Read in YAML guide

```
library(yaml)
yamls <- yaml.load_file("de.yml")
sample1 <- yamls$sample1
sample2 <- yamls$sample2

sample1
```

```
## [1] "wtcmbr"
```

```
sample2
```

```
## [1] "wtcother"
```

Read in Data

Read in raw count data per gene.

```
counts <- read.delim("../sam2countsResults.tsv",row.names=1)

#check the file
head(counts)
summary(counts)
colnames(counts)
#need to convert NA to 0 counts
counts[is.na(counts)] <- 0
```

Subset per DE expirement

I am going to start by subsetting the particular treatments I am looking at.

```
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)
```

```
##                     wtcmbr10 wtcmbr1.4.6 wtcmbr2 wtcmbr3 wtcmbr7 wtcmbr9
## Solyc00g005040.2.1         0           9       3       1       0       0
## Solyc00g005050.2.1         5          38      21      11       4       7
## Solyc00g005060.1.1         1           3       0       0       1       0
## Solyc00g005070.1.1         5          12       7       4       6       1
## Solyc00g005080.1.1         0           7      19      45       4       7
## Solyc00g005150.1.1         0           1       3       3       2       1
##                     wtcother1.3.4 wtcother2 wtcother6
## Solyc00g005040.2.1              0         0        12
## Solyc00g005050.2.1              2         6        37
## Solyc00g005060.1.1             13         0         0
## Solyc00g005070.1.1            169         6        24
## Solyc00g005080.1.1             11        26        35
## Solyc00g005150.1.1              2         1         5
```

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)
```

```
d$samples
```

```
##                   group lib.size norm.factors
## wtcmbr10         wtcmbr   459717            1
## wtcmbr1.4.6      wtcmbr  1158809            1
## wtcmbr2          wtcmbr  1130695            1
## wtcmbr3          wtcmbr  1560130            1
## wtcmbr7          wtcmbr   374882            1
## wtcmbr9          wtcmbr   386974            1
## wtcother1.3.4 wtcother   197345            1
## wtcother2     wtcother   319043            1
## wtcother6     wtcother  1525172            1
```

```
cpm.d <- cpm(d)
d <- d[rowSums(cpm.d>5)>=3,] #change to 5
d <- estimateCommonDisp(d,verbose=T)
```

```
## Disp = 0.3151 , BCV = 0.5614
```

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

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

```
##                       logFC logCPM    PValue
## Solyc00g005050.2.1  0.24832  4.288 7.080e-01
## Solyc00g005070.1.1  5.47739  6.832 9.953e-18
## Solyc00g005080.1.1  1.92902  4.908 1.338e-03
## Solyc00g005160.1.1  1.73665  3.041 2.264e-02
## Solyc00g005440.1.1 -0.01889  5.024 9.531e-01
## Solyc00g005840.2.1 -0.21612  4.981 7.299e-01
```

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

```
## Comparison of groups:  wtcother-wtcmbr
##                     logFC logCPM    PValue       FDR
## Solyc10g052420.1.1 8.110  9.592 1.187e-33 1.881e-29
## Solyc08g023400.1.1 8.170  8.802 1.488e-32 1.179e-28
## Solyc10g050260.1.1 7.762 10.220 2.352e-32 1.243e-28
## Solyc07g039270.2.1 7.822  9.553 3.323e-32 1.317e-28
## Solyc01g028970.1.1 7.723  9.608 8.692e-32 2.755e-28
## Solyc11g020560.1.1 7.485 11.445 1.156e-31 3.008e-28
```

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

```
## [1] 15850     4
```

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

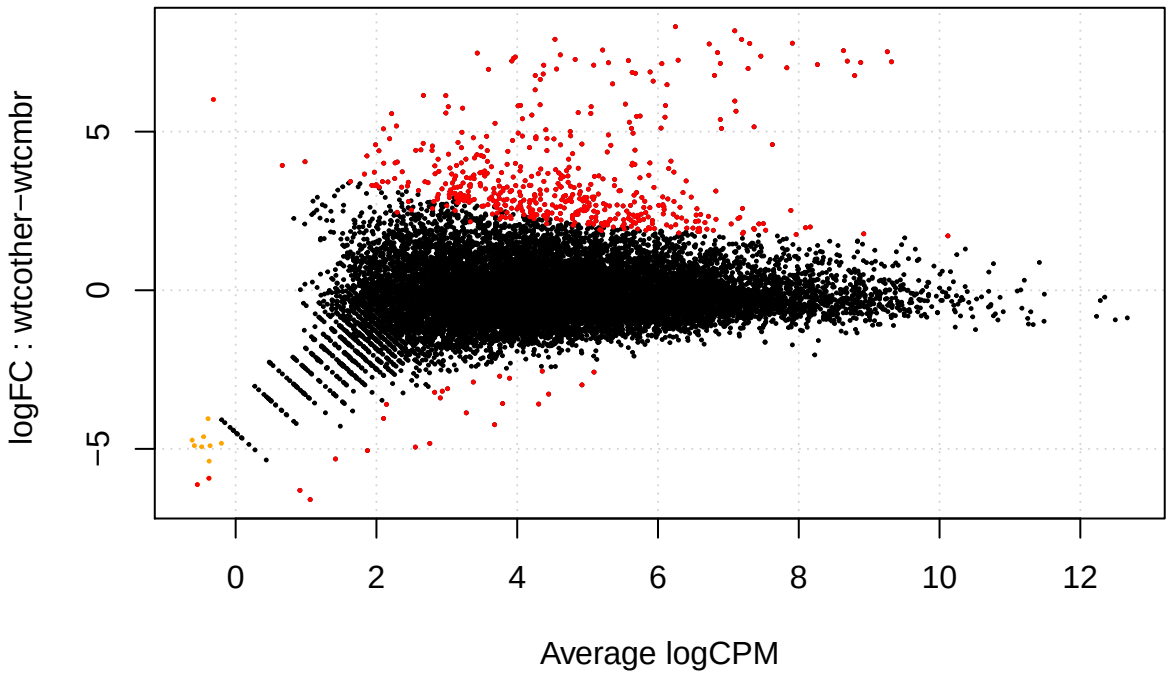
```
## [1] 538
```

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

```
##    [,1]
## -1    25
## 0  15312
## 1    513
```

```
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 ones with a significant 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("../ITAG2.3_all_Arabidopsis_ITAG_annotations.tsv", header=FALSE)  #Changed to
colnames(annotation1) <- c("ITAG", "SGN_annotation")
annotation2<- read.delim ("../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,"DE_all.txt",sep="\t",row.names=F)
write.table(results.sig.annotated,"DE_sig.txt",sep="\t",row.names=F)
```

library(rmarkdown) render("skeletonDE.Rmd", "pdf_document")