

# Shank3 Modulates Sleep and Expression of Circadian Transcription Factors differential expression

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## Description

This is the report of the analysis made for the paper *Shank3 Modulates Sleep and Expression of Circadian Transcription Factors* by Ashley M. Ingiosi, Taylor Wintler, Hannah Schoch, Kristan G. Singletary, Dario Righelli, Leandro G. Roser, Davide Risso, Marcos G. Frank and Lucia Peixoto.

Autism Spectrum Disorder (ASD) is the most prevalent neurodevelopmental disorder in the US that often co-presents with sleep problems. Sleep impairments in ASD predict the severity of ASD core diagnostic symptoms and have a considerable impact on the quality of life of caregivers. However, little is known about the underlying molecular mechanism(s) of sleep impairments in ASD. In this study we investigated the role of Shank3, a high confidence ASD gene candidate, in the regulation of sleep. We show that Shank3 mutant

mice have problems falling asleep despite accumulating sleep pressure. Using RNA-seq we show that sleep deprivation doubles the differences in gene expression between mutants and wild types and downregulates circadian transcription factors Per3, Dec2, and Rev-erba. Shank3 mutants also have trouble regulating locomotor activity in the absence of light. Overall, our study shows that Shank3 is an important modulator of sleep and circadian activity. # Differential Expression Analysis

## Importing data

Importing data and filtering out those genes with cpm lesser than 1. We use the *filtered.data* method in *NOISeq* package.

```
countMatrix <- ReadDataFrameFromTsv(file.name.path="./data/refSEQ_countMatrix.txt")

## ./data/refSEQ_countMatrix.txt read from disk!
# head(countMatrix)

designMatrix <- ReadDataFrameFromTsv(file.name.path="./design/all_samples_short_names.tsv")

## ./design/all_samples_short_names.tsv read from disk!
# head(designMatrix)

filteredCountsProp <- filterLowCounts(counts.dataframe=countMatrix,
                                     is.normalized=FALSE,
                                     design.dataframe=designMatrix,
                                     cond.col.name="gcondition",
                                     method.type="Proportion")

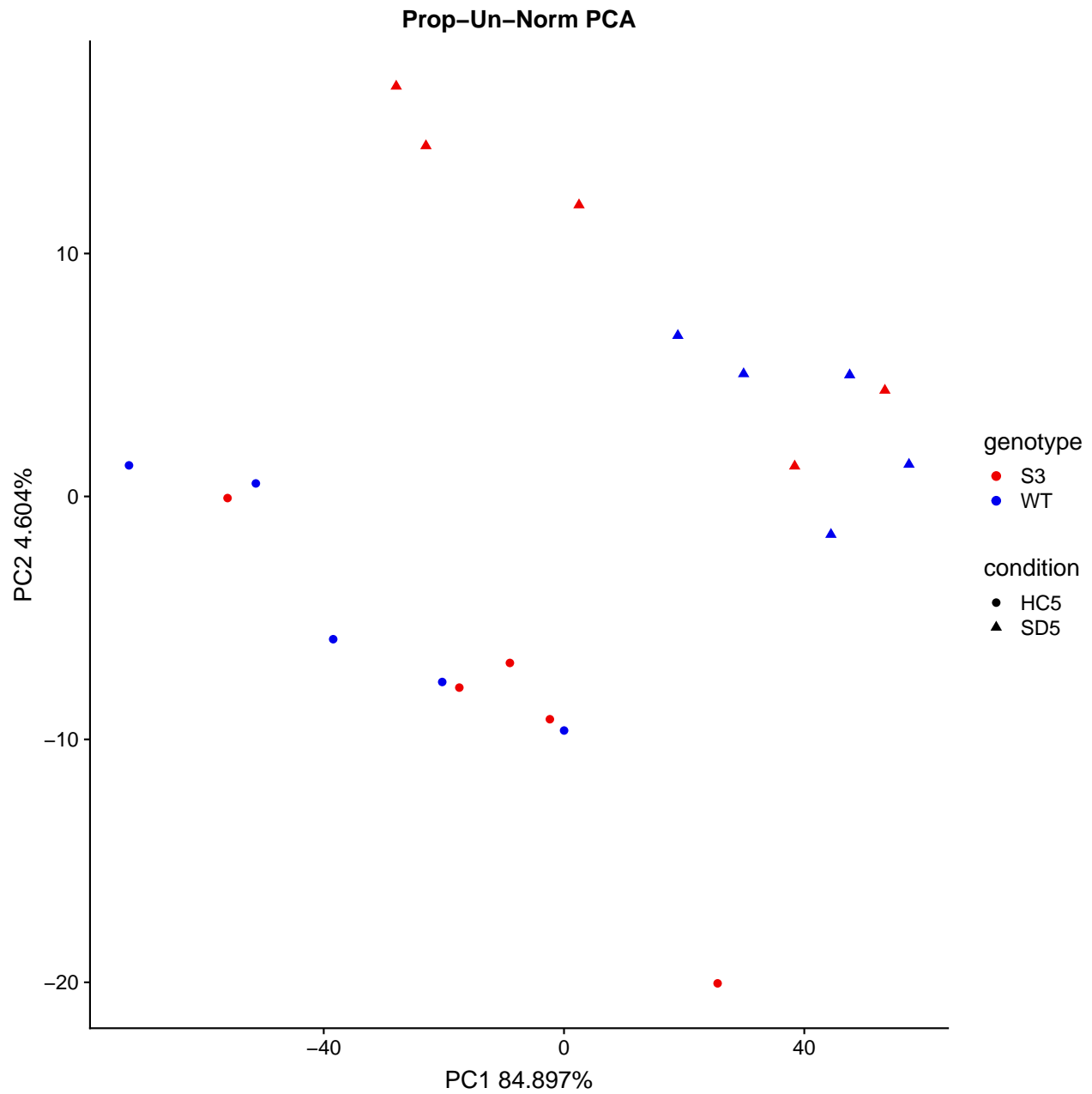
## features dimensions before normalization: 27179
## Filtering out low count features...
## 14454 features are to be kept for differential expression analysis with filtering method 3
```

## Plot PCA of log unnormalized data

PCA Plot of filtered not-normalized data.

```
PlotPCAPlotlyFunction(counts.data.frame=log1p(filteredCountsProp),
                      design.matrix=designMatrix,
                      shape.colname="condition", color.colname="genotype", xPCA="PC1", yPCA="PC2",
                      plotly.flag=FALSE, show.plot.flag=TRUE, prefix.plot="Prop-Un-Norm")

## [1] FALSE
```



## Control Genes

### Negative control genes

Loading Negative Control Genes to normalize data

```
library(readxl)
```

```
sd.ctrls <- read_excel(path="./data/controls/Additional File 4 full list of BMC genomics SD&RS2.xlsx", sheet="SD5")
sd.ctrls <- sd.ctrls[order(sd.ctrls$adj.P.Val),]
```

```
sd.neg.ctrls <- sd.ctrls[sd.ctrls$adj.P.Val > 0.9, ]
```

```

sd.neg.ctrls <- sd.neg.ctrls$`MGI Symbol`
sd.neg.ctrls <- sd.neg.ctrls[~which(is.na(sd.neg.ctrls))]

int.neg.ctrls <- sd.neg.ctrls
int.neg.ctrls <- unique(int.neg.ctrls)
neg.map <- convertGenesViaMouseDb(gene.list=int.neg.ctrls, fromType="SYMBOL",
                                "ENTREZID")
# sum(is.na(neg.map$ENTREZID))
neg.ctrls.entrez <- as.character(neg.map$ENTREZID)

ind.ctrls <- which(rownames(filteredCountsProp) %in% neg.ctrls.entrez)
counts.neg.ctrls <- filteredCountsProp[ind.ctrls,]

```

## positive control genes

Loading Positive Control Genes to detect them during the differential expression step.

```

## sleep deprivation
sd.lit.pos.ctrls <- read_excel("./data/controls/SD_RS_PosControls_final.xlsx",
                              sheet=1)
colnames(sd.lit.pos.ctrls) <- sd.lit.pos.ctrls[1,]
sd.lit.pos.ctrls <- sd.lit.pos.ctrls[-1,]

sd.est.pos.ctrls <- read_excel("./data/controls/SD_RS_PosControls_final.xlsx",
                              sheet=3)

sd.pos.ctrls <- cbind(sd.est.pos.ctrls$`MGI Symbol`, "est")
sd.pos.ctrls <- rbind(sd.pos.ctrls, cbind(sd.lit.pos.ctrls$Gene, "lit"))

sd.pos.ctrls <- sd.pos.ctrls[~which(duplicated(sd.pos.ctrls[,1])),]
sd.pos.ctrls <- sd.pos.ctrls[~which(is.na(sd.pos.ctrls[,1])),]

```

## Normalizations

### TMM Normalization

Normalizing data with *TMM*, as implemented in *edgeR* package, and plotting a PCA and an RLE plot of them.

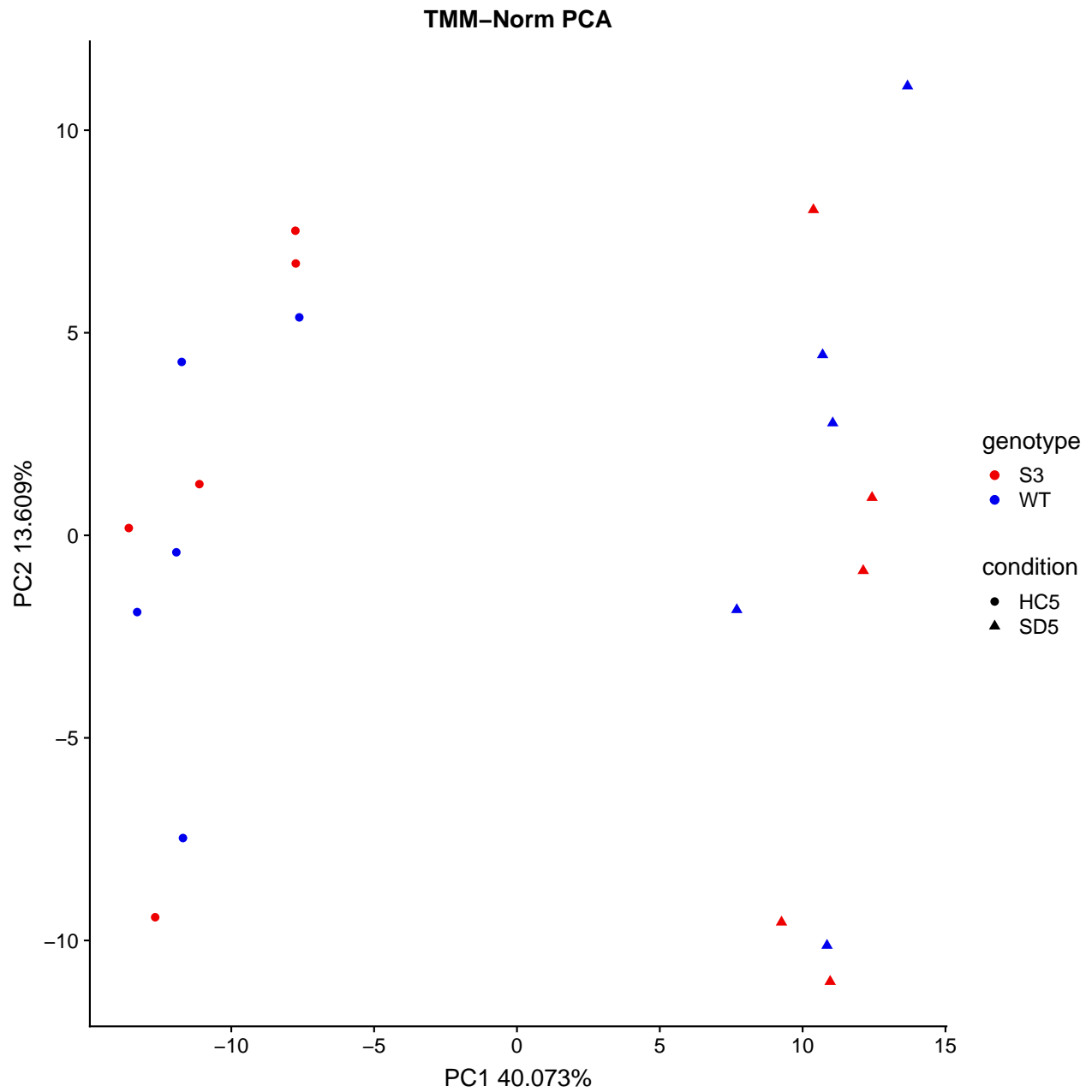
```

normPropCountsUqua <- NormalizeData(data.to.normalize=filteredCountsProp,
                                   norm.type="tmm",
                                   design.matrix=designMatrix)

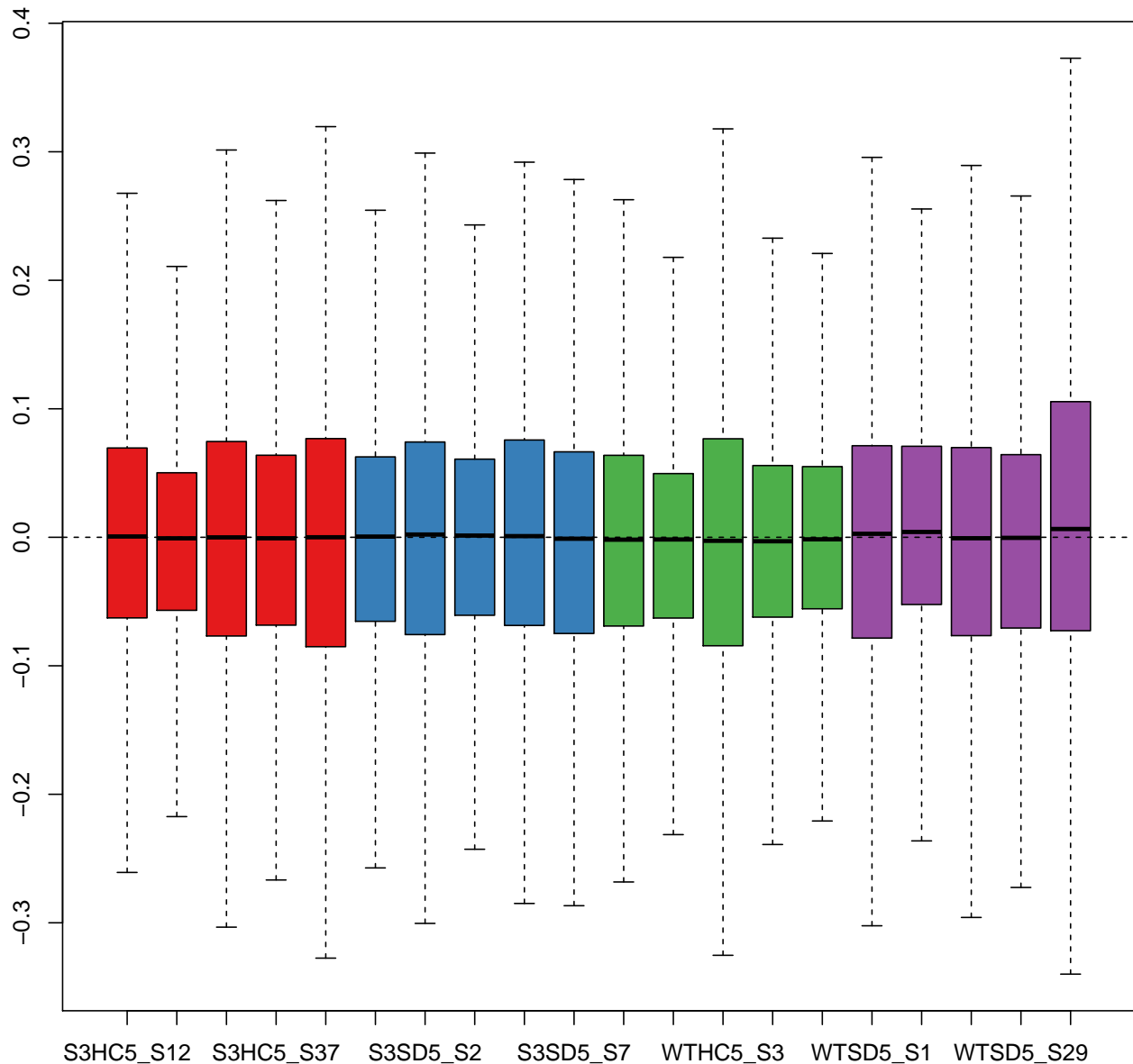
PlotPCAPlotlyFunction(counts.data.frame=log1p(normPropCountsUqua),
                      design.matrix=designMatrix, shapeColname="condition",
                      colorColname="genotype", xPCA="PC1", yPCA="PC2",
                      plotly.flag=FALSE, show.plot.flag=TRUE,
                      prefix.plot="TMM-Norm")

