

d#Skeleton Key for RNAseq analysis *Written By: Ciera Martinez*

About

This is the script used to perform differential gene expression analysis using **edgeR**. The tissue came from p4 leaves of *Solanum lycopersicum* using Laser Capture Microdissection.

Key to Samples

genotype: either wildtype of *tf2*

region: A. tip B. early emerging leaflet C. base

type: MBR = Marginal Blastozone Region, other = the rachis or midvein region

Samples:

```
print(sample1)
```

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

```
print(sample2)
```

```
## [1] "wtbmbmr"
```

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

Run the **render()** function below and everything will be run with report at end.

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

Analysis (Actual start)

libraries

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

Read in YAML guide

This reads in the information in the **de.yaml** file which has the two names of the samples you are interested in comparing.

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

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] "wtbmr"
```

Read in Data

Read in raw count data per gene.

```
counts <- read.delim("../data/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 experiment

Start by subsetting the particular treatments which are being compared. This might need to be modified depending on the naming of your samples. In my case each sample is named by sample and rep number, so the script is identifying any sample with the sample name given in the `de.yml` file.

```
colnames(counts)
```

```
## [1] "tf2ambr1"      "tf2ambr3"      "tf2ambr4"      "tf2ambr6"
## [5] "tf2aoth1"      "tf2aoth2"      "tf2aoth4"      "tf2aoth7"
## [9] "tf2bmr2"      "tf2bmr5"      "tf2bmr6"      "tf2bmr1"
## [13] "tf2bmr3"      "tf2bmr4"      "tf2bmr6"      "tf2cmbr1.4"
## [17] "tf2cmbr3"      "tf2cmbr6"      "tf2cmbr7"      "tf2coth2"
## [21] "tf2coth5"      "tf2coth6"      "tf2coth7"      "wtambr2"
## [25] "wtambr4"      "wtambr5"      "wtaoth1"      "wtaoth5"
## [29] "wtaoth6"      "wtaoth7"      "wtaoth8"      "wtbmr2"
## [33] "wtbmr3"      "wtbmr6"      "wtbmr8"      "wtbmr1.4"
## [37] "wtbmr3"      "wtbmr5"      "wtbmr8"      "wtcmbr10"
## [41] "wtcmbr1.4.6"  "wtcmbr2"      "wtcmbr3"      "wtcmbr7"
## [45] "wtcmbr9"      "wtcoth1.3.4"  "wtcoth2"      "wtcoth6"
```

```
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 of each edgeR command being used. The manual is amazing so read it *before* running the DE analysis below [edgeR manual](#). There are many options and they must be set to be appropriate for your analysis.

```
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  3.223 8.895e-01
## Solyc00g005070.1.1 -0.0197  5.497 7.960e-01
## Solyc00g005080.1.1 -1.2336  4.421 5.943e-02
## Solyc00g005440.1.1  0.4710  4.516 5.326e-01
## Solyc00g005840.2.1 -0.9441  4.784 1.210e-01
## Solyc00g006470.1.1 -5.6131 11.649 1.043e-12
```

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

```
## Comparison of groups: wtbmbr-wtambr
##           logFC logCPM    PValue      FDR
## Solyc00g011160.1.1 -7.949 11.320 7.094e-19 9.940e-15
## Solyc06g024230.1.1 -7.315 11.601 6.008e-18 4.209e-14
## Solyc11g027710.1.1 -7.243 12.716 1.586e-17 6.061e-14
## Solyc00g068970.2.1 -7.215 13.103 1.730e-17 6.061e-14
## Solyc06g024240.1.1 -7.960  8.692 4.075e-17 1.142e-13
## Solyc06g024350.1.1 -8.110  8.368 1.180e-16 2.755e-13
```

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

```
## [1] 14012      4
```

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

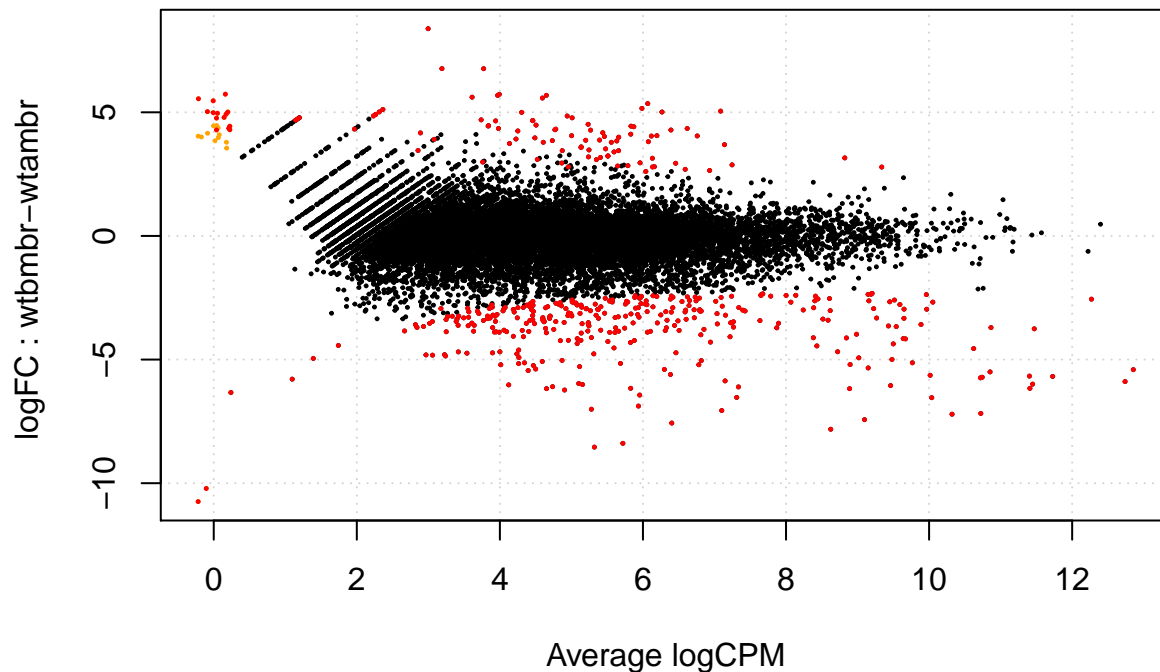
```
## [1] 436
```

```
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 all the genes with a significant FDR score less than .05.

```
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("../data/ITAG2.3_all_Arabidopsis_ITAG_annotations.tsv", header=FALSE) #Cha
colnames(annotation1) <- c("ITAG", "SGN_annotation")
annotation2<- read.delim("../data/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 ***- DOEM91_9ROSI
## 3
## 4
## 5 Oxygen-evolving enhancer protein 1, chloroplastic (AHRD V1 ***- PSBO_SOLTU); contains Interpro dom
## 6 Serine/threonine-protein phosphatase 6 regulatory subunit 3 (AHRD V1 ***- SAPS3_HUMAN); contain
##      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 only si

#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.