A

```
###Read in count data###
daf1_cts <- read.table("STAR_output/daf1_ReadsPerGene.out.tab", header = FALSE)
daf1 <- daf1_cts[-c(1:4),]
daf1$V1 <- gsub("\\..*","", daf1$V1)

daf2_cts <- read.table("STAR_output/daf2_ReadsPerGene.out.tab", header = FALSE)
daf2 <- daf2_cts[-c(1:4),]
daf2$V1 <- gsub("\\..*","", daf2$V1)
```

B

```
###Make dataframe with count data for all samples###
totcts <- data.frame(iluc1=iluc1$V2, iluc2=iluc2$V2, iluc3=iluc3$V2,
                     ief1=ief1$V2, ief2=ief2$V2, ief3=ief3$V2,
                     wtef1=wtef1$V2, wtef2=wtef2$V2, wtef3=wtef3$V2,
                     del221=del221$V2, del222=del222$V2, del223=del223$V2,
                     daf1=daf1$V2, daf2=daf2$V2, daf3=daf3$V2)

rownames(totcts) <- iluc1$V1
```

C

```
library("sva")
library(DESeq2)
library("AnnotationDbi")
library("org.Hs.eg.db")
library("pheatmap")
library("RColorBrewer")
library("genefilter")
library(Cairo)
library("ggplot2")
library("ggbiplot")
library("rgl")
library("reshape2")
```

D

```
###Filter out genes with low read counts###
n <- as.number(ncol(totcts))
keep <- (rowSums(totcts)>=n)
totcts_flt <- totcts[keep,]
```

E

```
###Define your experimental variables for batch normalization###
rows <- c("iluc1", "iluc2", "iluc3", "ief1", "ief2", "ief3", "wtef1", "wtef2", "wtef3",
          "dell221", "del222", "del223", "daf1", "daf2", "daf3")
smpl <- 1:15 ; smpl
btch <- c(1,2,3,1,2,3,1,2,3,1,2,3,1,2,3)
cond <- c("iluc", "iluc", "iluc", "ief", "ief", "ief", "wtef", "wtef", "wtef",
          "del22", "del22", "del22", "daf", "daf", "daf")

vars <- data.frame(sample = smpl, batch = btch, construct = cond)
rownames(vars) <- rows
```

F

```
###Run ComBat###
b <- vars$batch
modComBat <- model.matrix(~1, data = vars)
combat_cts <- ComBat(dat = totcts_flt, batch = b, mod = modComBat, par.prior = FALSE, prior.plots = TRUE)
```