```

```
## [1] FALSE
```



```
pal <- RColorBrewer::brewer.pal(9, "Set1")
plotRLE(as.matrix(normPropCountsUqua), outline=FALSE, col=pal[designMatrix$gcondition])
```



### TMM + RUVs Normalization

Applying a *RUVs* method of *RUVSeq* package on normalized data, in order to adjust the counts for the unwanted variation. And of course we plot a PCA and an RLE plot on these data.

```
library(RUVSeq)
neg.ctrl.list <- rownames(counts.neg.ctrls)
groups <- makeGroups(designMatrix$gcondition)
ruvedSEXPData <- RUVs(as.matrix(round(normPropCountsUqua)), cIdx=neg.ctrl.list,
                      scIdx=groups, k=5)

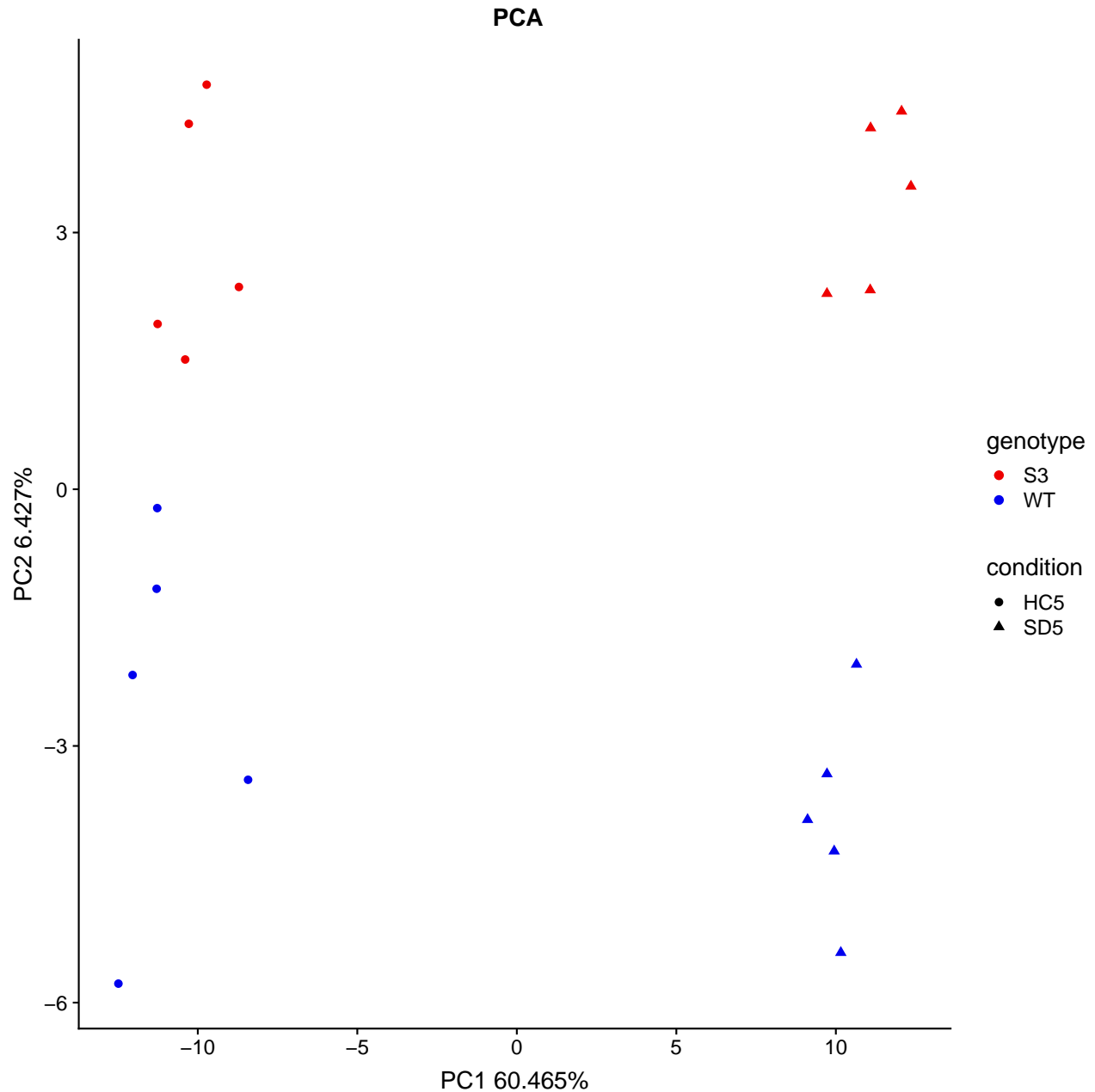
normExprData <- ruvedSEXPData$normalizedCounts

ggp <- PlotPCAPlotlyFunction(counts.data.frame=log1p(normExprData),
                             design.matrix=designMatrix, shapeColname="condition",
                             colorColname="genotype", xPCA="PC1", yPCA="PC2",
```

```
plotly.flag=FALSE, show.plot.flag=FALSE, save.plot=FALSE,
prefix.plot=NULL)
```

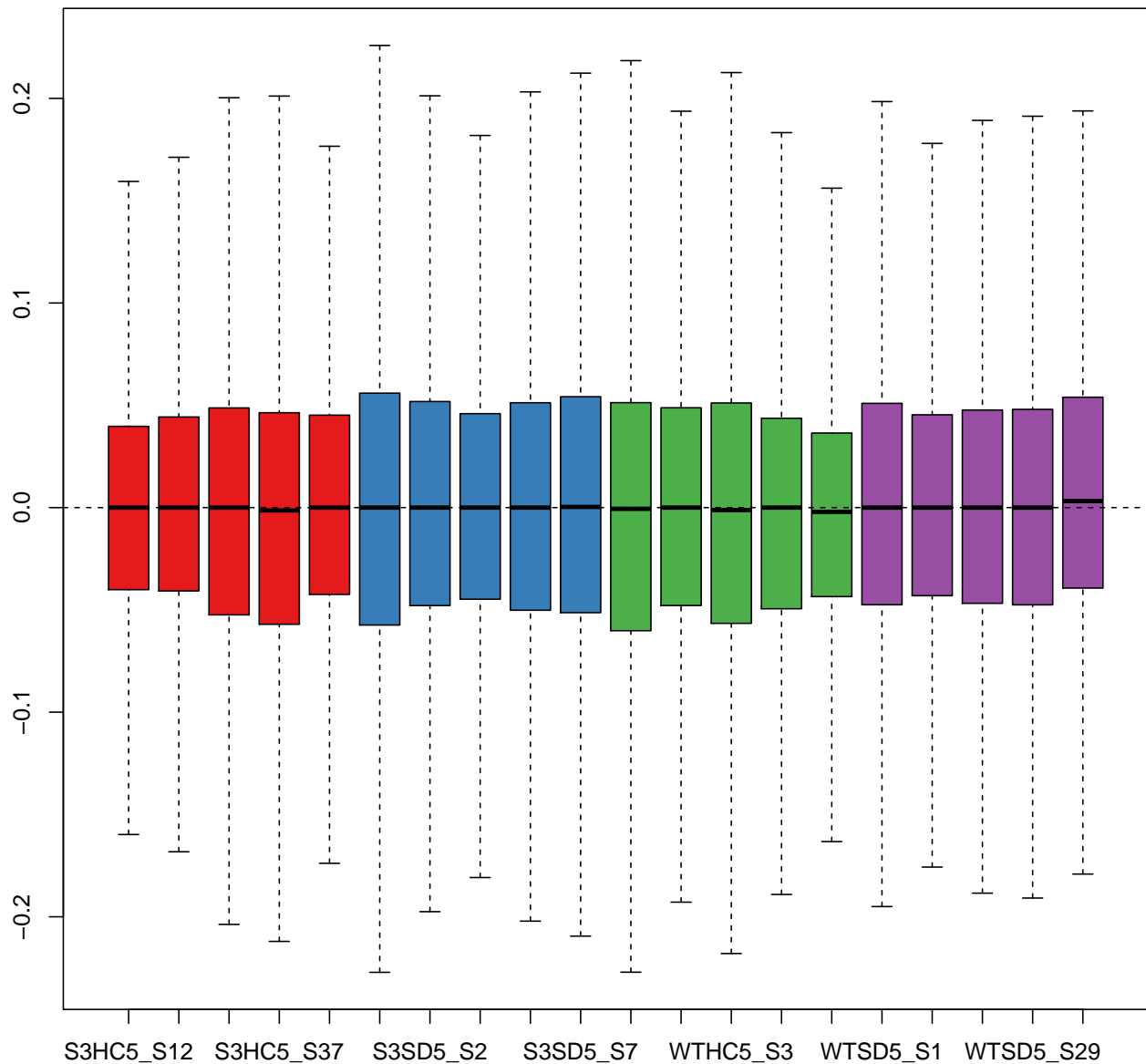
```
## [1] FALSE
```

```
print(ggp)
```



```
dir.create("plots")
save_plot(filename="plots/PCA.pdf", plot=ggp)

pal <- RColorBrewer::brewer.pal(9, "Set1")
plotRLE(normExprData, outline=FALSE, col=pal[designMatrix$gcondition])
```



## edgeR Differential Expression Analysis

Making differential expression analysis with *edgeR* package on four different contrasts.

