Skeleton Key for RNAseq analysis

Written By: Ciera Martinez

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] "tf2cmbr"
```

```
sample2
```

```
## [1] "tf2cother"
```

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)
```

```
##                    tf2cmbr1.4 tf2cmbr3 tf2cmbr6 tf2cmbr7 tf2cother2
## Solyc00g005040.2.1          0        6        8        4          3
## Solyc00g005050.2.1          1       34       17       12          4
## Solyc00g005060.1.1          0        1        0        0          1
## Solyc00g005070.1.1         23       11        8        9          4
## Solyc00g005080.1.1         22        7        8       12          9
## Solyc00g005150.1.1          1        3        0        0          1
##                    tf2cother5 tf2cother6 tf2cother7
## Solyc00g005040.2.1          8          4          1
## Solyc00g005050.2.1         10         16         12
## Solyc00g005060.1.1          1          2          1
## Solyc00g005070.1.1         11          5          5
## Solyc00g005080.1.1         21         14          3
## Solyc00g005150.1.1          6          1          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
## tf2cmbr1.4   tf2cmbr   443572            1
## tf2cmbr3     tf2cmbr  1337575            1
## tf2cmbr6     tf2cmbr   790129            1
## tf2cmbr7     tf2cmbr   832907            1
## tf2cother2 tf2cother   723602            1
## tf2cother5 tf2cother  1216379            1
## tf2cother6 tf2cother   838942            1
## tf2cother7 tf2cother   676969            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 = 0.5176 , BCV = 0.7194
```

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

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

```
##                       logFC logCPM  PValue
## Solyc00g005050.2.1 -0.32797  4.038 0.71623
## Solyc00g005070.1.1 -1.60929  4.084 0.05341
## Solyc00g005080.1.1 -0.71837  4.263 0.34628
## Solyc00g005440.1.1  0.07592  4.569 0.91337
## Solyc00g005840.2.1  1.30274  5.279 0.08277
## Solyc00g005880.1.1 -1.65569  3.206 0.05087
```

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

```
## Comparison of groups:  tf2cother-tf2cmbr
##                     logFC logCPM    PValue       FDR
## Solyc02g092060.1.1 -9.185  5.426 1.490e-11 1.990e-07
## Solyc09g009250.2.1 -6.853  6.440 2.621e-11 1.990e-07
## Solyc11g010940.1.1 -6.200  5.228 1.807e-09 9.146e-06
## Solyc02g023990.2.1 -5.498  6.685 3.507e-09 1.331e-05
## Solyc06g060410.2.1 -5.312  6.701 4.910e-09 1.491e-05
## Solyc03g118770.2.1 -5.509  5.635 1.381e-08 3.149e-05
```

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

```
## [1] 15183     4
```

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

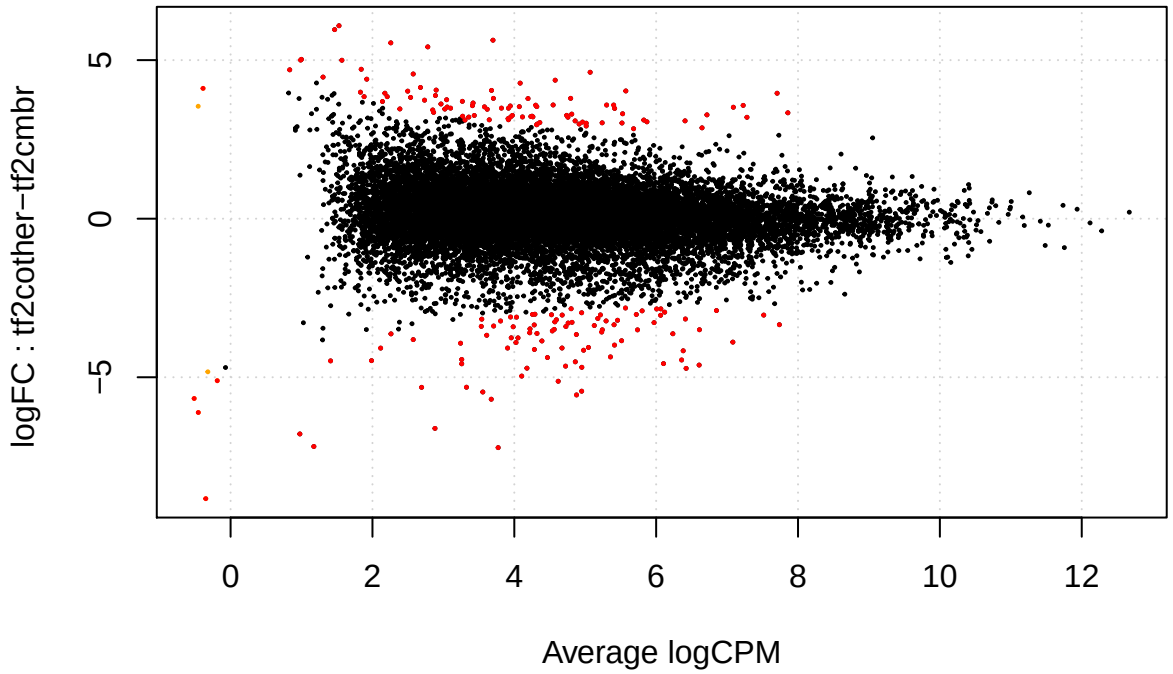
```
## [1] 190
```

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

```
##    [,1]
## -1    96
## 0  14993
## 1     94
```

```
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=""))
```