e+00       979               99.77
```

```
#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", all.x=TRUE) #This is merging to only

#Making all table

results$table$ITAG <- rownames(results$table)
results.all.annotated <- merge(results$table, annotation,by = "ITAG")
```

Write table with results.

```
write.table(results.all.annotated, file=paste(sample1,"_",sample2,"_","DE_all.txt",sep=""),sep="\t",row
write.table(results.sig.annotated, file=paste(sample1,"_",sample2,"_","DE_sig.txt",sep=""),sep="\t",row
```

Now run the script below for a full `knitr` report of what was run and leave this report in the folder that the analysis was done with output files.

```
library(rmarkdown)
render("skeletonDE.Rmd", "pdf_document", output_file = paste(sample1,"_",sample2,"_","DE.pdf",sep=""))
```