Here is a brief legend:

- WTHC5: Wild Type Home Cage Control 5 days
- WTSD5: Wild Type Sleep Deprivation 5 days.
- KOHC5: Knock Out Home Cage Control 5 days.
- KOSD5: Knock Out Sleep Deprivation 5 days.

```
padj.thr <- 0.05
venn.padj.thr <- 0.1
desMat <- cbind(designMatrix, ruvedSEExprData$W)
colnames(desMat) <- c(colnames(designMatrix), colnames(ruvedSEExprData$W))

cc <- c("S3HC5 - WTHC5", "S3SD5 - WTSD5")
```



```

rescList1 <- applyEdgeR(counts=filteredCountsProp, design.matrix=desMat,
                        factors.column="gcondition",
                        weight.columns=c("W_1", "W_2", "W_3", "W_4", "W_5"),
                        contrasts=cc, useIntercept=FALSE, p.threshold=1,
                        is.normalized=FALSE, verbose=TRUE)
names <- names(rescList1)
rescList1 <- lapply(seq_along(rescList1), function(i)
{
  attachMeans(normalized.counts=normExprData, design.matrix=desMat,
              factor.column="gcondition", contrast.name=names(rescList1)[i],
              de.results=rescList1[[i]])
})
names(rescList1) <- names

```

### Shank3 Home Cage control VS Wild Type Home Cage Controls

#### volcano plot

A volcano plot of differential expressed genes.

```

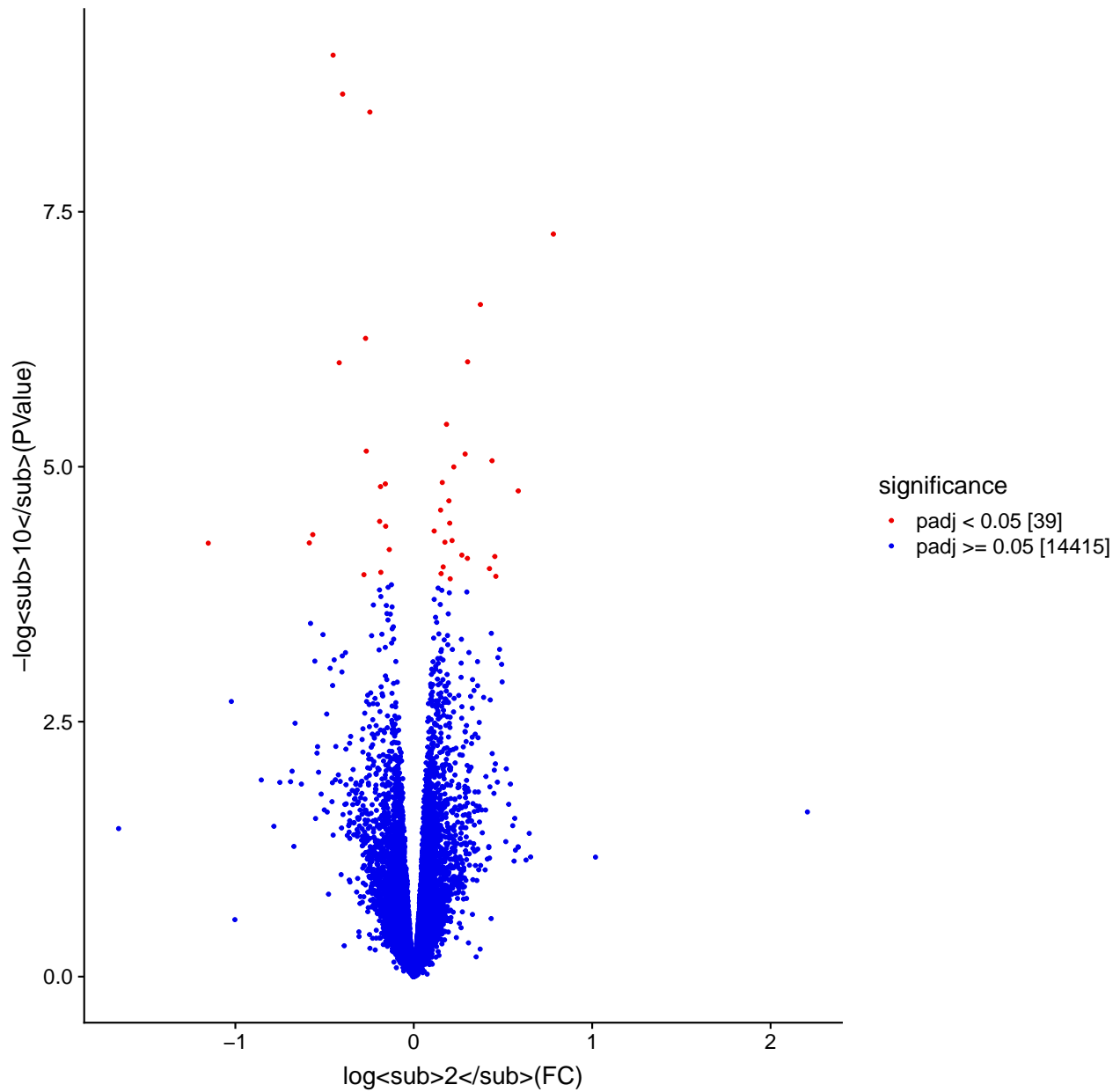
res.o.map1 <- convertGenesViaMouseDb(gene.list=rownames(rescList1[[1]]),
                                    fromType="ENTREZID")

res.o <- attachGeneColumnToDf(mainDf=rescList1[[1]],
                              genesMap=res.o.map1,
                              rowNamesIdentifier="ENTREZID",
                              mapFromIdentifier="ENTREZID",
                              mapToIdentifier="SYMBOL")
WriteDataFrameAsTsv(data.frame.to.save=res.o,
                   file.name.path=paste0(names(rescList1)[1], "_edgeR"))

vp <- luciaVolcanoPlot(res.o, prefix=names(rescList1)[1],
                      positive.controls.df=NULL,
                      threshold=padj.thr)
print(vp)

```

### S3HC5 – WTHC5 Volcano Plot



```
de <- sum(res.o$FDR < padj.thr)
nde <- sum(res.o$FDR >= padj.thr)
detable <- cbind(de,nde)
rownames(detable) <- names(rescList1)[1]
ddetable <- detable

tot.ctrls <- dim(sd.pos.ctrls)[1]
idx.pc <- which(tolower(res.o$gene) %in% tolower(sd.pos.ctrls[,1]))
tot.pc.de <- sum(res.o$FDR[idx.pc] < padj.thr)
tot.pc.nde <- length(idx.pc) - tot.pc.de

wt <- res.o[which(res.o$FDR < padj.thr),]
wt.sign.genes.entrez <- rownames(res.o)[which(res.o$FDR < venn.padj.thr)]
```

```
kowthc5 <- res.o[which(res.o$FDR < padj.thr),]
kowthc5.sign.genes.entrez <- rownames(res.o)[which(res.o$FDR < venn.padj.thr)]
```

## Shank3 Sled Deprivation VS Wild Type Sleep Deprivation

### volcano plot

A volcano plot of differential expressed genes.

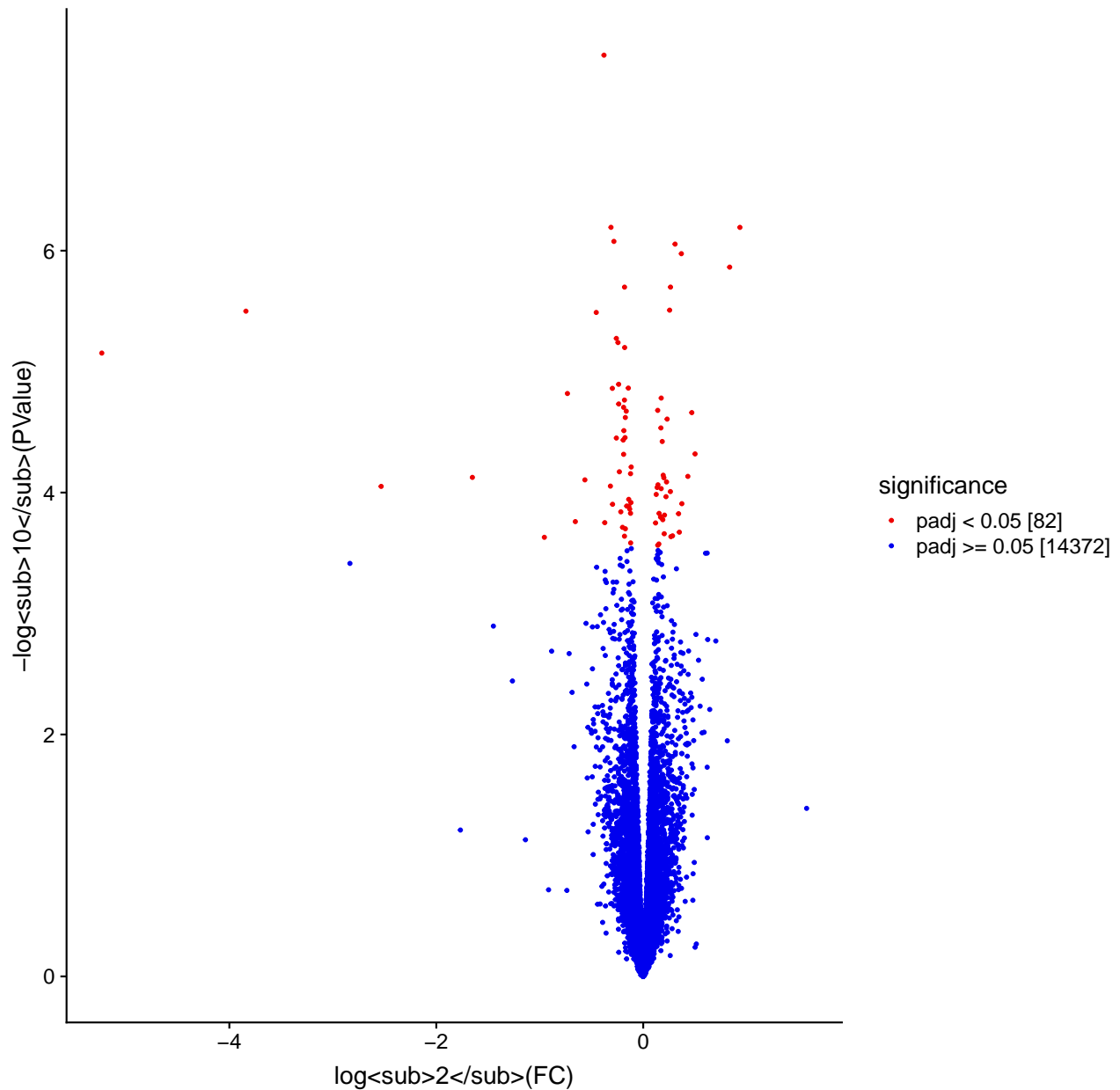
```
rs2.o.map <- convertGenesViaMouseDb(gene.list=rownames(rescList1[[2]]),
                                   fromType="ENTREZID")

res.rs2.o <- attachGeneColumnToDf(mainDf=rescList1[[2]],
                                   genesMap=rs2.o.map,
                                   rowNamesIdentifier="ENTREZID",
                                   mapFromIdentifier="ENTREZID",
                                   mapToIdentifier="SYMBOL")
WriteDataframeAsTsv(data.frame.to.save=res.rs2.o,
                    file.name.path=paste0(names(rescList1)[2], "_edgeR"))

vp <- luciaVolcanoPlot(res.rs2.o, positive.controls.df=NULL,
                      prefix=names(rescList1)[2],
                      threshold=padj.thr)

print(vp)
```

### S3SD5 – WTSD5 Volcano Plot



```
de <- sum(res.rs2.o$FDR < padj.thr)
nde <- sum(res.rs2.o$FDR >= padj.thr)
detable <- cbind(de,nde)
rownames(detable) <- names(rescList1)[2]
ddetable <- rbind(ddetable, detable)
pos.df <- cbind(tot.ctrls, tot.pc.de, tot.pc.nde)
colnames(pos.df) <- c("total_p.ctrl", "p.ctrl_de_mapped",
                     "p.ctrl_notde_mapped")
rownames(pos.df) <- names(rescList1)[2]

kowtsd5 <- res.rs2.o[which(res.rs2.o$FDR < padj.thr),]
kowtsd5.sign.genes.entrez <- rownames(res.rs2.o)[which(res.rs2.o$FDR < venn.padj.thr)]
```

## DE TABLE + Positive Controls table

We present a summarization of the results. The first table is a summarization on how many genes are Differentially Expressed. The second table explains on the first column how many positive controls we have, on the second column how many positive controls have been identified over the differentially expressed genes, and, finally, on the third column how many positive controls have been identified on the NOT differentially expressed genes.

```
ddetable
```

```
##           de   nde
## S3HC5 - WTHC5 39 14415
## S3SD5 - WTSD5 82 14372
```

```
pos.df
```

```
##           total_p.ctrl p.ctrl_de_mapped p.ctrl_notde_mapped
## S3SD5 - WTSD5           579              3              553
```

