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] "tf2aother"
```

```
sample2
```

```
## [1] "tf2bother"
```

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 tf2bother1
## Solyc00g005040.2.1          0          1          0          2          6
## Solyc00g005050.2.1          0          2          3          0         46
## Solyc00g005060.1.1          0          0          0          0          0
## Solyc00g005070.1.1          3          6         33          2         25
## Solyc00g005080.1.1          0         12         10          3         52
## Solyc00g005150.1.1          0          0          2          1         11
##                      tf2bother3 tf2bother4 tf2bother6
## Solyc00g005040.2.1          3          5          2
## Solyc00g005050.2.1          9         23         22
## Solyc00g005060.1.1          1          1          1
## Solyc00g005070.1.1          4         11         11
## Solyc00g005080.1.1         12         15         12
## Solyc00g005150.1.1          0          0          1
```

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
## tf2bother1 tf2bother  2415227            1
## tf2bother3 tf2bother   626786            1
## tf2bother4 tf2bother  1003586            1
## tf2bother6 tf2bother   854903            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 = 0.3454 , BCV = 0.5877
```

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

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

```
##                      logFC logCPM   PValue
## Solyc00g005050.2.1  2.6711  3.641 0.001269
## Solyc00g005070.1.1 -1.3362  4.172 0.067383
## Solyc00g005080.1.1  0.1799  4.138 0.779627
## Solyc00g005440.1.1  0.4710  4.585 0.530578
## Solyc00g005840.2.1 -1.7567  6.313 0.007125
## Solyc00g005880.1.1  1.0866  2.504 0.568766
```

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

```
## Comparison of groups:  tf2bother-tf2aother
##                     logFC logCPM    PValue       FDR
## Solyc12g014030.1.1 -5.528  7.977 3.684e-13 5.561e-09
## Solyc06g009710.2.1 -6.613  5.798 1.030e-12 7.772e-09
## Solyc06g068350.1.1 -5.085  7.043 2.136e-11 1.075e-07
## Solyc03g112640.2.1 -4.971  6.654 7.208e-11 2.720e-07
## Solyc06g083200.1.1 -4.925  6.251 2.368e-10 7.148e-07
## Solyc02g086460.2.1  5.668  5.764 3.741e-10 7.328e-07
```

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

```
## [1] 15096     4
```

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

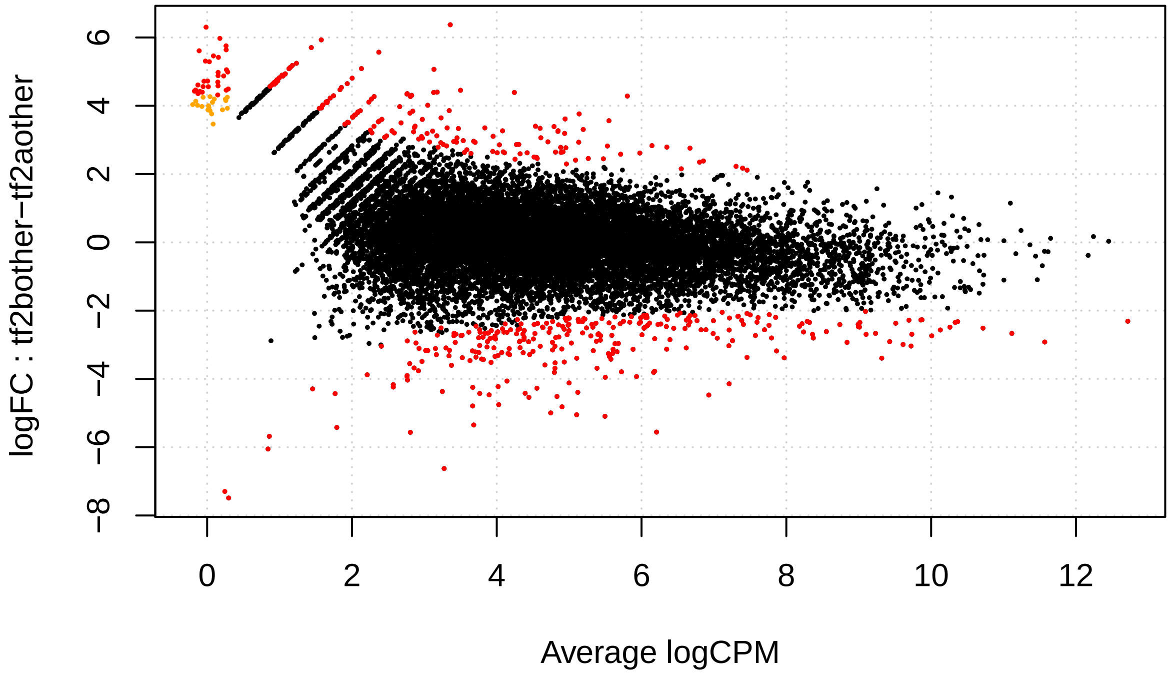
```
## [1] 481
```

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

```
##     [,1]
## -1    290
## 0   14615
## 1     191
```

```
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
```

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=""))
```