Skeleton Key for RNAseq analysis

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

Analysis

libraries

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

Read in YAML guide

```r
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.

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

sample1
```

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

```r
sample2
```

```
## [1] "wtaother"
```

Read in Data

Read in raw count data per gene.

```r
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 wtaother1 wtaother5 wtaother6
## Solyc00g005040.2.1       0       2       8         1         1         1
## Solyc00g005050.2.1       0       6       6        17        16         9
## Solyc00g005060.1.1       0       0       1         0         0         0
## Solyc00g005070.1.1      24       3       9         8         6         5
## Solyc00g005080.1.1       9      15      19        18        37         6
## Solyc00g005150.1.1       0       1       2         2         5         0
##                    wtaother7 wtaother8
## Solyc00g005040.2.1         0         2
## Solyc00g005050.2.1         2         3
## Solyc00g005060.1.1         0         2
## Solyc00g005070.1.1         5         6
## Solyc00g005080.1.1        10         7
## Solyc00g005150.1.1         0         2
```

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
## wtaother1 wtaother   929017            1
## wtaother5 wtaother  1555921            1
## wtaother6 wtaother   498294            1
## wtaother7 wtaother   479003            1
## wtaother8 wtaother   510148            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 = 0.3091 , BCV = 0.556
```

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

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

```
##                      logFC logCPM    PValue
## Solyc00g005050.2.1  0.5863  3.636 3.961e-01
## Solyc00g005070.1.1 -2.4393  4.357 2.113e-04
## Solyc00g005080.1.1 -0.8616  4.558 1.405e-01
## Solyc00g005440.1.1  0.1874  4.544 7.346e-01
## Solyc00g005840.2.1 -0.6429  4.978 2.500e-01
## Solyc00g006470.1.1 -2.9538 11.691 4.068e-08
```

```
results <- topTags(DEtest, n=Inf)
head(results)
```

```
## Comparison of groups:  wtaother-wtambr
##                     logFC logCPM    PValue       FDR
## Solyc03g062850.1.1 -6.838  6.956 4.894e-23 7.442e-19
## Solyc08g079850.1.1 -5.678  9.228 3.985e-21 2.325e-17
## Solyc06g024350.1.1 -5.724  8.162 4.588e-21 2.325e-17
## Solyc09g091110.2.1 -5.444  7.953 1.039e-19 3.948e-16
## Solyc01g058490.1.1 -6.000  6.360 1.378e-19 4.191e-16
## Solyc07g025190.1.1 -5.725  6.911 1.970e-19 4.992e-16
```

```r
dim(results$table)
```

```
## [1] 15204     4
```

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

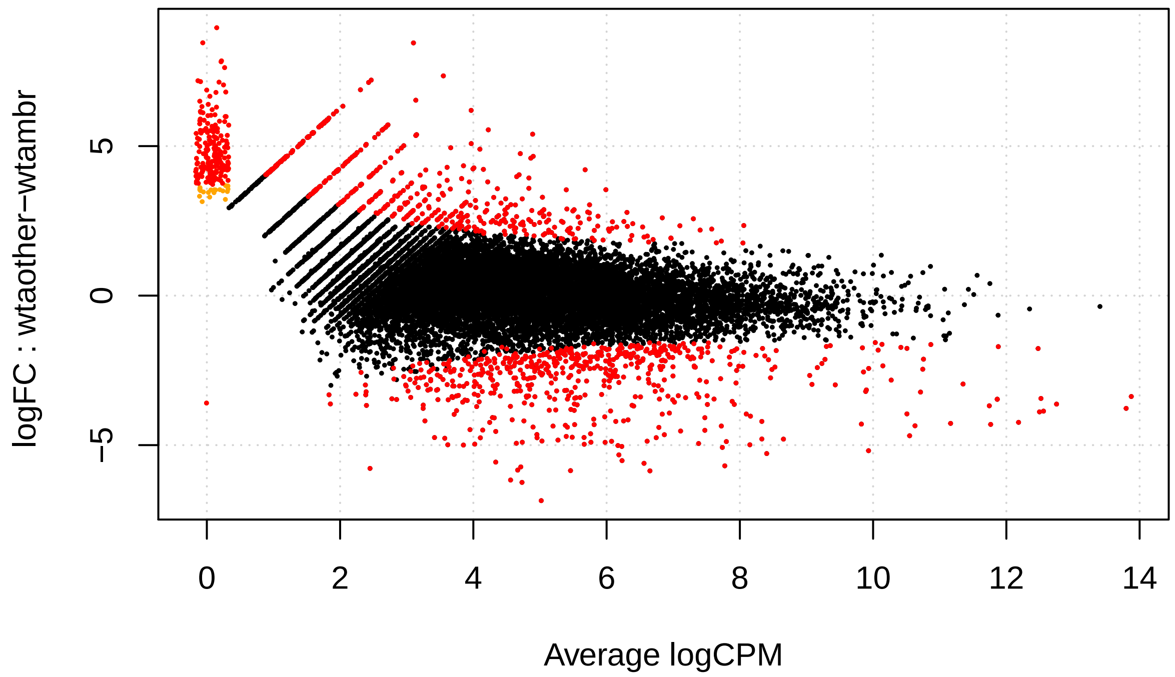
```
## [1] 1251
```

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

```
##     [,1]
## -1   602
## 0  13953
## 1    649
```

```r
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.

```r
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=""))
```