## Venn Diagram

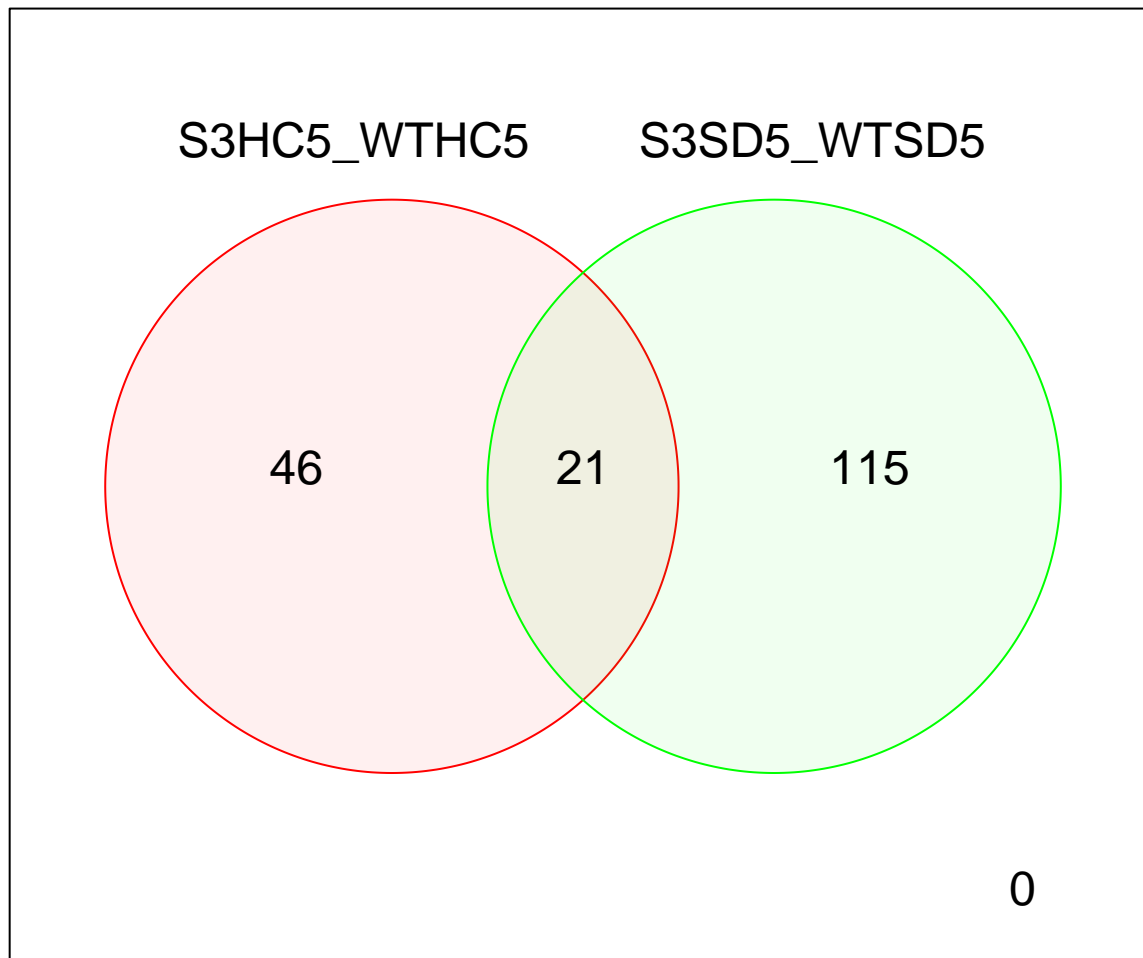
### KOHC5-WTHC5 vs KOSD5-WTSD5

We take the results of the two contrasts. *Knock Out Sleep Deprivation VS Wild Type Sleep Deprivation* and *Knock Out Home Cage control VS Wild Type Home Cage Controls*. And plot the results in a Venn Diagram

```
source("./R/venn2.R")
```

```
gene.map <- convertGenesViaMouseDb(gene.list=rownames(normExprData),
                                   fromType="ENTREZID", toType="SYMBOL")
venn <- Venn2de(x=kowthc5.sign.genes.entrez, y=kowtsd5.sign.genes.entrez,
               label1="S3HC5_WTHC5", label2="S3SD5_WTSD5",
               title="S3HC5_WTHC5 venn S3SD5_WTSD5", plot.dir=".",
               conversion.map=gene.map)
```

## S3HC5\_WTHC5 venn S3SD5\_WTSD5



### Heatmaps

Setting up the data structures for the heatmps.

```
source("./R/heatmapFunctions.R")
de.genes.entr <- union(rownames(venn$int), rownames(venn$XnoY))
de.genes.entr <- union(de.genes.entr, rownames(venn$YnoX))

gene.map <- convertGenesViaMouseDb(gene.list=de.genes.entr,
                                   fromType="ENTREZID")
```

```

de.genes.symb <- attachGeneColumnToDf(as.data.frame(de.genes.entr,
                                                    row.names=de.genes.entr),
                                     genesMap=gene.map,
                                     rowNamesIdentifier="ENTREZID",
                                     mapFromIdentifier="ENTREZID",
                                     mapToIdentifier="SYMBOL")

# de.genes.symb[which(is.na(de.genes.symb$gene)),]
de.genes.symb$gene[which(de.genes.symb$de.genes.entr=="100039826")] <- "Gm2444" ## not annotated in ncl
de.genes.symb$gene[which(de.genes.symb$de.genes.entr=="210541")] <- "Entrez:210541" ## not annotated in ncl

de.genes.counts <- normExprData[match(de.genes.symb$de.genes.entr, rownames(normExprData)),]
rownames(de.genes.counts) <- de.genes.symb$gene

de.gene.means <- computeGeneMeansOverGroups(counts=de.genes.counts,
                                             design=designMatrix, groupColumn="gcondition")

library(gplots)
library(clusterExperiment)
color.palette = clusterExperiment::seqPal3#c("black", "yellow")
pal <- colorRampPalette(color.palette)(n = 1000)

library(pheatmap)
filter2 <- rowMeans(de.gene.means)>0
filter <- apply(de.gene.means, 1, function(x) log(x[4]/x[3]) * log(x[2]/x[1]) < 0)
filter[is.na(filter)] <- FALSE

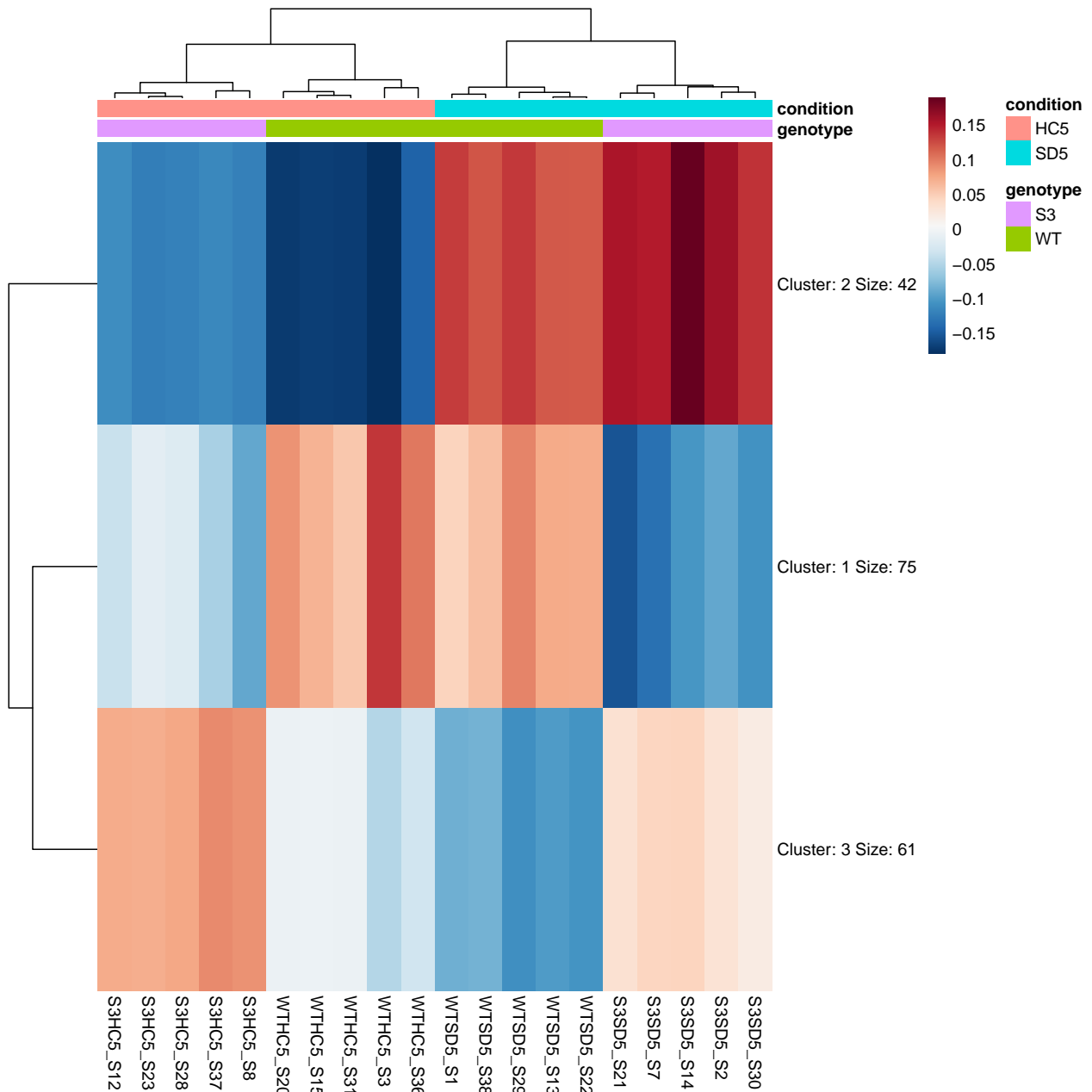
```

## Heatmap gene by bene

```

gene_names <- c("Nr1d1", "Fabp7", "Per3", "Jun", "Elk1", "Fosl2", "Mapk1",
               "Mapk3", "Mapk11", "Hmgcr", "Insig1", "Nfil3", "Stat4",
               "Kcnv1", "Kcnk1", "Kcnk2", "Dusp10", "Dusp3", "Ptprj",
               "Cntn1", "Ntrk2", "Reln", "Sema3a", "Tef", "Hlf", "Nr1d1",
               "Prkab2", "Bhlhe41", "Slc6a15", "Slc22a4", "Slc24a4")
idx <- which(!(rownames(de.genes.counts) %in% gene_names))
de.genes.counts1 <- de.genes.counts
rownames(de.genes.counts1)[idx] <- ""
ann.col <- desMat[, c(1:2)]
de.heatmap <- de.genes.counts[filter2,]
set.seed(0)
heatmap_data <- t(scale(t(log(de.heatmap+1)), center = TRUE, scale = FALSE))
ph1 <- pheatmap(heatmap_data, cluster_cols=TRUE, scale="none",
               color=pal, border_color=NA, fontsize_row=10, kmeans_k=3, annotation_col=ann.col)

