Skeleton Key for RNAseq analysis

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

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=""))
```

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] "tf2aother"
```

```
sample2
```

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

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)
```

```
##                   tf2aother1 tf2aother2 tf2aother4 tf2aother7 wtaother1
## Solyc00g005040.2.1          0          1          0          2         1
## Solyc00g005050.2.1          0          2          3          0        17
## Solyc00g005060.1.1          0          0          0          0         0
## Solyc00g005070.1.1          3          6         33          2         8
## Solyc00g005080.1.1          0         12         10          3        18
## Solyc00g005150.1.1          0          0          2          1         2
##                   wtaother5 wtaother6 wtaother7 wtaother8
## Solyc00g005040.2.1         1         1         0         2
## Solyc00g005050.2.1        16         9         2         3
## Solyc00g005060.1.1         0         0         0         2
## Solyc00g005070.1.1         6         5         5         6
## Solyc00g005080.1.1        37         6        10         7
## Solyc00g005150.1.1         5         0         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
## tf2aother1 tf2aother   263117            1
## tf2aother2 tf2aother   698710            1
## tf2aother4 tf2aother   792325            1
## tf2aother7 tf2aother   142504            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 = 0.36 , BCV = 0.6
```

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

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

```
##                       logFC logCPM    PValue
## Solyc00g005050.2.1  1.8811  3.231 0.0191880
## Solyc00g005070.1.1 -1.5353  4.200 0.0376167
## Solyc00g005080.1.1  0.2928  4.395 0.7026833
## Solyc00g005160.1.1 -1.4653  2.605 0.1392161
## Solyc00g005440.1.1  0.1746  4.696 0.8443639
## Solyc00g005840.2.1 -2.1046  6.016 0.0008482
```

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

```
## Comparison of groups:  wtaother-tf2aother
##                       logFC logCPM    PValue       FDR
## Solyc01g103860.2.1 -6.978  7.101 1.828e-18 2.849e-14
## Solyc11g013430.1.1 -9.119  5.682 2.683e-16 2.091e-12
## Solyc02g065610.2.1 -7.607  5.154 8.097e-15 4.206e-11
## Solyc02g030220.1.1 -6.199  6.294 1.264e-14 4.925e-11
## Solyc12g014030.1.1 -5.102  7.776 1.298e-13 4.047e-10
## Solyc06g083200.1.1 -5.115  6.060 2.687e-12 6.979e-09
```

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

```
## [1] 15585     4
```

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

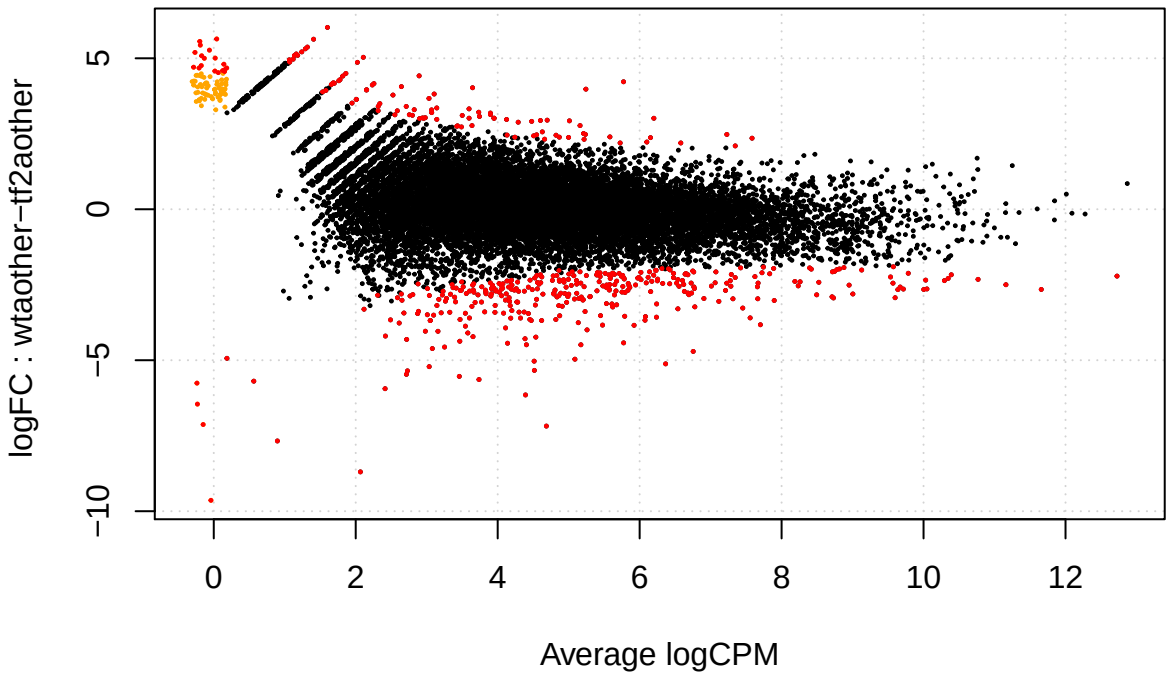
```
## [1] 463
```

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

```
##    [,1]
## -1   349
## 0  15122
## 1    114
```

```
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)
```

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")

#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")
```

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
```