

# Finding shade-responsive genes

edgeR, glm function

```
for(i in Genotype[-c(3,8)]) { # error in i=11,16
  print(paste("Genotype is ",i))
  # subset genotype of interests from DGEList (with normalized). samples.nolow.s<-
  samples.nolow[samples.nolow$genotype==i,]
  dge.nolow.s<-dge.nolow[,samples.nolow$genotype==i]
  # eliminating low expressed genes in dge.nolow.s
  dge.nolow.s<-dge.nolow.s[rowSums(dge.nolow.s$counts > 5) >= 3,]
  # model
  design.nolow.s.batch<- model.matrix(~samples.nolow.s$time*samples.nolow.s$trt +
  samples.nolow.s$batch) # we have batch C, D, E
  colnames(design.nolow.s.batch) <- gsub("samples.nolow.s
  $", "", colnames(design.nolow.s.batch), fixed=TRUE) # nicer column names
  colnames(design.nolow.s.batch)
  print(design.nolow.s.batch)
  dge.nolow.s.batch.glm <- estimateGLMCommonDisp(dge.nolow.s, design.nolow.s.batch,
  verbose=TRUE)
  dge.nolow.s.batch.glm <- estimateGLMTrendedDisp(dge.nolow.s.batch.glm, design.nolow.s.batch,
  verbose=TRUE)
  dge.nolow.s.batch.glm <- estimateGLMTagwiseDisp(dge.nolow.s.batch.glm, design.nolow.s.batch)
  dge.nolow.s.batch.glm.fit <- glmFit(dge.nolow.s.batch.glm, design.nolow.s.batch)
  dge.nolow.s.batch.glm.lrt <-
  glmLRT(dge.nolow.s.batch.glm.fit, coef=c("trtL", "time4:trtL", "time16:trtL", "time25:trtL", "time4
  9:trtL"))
  DE.batch.anytime<-
  topTags(dge.nolow.s.batch.glm.lrt, n=2000)$table[topTags(dge.nolow.s.batch.glm.lrt, n=2000)$tabl
  e$FDR<0.05,]
  dim(DE.batch.anytime) # 416 (Col, w/o eliminating low expressed genes). 518 (Col, after
  eliminating low expressed genes)
  save(DE.batch.anytime, file=paste("DE.batch.anytime_genotype", i, ".Rdata", sep=""))
}
```

# Finding genotype-responsive genes

edgeR, glm function

```
for(i in Genotype[-1]) {# Genotype 17, 3,4,5,6,7,9 exceed 2000 genes. List up 5000 genes
  samples.nolow5.s<-samples.nolow5[samples.nolow5$genotype=="1"|samples.nolow5$genotype==i,]
#pairwise comparison between Col and genotype "i"
  samples.nolow5.s$genotype<-as.character(samples.nolow5.s$genotype)
  dge.nolow.s<-dge.nolow5[,colnames(dge.nolow5) %in% samples.nolow5.s$file]
  # eliminating low expressed genes in dge.nolow.s
  dge.nolow.s<-dge.nolow.s[rowSums(dge.nolow.s$counts > 5) >= 3,]
  #
  design.nolow.s.gt.batch<- model.matrix(~samples.nolow5.s$genotype + samples.nolow5.s
  $trt*samples.nolow5.s$time + samples.nolow5.s$batch) # new model to detect genes with gt
  effects (081614)
  colnames(design.nolow.s.gt.batch) <- gsub("samples.nolow5.s
  $", "",colnames(design.nolow.s.gt.batch),fixed=TRUE) # nicer column names
  colnames(design.nolow.s.gt.batch)
  print(design.nolow.s.gt.batch)
  dge.nolow.s.batch.glm <- estimateGLMCommonDisp(dge.nolow.s,design.nolow.s.gt.batch,
  verbose=TRUE)
  dge.nolow.s.batch.glm <-
  estimateGLMTrendedDisp(dge.nolow.s.batch.glm,design.nolow.s.gt.batch, verbose=TRUE)
  dge.nolow.s.batch.glm<-estimateGLMTagwiseDisp(dge.nolow.s.batch.glm,design.nolow.s.gt.batch)
  dge.nolow.s.batch.glm.fit <- glmFit(dge.nolow.s.batch.glm,design.nolow.s.gt.batch)
  dge.nolow.s.batch.glm.lrt <-
  glmLRT(dge.nolow.s.batch.glm.fit,coef=paste("genotype",i,sep="")) # this is for comparison
  between Col and genotype "i".
  DE.batch.gt<-
  topTags(dge.nolow.s.batch.glm.lrt,n=10000)$table[topTags(dge.nolow.s.batch.glm.lrt,n=10000)$ta
  ble$FDR<0.05,]
  dim(DE.batch.gt) #
  save(DE.batch.gt,file=paste("DE.genotype.batch.gt.wol6h",i,".Rdata",sep=""))
}
```