```



```
clusterized.genes <- as.data.frame(ph1$kmeans$cluster)

gene.map <- convertGenesViaMouseDb(gene.list=rownames(clusterized.genes), fromType="SYMBOL")
converted.clusterized.gens <- attachGeneColumnToDf(mainDf=clusterized.genes, genesMap=gene.map,
  rowNamesIdentifier="SYMBOL", mapFromIdentifier="SYMBOL", mapToIdentifier="ENTREZID")

converted.clusterized.gens$gene[which(rownames(converted.clusterized.gens)=="Gm2444")] <- "100039826" ;
converted.clusterized.gens$gene[which(rownames(converted.clusterized.gens)=="Entrez:210541")] <- "210541" ;
converted.clusterized.gens <- converted.clusterized.gens[order(converted.clusterized.gens$ph1$kmeans$cluster)]

save_pheatmap_pdf(filename="plots/heatmap_kmeans_k3.pdf", plot=ph1, width=20, height=20)
```

```
## pdf
## 2
```



```

WriteDataFrameAsTsv(data.frame.to.save=converted.clusterized.gens, file.name.path="plots/clustered_genes

ord.de.genes.counts <- de.heatmap[match(rownames(converted.clusterized.gens), rownames(de.heatmap)),]
idx <- which(!(rownames(ord.de.genes.counts) %in% gene_names))

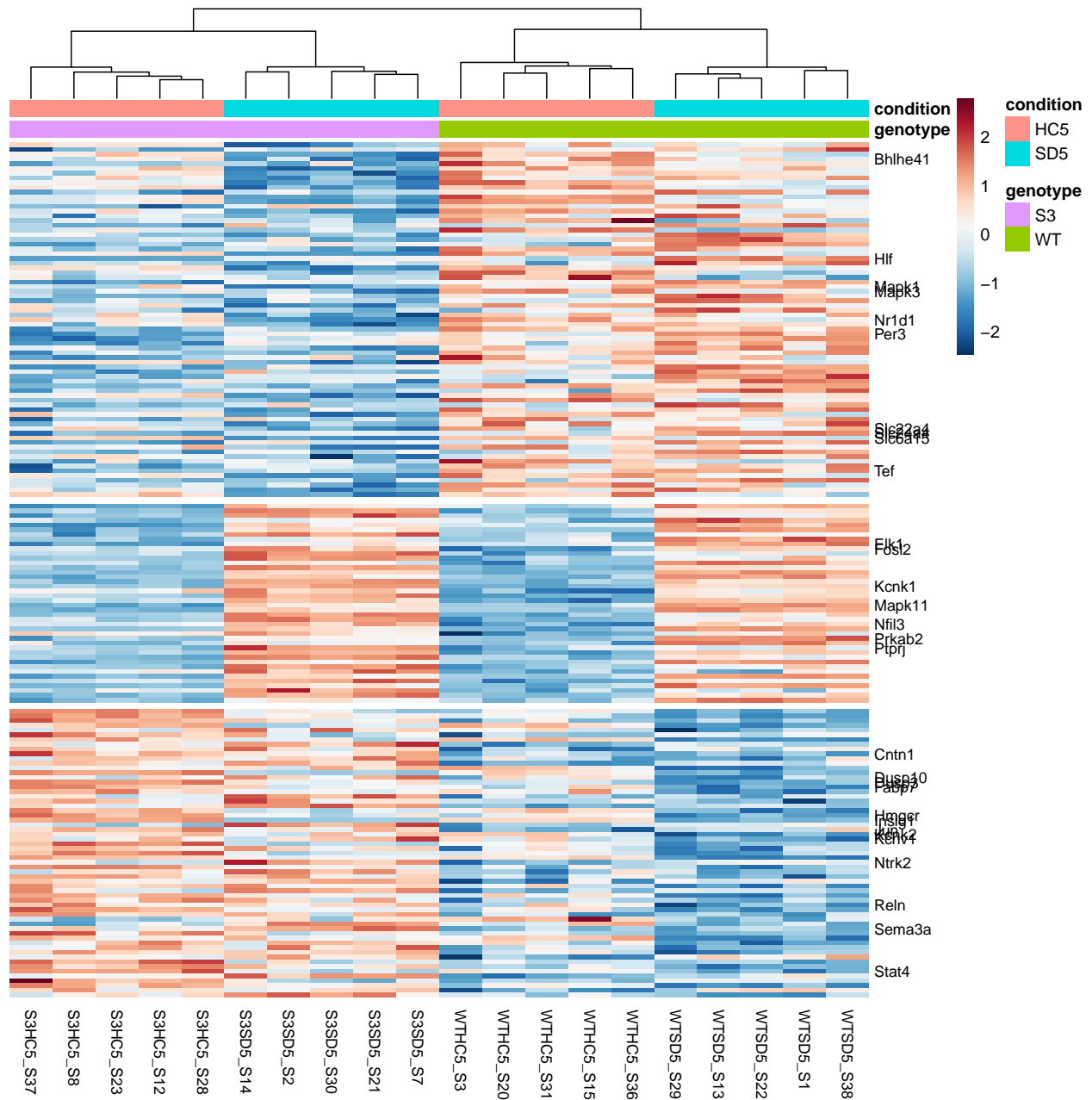
rownames(ord.de.genes.counts)[idx] <- ""
gaps.row <- c()
for(i in c(1:3))
{
  li <- length(which(converted.clusterized.gens$`ph1$kmeans$cluster`==i))
  l <- ifelse(i!=1, gaps.row[i-1]+li, li)
  gaps.row <- c(gaps.row, l)
}

heatmap_data_scaled <- t(scale(t(log(ord.de.genes.counts+1)), center = TRUE, scale = TRUE))

library(dendextend)
column_dend <- as.dendrogram(hclust(dist(t(heatmap_data_scaled))))
ord <- labels(column_dend)
ord[11:15] <- labels(column_dend)[16:20]
ord[16:20] <- labels(column_dend)[11:15]
column_dend <- rotate(column_dend, ord)

ph1 <- pheatmap(heatmap_data_scaled, cluster_cols=as.hclust(column_dend), scale="none",
  color=pal, border_color=NA, fontsize_row=9, fontsize_col=9, cluster_rows=FALSE,
  annotation_col=ann.col, gaps_row=gaps.row)

```



```
save_pheatmap_pdf(filename="plots/heatmap_gg_k3.pdf", plot=ph1, width=20, height=20)
```

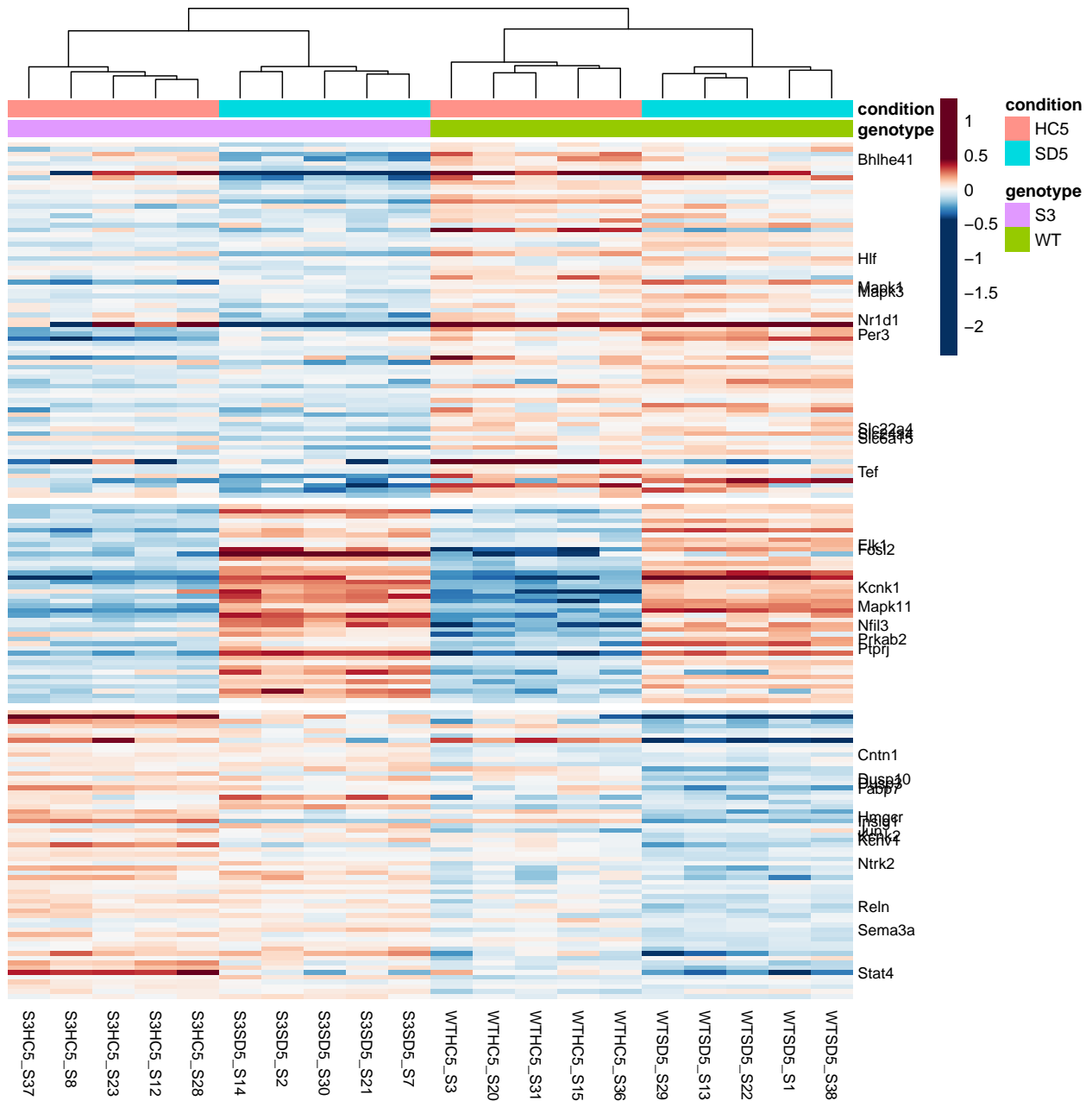
```
## pdf
## 2
```

### other heatmaps

```
heatmap_data <- t(scale(t(log(ord.de.genes.counts+1)), center = TRUE, scale = FALSE))

ph1 <- pheatmap(heatmap_data, cluster_cols=as.hclust(column_dend), scale="none",
  color=pal, border_color=NA, fontsize_row=9, fontsize_col=9, cluster_rows=FALSE,
  annotation_col=ann.col, gaps_row=gaps.row,
```

```
breaks = c(min(heatmap_data), seq(quantile(as.vector(heatmap_data), .01), quantile(as.vector(heatmap_data), .99))),
```



```
save_pheatmap_pdf(filename="plots/heatmap_gg_k3_no_scale.pdf", plot=ph1, width=20, height=20)
```

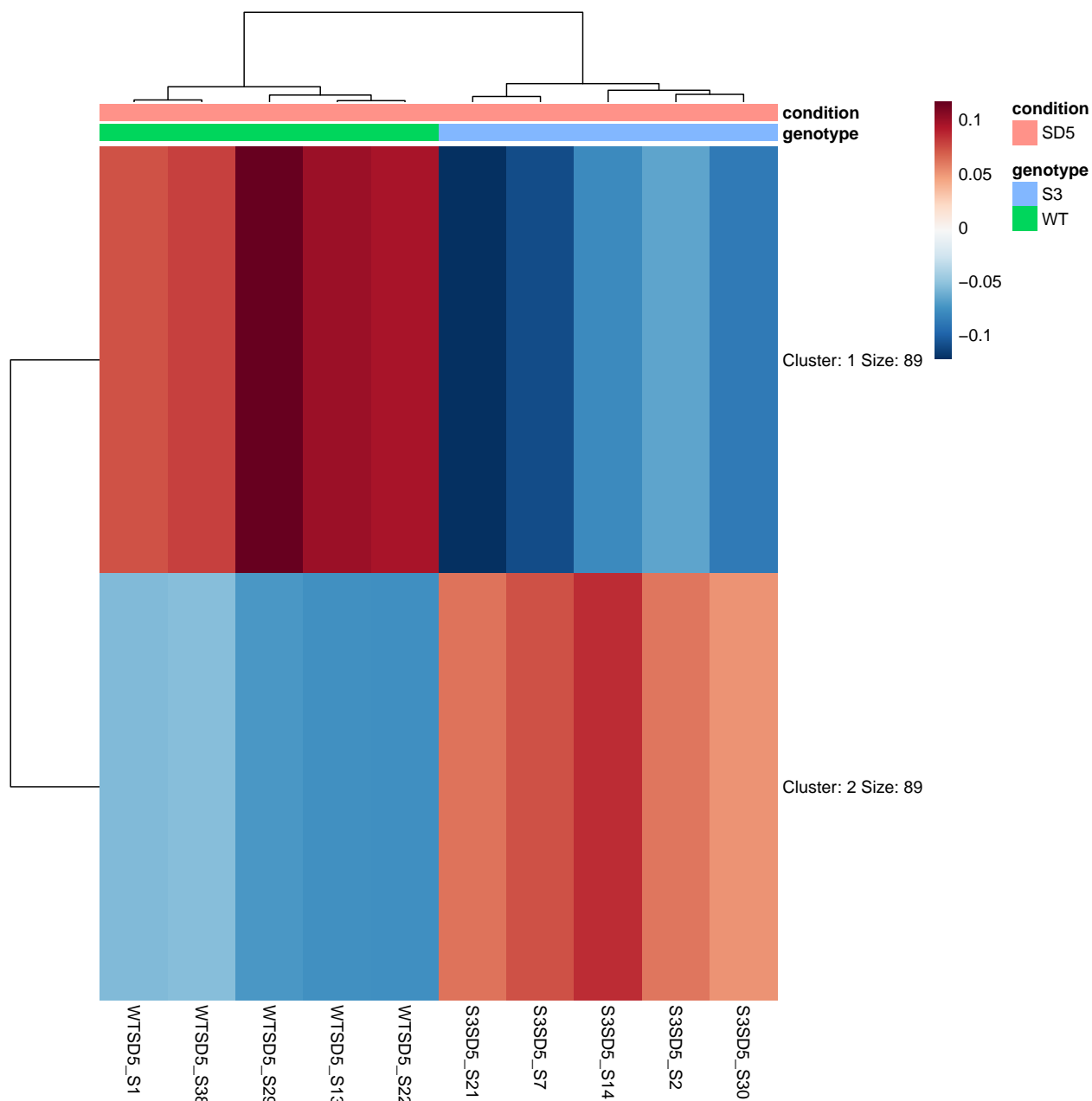
```
## pdf
```

```
## 2
```

```
## Only SD samples
```

```
heatmap_data <- t(scale(t(log(de.heatmap[, grep("^SD", desMat[,2]))+1)), center = TRUE, scale = FALSE))
```

```
ph1 <- pheatmap(heatmap_data, cluster_cols=TRUE, scale="none",  
color=pal, border_color=NA, fontsize_row=10, kmeans_k=2, annotation_col=ann.col)
```



```
clusterized.genes <- as.data.frame(ph1$kmeans$cluster)

gene.map <- convertGenesViaMouseDb(gene.list=rownames(clusterized.genes), fromType="SYMBOL")
converted.clusterized.gens <- attachGeneColumnToDf(mainDf=clusterized.genes, genesMap=gene.map,
  rowNamesIdentifier="SYMBOL", mapFromIdentifier="SYMBOL", mapToIdentifier="ENTREZID")

converted.clusterized.gens$gene[which(rownames(converted.clusterized.gens)=="Gm2444")] <- "100039826" ;
converted.clusterized.gens$gene[which(rownames(converted.clusterized.gens)=="Entrez:210541")] <- "210541" ;
converted.clusterized.gens <- converted.clusterized.gens[order(converted.clusterized.gens$ph1$kmeans$cluster),]

ord.de.genes.counts <- de.heatmap[match(rownames(converted.clusterized.gens), rownames(de.heatmap)),]
idx <- which(!(rownames(ord.de.genes.counts) %in% gene_names))
```

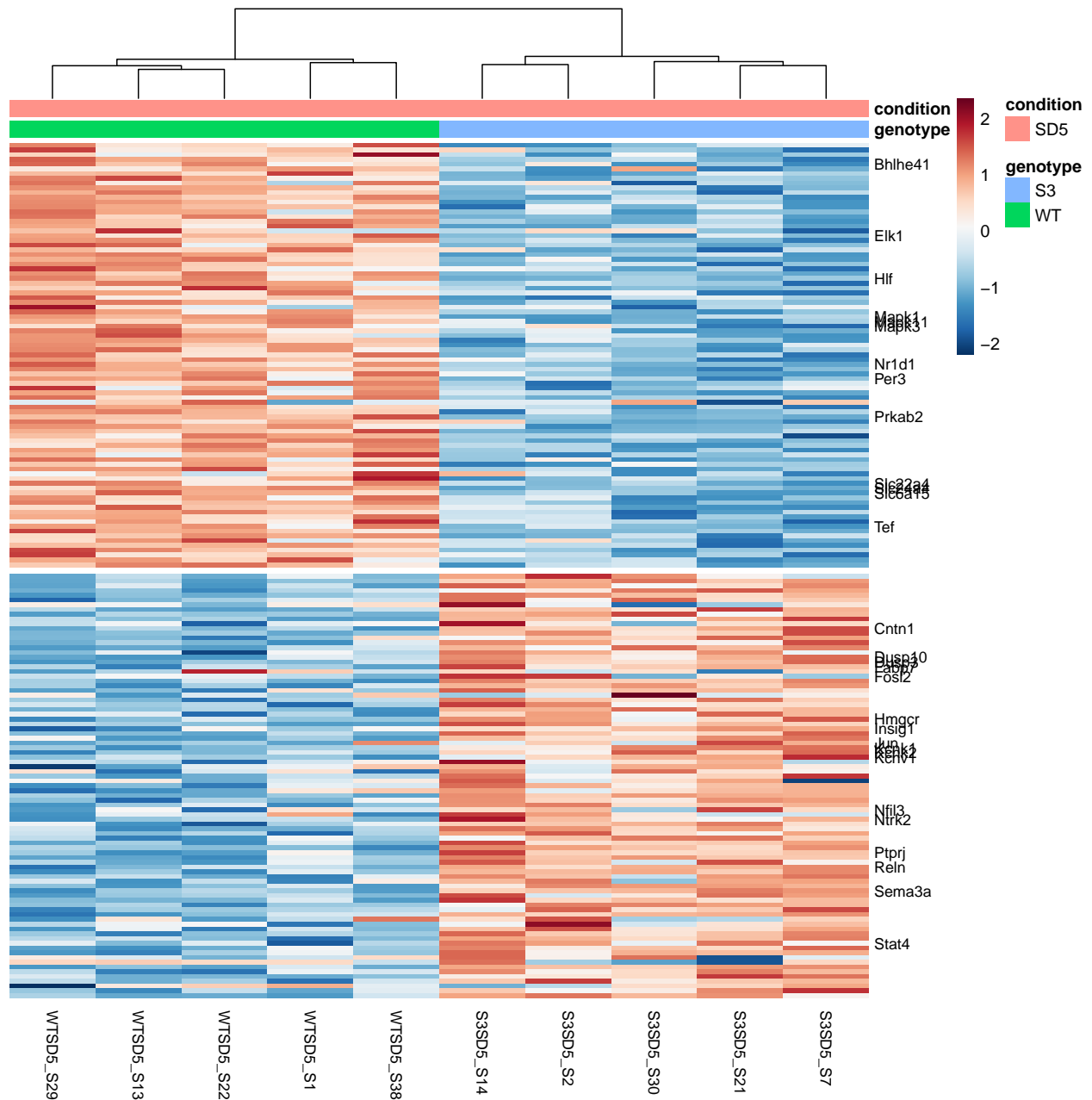
```

rownames(ord.de.genes.counts)[idx] <- ""
gaps.row <- c()
for(i in c(1:2))
{
  li <- length(which(converted.clusterized.genes$`ph1$kmeans$cluster`==i))
  l <- ifelse(i!=1, gaps.row[i-1]+li, li)
  gaps.row <- c(gaps.row, l)
}

heatmap_data <- t(scale(t(log(ord.de.genes.counts[, grep("^SD", desMat[,2]))+1)), center = TRUE, scale =

ph1 <- pheatmap(heatmap_data, cluster_cols=TRUE, scale="none",
  color=pal, border_color=NA, fontsize_row=9, fontsize_col=9, cluster_rows=FALSE,
  annotation_col=ann.col, gaps_row=gaps.row)

```



```
save_pheatmap_pdf(filename="plots/heatmap_gg_sd_only.pdf", plot=ph1, width=20, height=20)
```

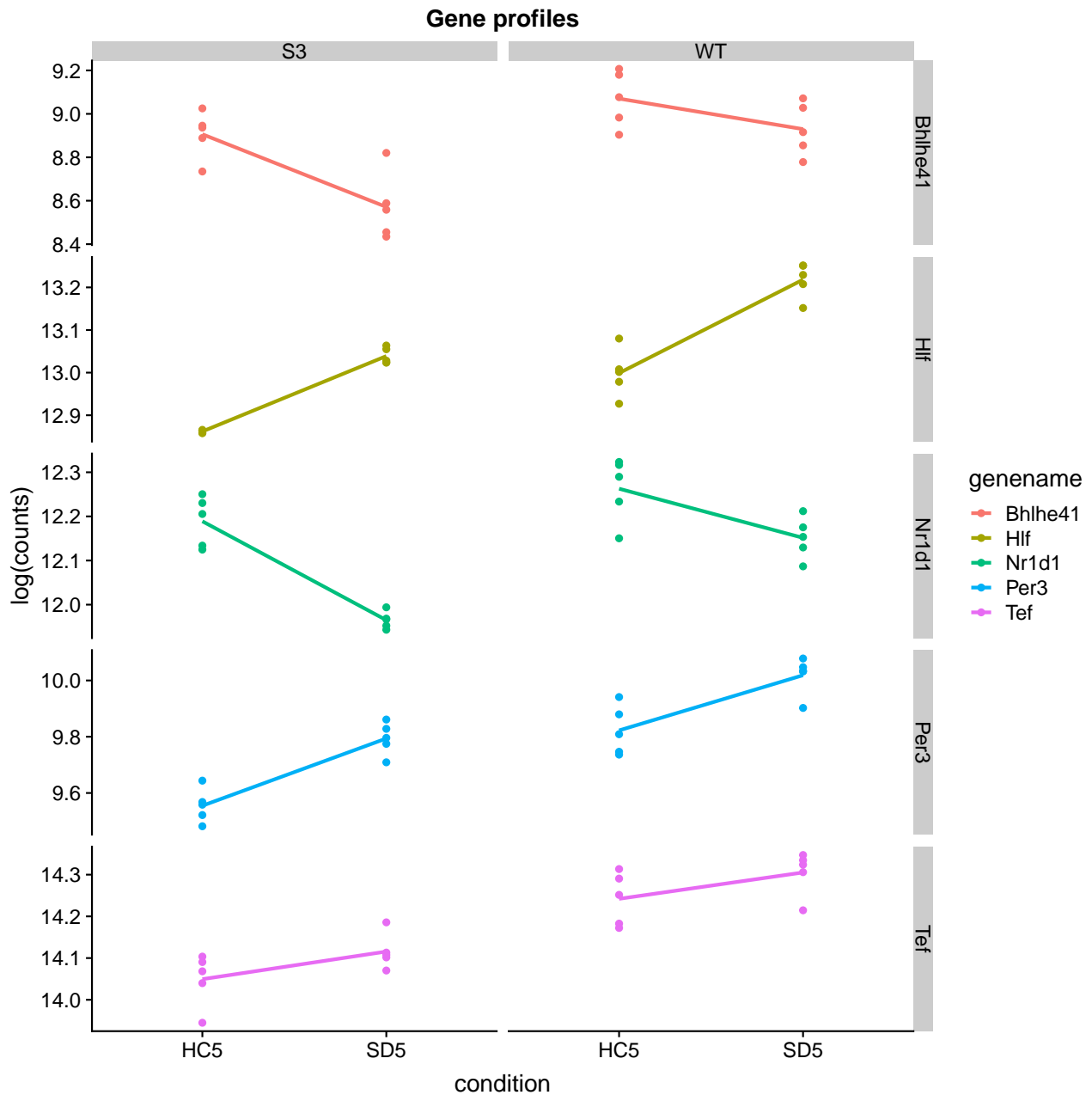
```
## pdf
## 2
```

## Group gene profiles

### Group gene profiles by genotype

```
g <- geneGroupProfileRows(normalized.counts=normExprData, design.matrix=designMatrix,
  gene.names=c("Nr1d1", "Hlf", "Per3", "Bhlhe41", "Tef"),
  res.o=de.genes.symb, show.plot=TRUE, plotly.flag=FALSE, log.flag=TRUE)
```

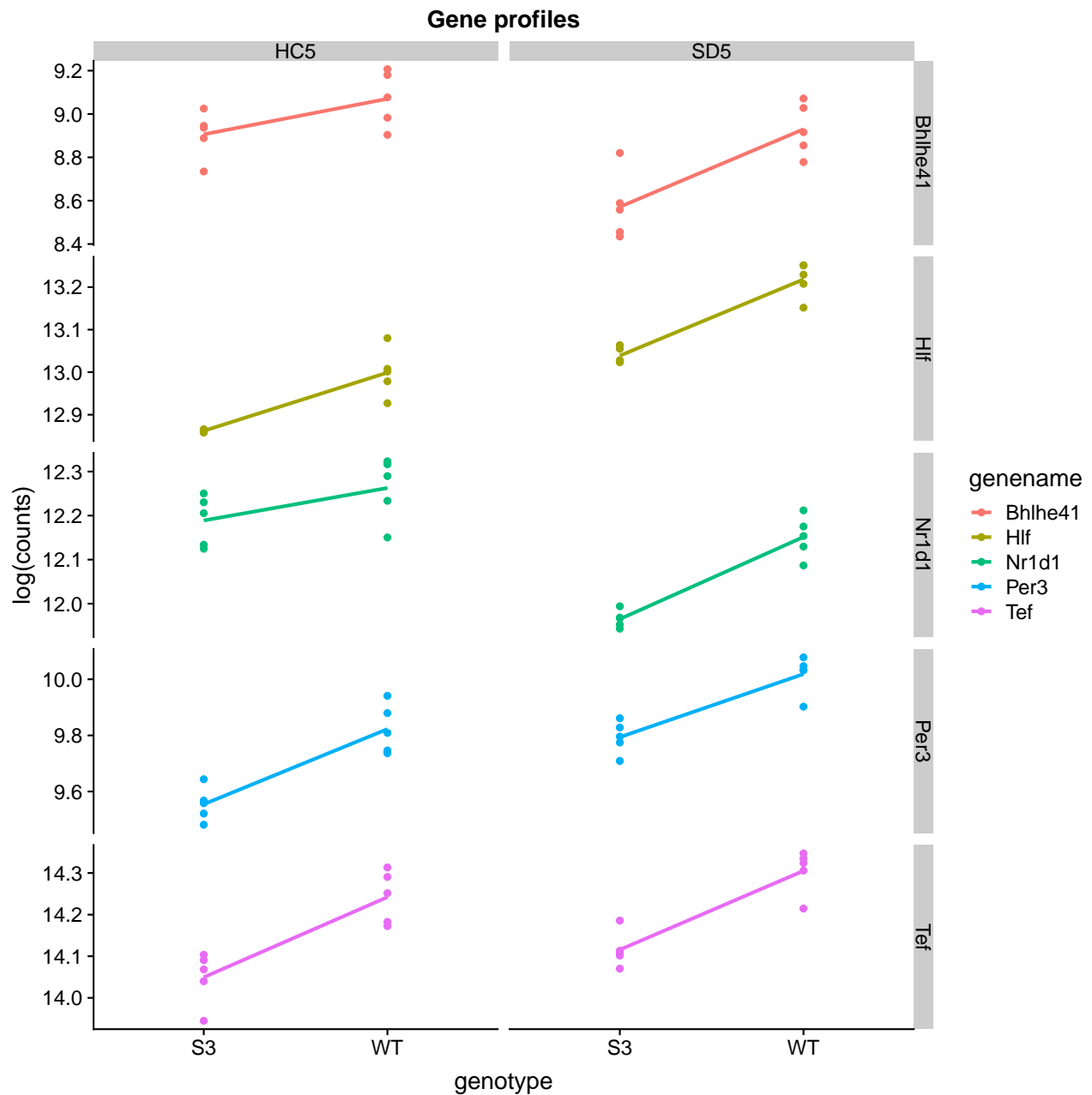
```
print(g)
```



```
save_plot(filename=paste0("plots/", "Nr1d1_Hlf_Per3_Bhlhe41_Tef", "_log_gene_profile_genotype.pdf"), plotly=TRUE,
           base_height=15, base_width=15)
```

Group gene profiles by condition

```
g <- geneGroupProfileRowsRev(normalized.counts=normExprData, design.matrix=designMatrix,
                             gene.names=c("Nr1d1", "Hlf", "Per3", "Bhlhe41", "Tef"),
                             res.o=de.genes.symb, show.plot=TRUE, plotly.flag=FALSE, log.flag=TRUE)
print(g)
```



```
save_plot(filename=paste0("plots/", "Nr1d1_Hlf_Per3_Bhlhe41_Tef", "_log_gene_profile_condition.pdf"), p
          base_height=15, base_width=15)
```

## Circadian Analysis

### Analysis for activity

```
wt <- read_xlsx("data/Activity_analysis_4_R.xlsx", sheet = 1)
mut <- read_xlsx("data/Activity_analysis_4_R.xlsx", sheet = 2)

wt <- wt %>%
```



```

bind_cols(WT.M=rep("WT", nrow(wt)), time = decimal_date(ymd(wt$`Total_revolutions/day`)), .) %>%
gather(mice, activity, -c(1:5)) %>%
mutate(time = time-min(time)) %>%
dplyr::select(-`Total_revolutions/day`)

mut <- mut %>%
  bind_cols(WT.M=rep("M", nrow(mut)), time = decimal_date(ymd(mut$`Total_revolutions/day`)), .) %>%
gather(mice, activity, -c(1:5)) %>%
mutate(time = time-min(time)) %>%
dplyr::select(-`Total_revolutions/day`)

data <- wt %>% bind_rows(mut)

data <- data %>% filter(week>=3)

data$mice <- factor(data$mice, levels= unique(data$mice))
data$time_scaled <- scale(data$time, scale=FALSE)
data$period <- factor(data$period, levels= unique(data$period))
data$WT.M <-factor(data$WT.M, levels=c("WT", "M"))

mod <- lme(activity ~ time_scaled * WT.M, random=~1|mice, data = data)

cat("Estimates, errors and the significance")

## Estimates, errors and the significance
summary(mod)

## Linear mixed-effects model fit by REML
## Data: data
##      AIC      BIC    logLik
## 8681.339 8705.303 -4334.67
##
## Random effects:
## Formula: ~1 | mice
##      (Intercept) Residual
## StdDev:    14936.81 11161.46
##
## Fixed effects: activity ~ time_scaled * WT.M
##              Value Std.Error DF   t-value p-value
## (Intercept)   38778.45   5335.29 388   7.268296  0.0000
## time_scaled  -20486.52  35588.68 388  -0.575647  0.5652
## WT.MM        -20324.26   7810.06  13  -2.602317  0.0219
## time_scaled:WT.MM -289880.69  52096.49 388 -5.564304  0.0000
## Correlation:
##              (Intr) tm_scl WT.MM
## time_scaled      0.000
## WT.MM          -0.683  0.000
## time_scaled:WT.MM 0.000 -0.683  0.000
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -2.69133816 -0.63470177  0.03277689  0.63109234  3.19701990
##

```

```
## Number of Observations: 405
## Number of Groups: 15

cat("Bootstrap confidence intervals for the estimates")

## Bootstrap confidence intervals for the estimates
mod_lmer <- lmer(activity ~ time_scaled * WT.M + (1|mice), data = data)
confint.merMod(mod_lmer, method = "boot", nsim = 999)

##              2.5 %      97.5 %
## .sig01          8955.95  20503.825
## .sigma         10389.64  12032.640
## (Intercept)     28479.16  48421.280
## time_scaled    -87582.69  48954.982
## WT.MM          -36065.65 -4532.543
## time_scaled:WT.MM -384418.16 -187596.734

cat("ANOVA table")

## ANOVA table
anova.lme(mod, type = "marginal", adjustSigma = F)

##              numDF denDF  F-value p-value
## (Intercept)         1   388  52.82812 <.0001
## time_scaled         1   388   0.33137  0.5652
## WT.M                 1    13   6.77206  0.0219
## time_scaled:WT.M     1   388  30.96148 <.0001
```

## Analysis for alpha

```
wt <- read_xlsx("data/Alpha_Activity_analysis_4_R.xlsx", sheet = 1, na = "NA")
mut <- read_xlsx("data/Alpha_Activity_analysis_4_R.xlsx", sheet = 2, na = "NA")

wt <- wt %>%
  bind_cols(WT.M = rep("WT", nrow(wt)), time = decimal_date(ymd(wt$`Total_revolutions/day`)), .)%>%
  gather(mice, alpha, -c(1:5)) %>%
  mutate(time = time-min(time)) %>%
  dplyr::select(-`Total_revolutions/day`)

mut <- mut %>%
  bind_cols(WT.M=rep("M", nrow(mut)), time = decimal_date(ymd(mut$`Total_revolutions/day`)), .)%>%
  gather(mice, alpha, -c(1:5)) %>%
  mutate(time = time-min(time)) %>%
  dplyr::select(-`Total_revolutions/day`)

alpha_data <- wt %>% bind_rows(mut)

alpha_data <- alpha_data %>% filter(week>=3)
alpha_data<- na.omit(alpha_data)

alpha_data$mice <- factor(alpha_data$mice, levels= unique(alpha_data$mice))
alpha_data$time_scaled <- scale(alpha_data$time, scale=FALSE)
alpha_data$period <- factor(alpha_data$period, levels= unique(alpha_data$period))
alpha_data$WT.M <- factor(alpha_data$WT.M, levels=c("WT", "M"))
```

```

alpha_data$alpha <- as.numeric(alpha_data$alpha)

mod1 <- lme(alpha ~ time_scaled * WT.M, random=~1|mice, data = alpha_data, na.action = na.omit)

cat("Estimates, errors and the significance")

## Estimates, errors and the significance
summary(mod1)

## Linear mixed-effects model fit by REML
## Data: alpha_data
##      AIC      BIC    logLik
## 2068.243 2091.978 -1028.121
##
## Random effects:
## Formula: ~1 | mice
##      (Intercept) Residual
## StdDev:    0.6720236 3.405597
##
## Fixed effects: alpha ~ time_scaled * WT.M
##              Value Std.Error DF   t-value p-value
## (Intercept)    10.101010  0.334981 373  30.154013  0.0000
## time_scaled   -16.267322 11.031558 373  -1.474617  0.1412
## WT.MM         -0.714526  0.490361  13  -1.457142  0.1688
## time_scaled:WT.MM  2.360361 16.148547 373   0.146166  0.8839
## Correlation:
##              (Intr) tm_scl WT.MM
## time_scaled      0.000
## WT.MM           -0.683  0.000
## time_scaled:WT.MM 0.000 -0.683  0.000
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -2.73631345 -0.48276146  0.06646042  0.49709145  3.94949030
##
## Number of Observations: 390
## Number of Groups: 15

cat("Bootstrap confidence intervals for the estimates")

## Bootstrap confidence intervals for the estimates
mod1_lmer <- lmer(alpha ~ time_scaled * WT.M + (1|mice), data = alpha_data)
confint.merMod(mod1_lmer, method = "boot", nsim = 999)

##              2.5 %    97.5 %
## .sig01         0.000000  1.126553
## .sigma         3.147126  3.655970
## (Intercept)     9.461287 10.763733
## time_scaled    -38.593893  4.970986
## WT.MM          -1.680165  0.235239
## time_scaled:WT.MM -29.410802 34.100068

cat("ANOVA table")

## ANOVA table

```

```
anova.lme(mod1, type = "marginal", adjustSigma = F)
```

```
##               numDF denDF  F-value p-value
## (Intercept)         1   373 909.2645 <.0001
## time_scaled         1   373  2.1745  0.1412
## WT.M               1    13  2.1233  0.1688
## time_scaled:WT.M    1   373  0.0214  0.8839
```

## Analysis for period

```
wt <- read_xlsx("data/Period_analysis_4_R.xlsx", sheet = 1) %>% gather(mice, value, -1)
wt <- data.frame(WT.M=rep("WT", nrow(wt))) %>% bind_cols(wt)
mut <- read_xlsx("data/Period_analysis_4_R.xlsx", sheet = 2) %>% gather(mice, value, -1)
mut <- data.frame(WT.M=rep("M", nrow(mut))) %>% bind_cols(mut)
```

```
period_data <- wt %>% bind_rows(mut)
period_data$value <- as.numeric(period_data$value)
```

```
mod2 <- lme(value~ week * WT.M, random = ~1|mice, data = period_data)
```

```
cat("Estimates, errors and the significance")
```

```
## Estimates, errors and the significance
```

```
summary(mod2)
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: period_data
```

```
##      AIC   BIC   logLik
```

```
## 309.1875 328.7 -144.5938
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | mice
```

```
##      (Intercept) Residual
```

```
## StdDev:  0.7251103 3.268825
```

```
##
```

```
## Fixed effects: value ~ week * WT.M
```

```
##               Value Std.Error DF   t-value p-value
```

```
## (Intercept)  23.721429  1.265532 39 18.744234  0.0000
```

```
## weekDD_Week_2 -3.381429  1.747260 39 -1.935275  0.0602
```

```
## weekDD_Week_3  2.055714  1.747260 39  1.176536  0.2465
```

```
## weekLD_Week_3  0.208571  1.747260 39  0.119371  0.9056
```

```
## WT.MWT         0.137321  1.732901 13  0.079244  0.9380
```

```
## weekDD_Week_2:WT.MWT 3.251429  2.392535 39  1.358989  0.1820
```

```
## weekDD_Week_3:WT.MWT -2.426964  2.392535 39 -1.014390  0.3166
```

```
## weekLD_Week_3:WT.MWT -0.063571  2.392535 39 -0.026571  0.9789
```

```
## Correlation:
```

```
##               (Intr) wkDD_W_2 wkDD_W_3 wkLD_W_3 WT.MWT wDD_W_2:
```

```
## weekDD_Week_2 -0.690
```

```
## weekDD_Week_3 -0.690  0.500
```

```
## weekLD_Week_3 -0.690  0.500  0.500
```

```
## WT.MWT        -0.730  0.504  0.504  0.504
```

```
## weekDD_Week_2:WT.MWT 0.504 -0.730 -0.365 -0.365 -0.690
```

```
## weekDD_Week_3:WT.MWT  0.504 -0.365   -0.730   -0.365   -0.690   0.500
## weekLD_Week_3:WT.MWT  0.504 -0.365   -0.365   -0.730   -0.690   0.500
##                               wDD_W_3:
## weekDD_Week_2
## weekDD_Week_3
## weekLD_Week_3
## WT.MWT
## weekDD_Week_2:WT.MWT
## weekDD_Week_3:WT.MWT
## weekLD_Week_3:WT.MWT  0.500
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -5.938352181 -0.067963537 -0.001414478  0.093674882  1.778532447
##
## Number of Observations: 60
## Number of Groups: 15

cat("Bootstrap confidence intervals for the estimates")

## Bootstrap confidence intervals for the estimates
mod2_lmer <- lmer(value ~ week * WT.M + (1|mice), data = period_data)
confint.merMod(mod2_lmer, method = "boot", nsim = 999)

##           2.5 %           97.5 %
## .sig01           0.000000  2.14316485
## .sigma           2.513938  3.84935166
## (Intercept)      21.295014  26.08264006
## weekDD_Week_2     -6.777346  0.06162009
## weekDD_Week_3     -1.202367  5.34826111
## weekLD_Week_3     -3.176616  3.68604026
## WT.MWT            -3.217573  3.26535504
## weekDD_Week_2:WT.MWT -1.222345  8.16487237
## weekDD_Week_3:WT.MWT -6.663714  1.92943012
## weekLD_Week_3:WT.MWT -4.658113  4.21914440

cat("ANOVA table")

## ANOVA table
anova.lme(mod2, type = "marginal", adjustSigma = F)

##           numDF denDF  F-value p-value
## (Intercept)      1    39 351.3463  <.0001
## week              3    39   3.3611  0.0282
## WT.M              1    13   0.0063  0.9380
## week:WT.M         3    39   1.9008  0.1454
```

## Analysis for Spectral Data

```
# GAM plots
library(mgcv)

data<-read_xlsx("data/BL_spectral.xlsx")
```

```

data <- data %>% gather(Hertz, value, -c(1:3))
data <- data %>%
  mutate(GT=replace(GT,GT == 1, "WT")) %>%
  mutate(GT=replace(GT,GT == 2, "MT")) %>%
  mutate(LD=replace(LD,LD == 1, "LIGHT")) %>%
  mutate(LD=replace(LD,LD == 2, "DARK")) %>%
  mutate(hz= as.numeric(Hertz)) %>%
  mutate(STATE = factor(STATE, levels = unique(STATE))) %>%
  mutate(GT = factor(GT, levels = unique(GT))) %>%
  mutate(LD = factor(LD, levels = unique(LD))) %>%
  mutate(value = replace(value,value == -99, NA))

temp<-data %>% filter(STATE == "WAKEFULNESS" & LD == "LIGHT")
index <-paste(data$STATE, data$LD, sep = "")
index_lev <- unique(index)

layout(matrix(seq_len(6), nrow = 3, ncol = 2, byrow = TRUE))
shadow_col <- c(rgb(109, 109, 109, max = 255, alpha = 80),
               rgb(244, 66, 66, max = 255, alpha = 80))

for(this_index in index_lev) {
  state <- unique(data[index == this_index, ][, 1])
  state <- as.character(unlist(state))
  light <- unique(data[index == this_index, ][, 3])
  light <- as.character(unlist(light))

  temp2 <- data[index == this_index, ]
  plot(x = temp2$hz, y = temp2$value, type = "n",
       ylab = "% of Total Power", ylim = c(0,20),
       xlab = 'Hertz', lwd = 3, cex = 1.2,
       main = paste0(state, "-", light), axes = FALSE)
  axis(1, at = seq(0, 20, by = 5), las = 1, pos = 0, lwd = 3)
  axis(2, at = seq(0, 15, by = 3), las = 2, pos = 0, lwd = 3)

  mod <- list(wt = gam(value~s(hz), data = temp2[temp2$GT == "WT",]),
             mt = gam(value~s(hz), data = temp2[temp2$GT == "MT",]))

  for(i in seq_along(mod)) {
    ss <- seq(min(temp2$hz) + 0.1, max(temp2$hz) - 0.1, 0.1)
    pred <- predict(mod[[i]], data.frame(hz = ss), se = TRUE)
    fit <- pred$fit
    se <- pred$se.fit
    lower <- fit - 1.96 * se
    upper <- fit + 1.96 * se
    to_plot <- data.frame(hz = ss, fit, lower, upper)

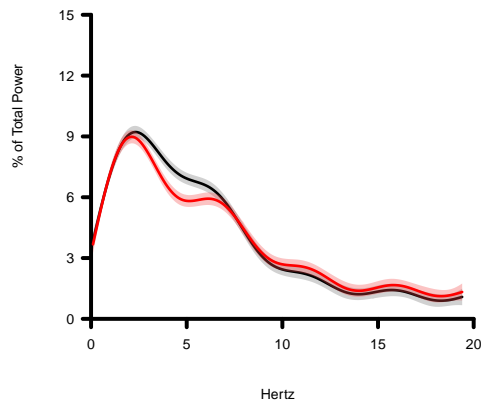
    polygon(c(to_plot$hz, rev(to_plot$hz)),
           c(to_plot$lower, rev(to_plot$upper)),
           col = shadow_col[i],
           border = NA)

    lines(to_plot$hz, fit, lwd=2, col = c("black", "red")[i])
  }
}

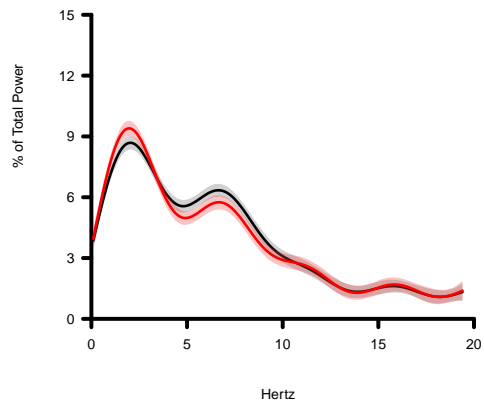
```

```
legend(x = "topright",horiz = TRUE, c("MT", "WT"),
      pch = c(NA, NA), col = c("black", "red"),
      inset = c(0, -0.08), cex = 1, bty = 'n',
      box.lty = c('NA', 'NA'), lwd = 3 )
}
```

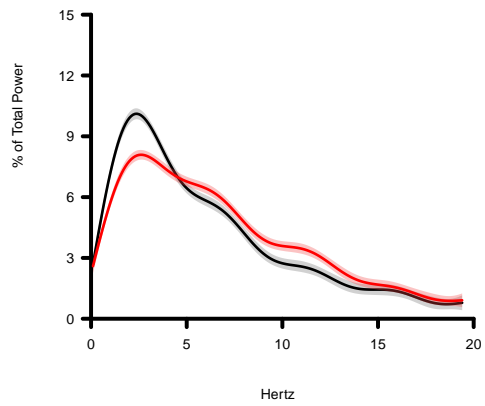
**WAKEFULNESS-LIGHT**



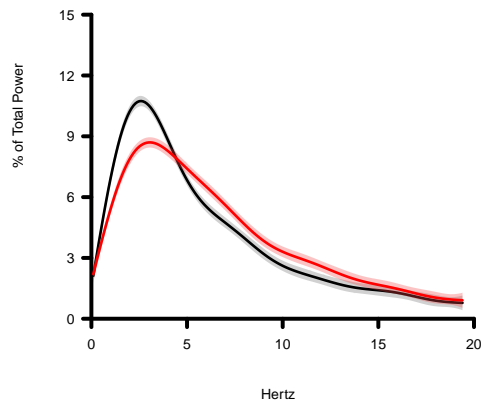
**WAKEFULNESS-DARK**



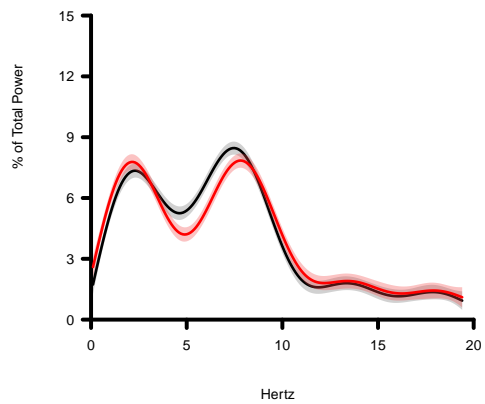
**NREM-LIGHT**



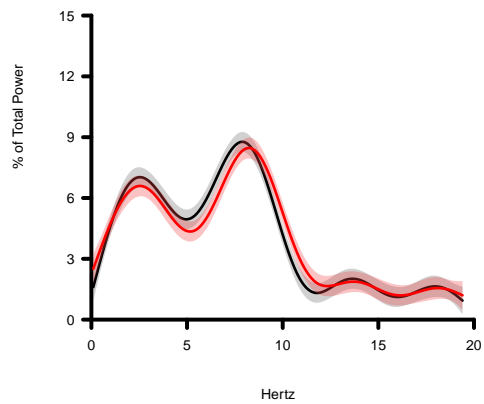
**NREM-DARK**



**REM-LIGHT**



**REM-DARK**





## Session Info

```
sessionInfo()
```

```
## R version 3.4.3 (2017-11-30)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.5 LTS
##
## Matrix products: default
## BLAS: /usr/local/lib/R/lib/libRblas.so
## LAPACK: /usr/local/lib/R/lib/libRlapack.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_GB.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_GB.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_GB.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4      parallel  stats      graphics  grDevices  utils      datasets
## [8] methods     base
##
## other attached packages:
##  [1] mgcv_1.8-24          bindrcpp_0.2.2
##  [3] dendextend_1.8.0     pheatmap_1.0.10
##  [5] clusterExperiment_1.4.0 gplots_3.0.1
##  [7] plyr_1.8.4           org.Mm.eg.db_3.5.0
##  [9] AnnotationDbi_1.40.0 readxl_1.1.0
## [11] lme4_1.1-18-1        Matrix_1.2-14
## [13] nlme_3.1-137         tidyr_0.8.1
## [15] dplyr_0.7.6          lubridate_1.7.4
## [17] cowplot_0.9.3        RUVSeq_1.12.0
## [19] edgeR_3.20.9         limma_3.34.9
## [21] EDASeq_2.12.0        ShortRead_1.36.1
## [23] GenomicAlignments_1.14.2 SummarizedExperiment_1.8.1
## [25] DelayedArray_0.4.1   matrixStats_0.53.1
## [27] Rsamtools_1.30.0     GenomicRanges_1.30.3
## [29] GenomeInfoDb_1.14.0  Biostrings_2.46.0
## [31] XVector_0.18.0       IRanges_2.12.0
## [33] S4Vectors_0.16.0     BiocParallel_1.12.0
## [35] Biobase_2.38.0       BiocGenerics_0.24.0
## [37] plotly_4.7.1         ggplot2_3.0.0
##
## loaded via a namespace (and not attached):
##  [1] uuid_0.1-2           backports_1.1.2       aroma.light_3.8.0
##  [4] NMF_0.21.0           lazyeval_0.2.1        splines_3.4.3
##  [7] rnc1_0.8.2           NOISeq_2.22.1         gridBase_0.4-7
## [10] digest_0.6.15        foreach_1.4.4         htmltools_0.3.6
## [13] viridis_0.5.1        gdata_2.18.0          magrittr_1.5
## [16] memoise_1.1.0        cluster_2.0.7-1       doParallel_1.0.11
## [19] annotate_1.56.2      R.utils_2.6.0         prettyunits_1.0.2
```

## [22] colorspace_1.3-2	blob_1.1.1	crayon_1.3.4
## [25] RCurl_1.95-4.11	jsonlite_1.5	genefilter_1.60.0
## [28] phylobase_0.8.4	bindr_0.1.1	survival_2.42-6
## [31] iterators_1.0.10	ape_5.1	glue_1.3.0
## [34] registry_0.5	gtable_0.2.0	zlibbioc_1.24.0
## [37] kernlab_0.9-26	DEoptimR_1.0-8	prabclus_2.2-6
## [40] scales_0.5.0.9000	DESeq_1.30.0	mvtnorm_1.0-8
## [43] DBI_1.0.0	rngtools_1.3.1	bibtex_0.4.2
## [46] Rcpp_0.12.17	viridisLite_0.3.0	xtable_1.8-2
## [49] progress_1.2.0	bit_1.1-14	mclust_5.4.1
## [52] htmlwidgets_1.2	httr_1.3.1	RColorBrewer_1.1-2
## [55] fpc_2.1-11	modeltools_0.2-22	flexmix_2.3-14
## [58] pkgconfig_2.0.1	XML_3.98-1.12	R.methodsS3_1.7.1
## [61] nnet_7.3-12	locfit_1.5-9.1	howmany_0.3-1
## [64] tidyselect_0.2.4	labeling_0.3	rlang_0.2.1
## [67] reshape2_1.4.3	munsell_0.5.0	cellranger_1.1.0
## [70] tools_3.4.3	RSQLite_2.1.1	ade4_1.7-11
## [73] evaluate_0.11	stringr_1.3.1	yaml_2.1.19
## [76] knitr_1.20	bit64_0.9-7	robustbase_0.93-1.1
## [79] caTools_1.17.1	purrr_0.2.5	whisker_0.3-2
## [82] R.oo_1.22.0	xml2_1.2.0	biomaRt_2.34.2
## [85] compiler_3.4.3	tibble_1.4.2	statmod_1.4.30
## [88] geneplotter_1.56.0	RNeXML_2.1.1	stringi_1.2.3
## [91] GenomicFeatures_1.30.3	RSpectra_0.13-1	trimcluster_0.1-2
## [94] lattice_0.20-35	nloptr_1.0.4	pillar_1.3.0
## [97] data.table_1.11.4	bitops_1.0-6	rtracklayer_1.38.3
## [100] R6_2.2.2	latticeExtra_0.6-28	hwriter_1.3.2
## [103] RMySQL_0.10.15	gridExtra_2.3	KernSmooth_2.23-15
## [106] codetools_0.2-15	boot_1.3-20	MASS_7.3-50
## [109] gtools_3.8.1	assertthat_0.2.0	pkgmaker_0.27
## [112] rprojroot_1.3-2	withr_2.1.2	locfdr_1.1-8
## [115] GenomeInfoDbData_1.0.0	diptest_0.75-7	hms_0.4.2
## [118] grid_3.4.3	class_7.3-14	minqa_1.2.4
## [121] rmarkdown_1.10		