

Lab 15

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Bioconductor and DESeq2 Setup

```
library(BiocManager)
library(DESeq2)
library(dplyr)
library(ggplot2)
library("AnnotationDbi")
library("org.Hs.eg.db")
library("EnhancedVolcano")
library(pathview)
library(gage)
library(gageData)
```

Import countData and colData

```
# Read in the scaled counts data as counts and the metadata data as metadata
counts <- read.csv("airway_scaledcounts.csv", row.names = 1)
metadata <- read.csv("airway_metadata.csv")

head(counts)

##          SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516
## ENSG00000000003     723      486      904      445     1170
## ENSG00000000005      0       0       0       0       0
## ENSG00000000419    467      523      616      371     582
## ENSG00000000457    347      258      364      237     318
## ENSG00000000460     96       81       73       66     118
## ENSG00000000938     0       0       1       0       2
##          SRR1039517 SRR1039520 SRR1039521
## ENSG00000000003    1097      806      604
## ENSG00000000005      0       0       0
## ENSG00000000419    781      417      509
## ENSG00000000457    447      330      324
## ENSG00000000460     94      102      74
## ENSG00000000938     0       0       0

head(metadata)

##      id   dex celltype geo_id
## 1 SRR1039508 control N61311 GSM1275862
## 2 SRR1039509 treated N61311 GSM1275863
## 3 SRR1039512 control N052611 GSM1275866
## 4 SRR1039513 treated N052611 GSM1275867
```

```
## 5 SRR1039516 control N080611 GSM1275870
## 6 SRR1039517 treated N080611 GSM1275871
```

Check if metadata and count data match:

```
all(metadata$id == colnames(counts))
```

```
## [1] TRUE
```

```
nrow(counts)
```

[Q1]: How many genes are in this data set?

```
## [1] 38694
```

```
table(metadata$dex)
```

[Q2]: How many ‘control’ cell lines do we have?

```
##
## control treated
##      4      4
```

Toy Differential Gene Expression

```
control_md <- metadata[metadata$dex == "control",]
control_counts <- counts[,control_md$id]
control_mean <- rowSums(control_counts)/4
head(control_mean)
```

```
## ENSG0000000003 ENSG0000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
##         900.75          0.00        520.50        339.75        97.25
## ENSG0000000938
##         0.75
```

Same as above, but using dplyr.

```
control_md <- metadata %>% filter(dex=="control")
control_counts <- counts %>% dplyr::select(control_md$id)
control_mean <- rowSums(control_counts)/4
head(control_mean)
```

```
## ENSG0000000003 ENSG0000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
##         900.75          0.00        520.50        339.75        97.25
## ENSG0000000938
##         0.75
```

[Q3]: How would you make the above code in either approach more robust? Change rowSums to rowMeans to avoid hard-coding number of samples.

```
control_md <- metadata %>% filter(dex=="control")
control_counts <- counts %>% dplyr::select(control_md$id)
control_mean <- rowMeans(control_counts)
head(control_mean)
```

```

## ENSG00000000003 ENSG00000000005 ENSG000000000419 ENSG000000000457 ENSG000000000460
##          900.75           0.00        520.50       339.75         97.25
## ENSG000000000938
##          0.75

```

```

treated_md <- metadata %>% filter(dex=="treated")
treated_counts <- counts %>% dplyr::select(treated_md$id)
treated_mean <- rowMeans(treated_counts)
head(treated_mean)

```

[Q4]: Follow the same procedure for the treated samples.

```

## ENSG00000000003 ENSG00000000005 ENSG000000000419 ENSG000000000457 ENSG000000000460
##          658.00           0.00        546.00       316.50         78.75
## ENSG000000000938
##          0.00
mean_counts <- data.frame("control" = control_mean, "treated" = treated_mean)
colSums(mean_counts)

##   control   treated
## 23005324 22196524

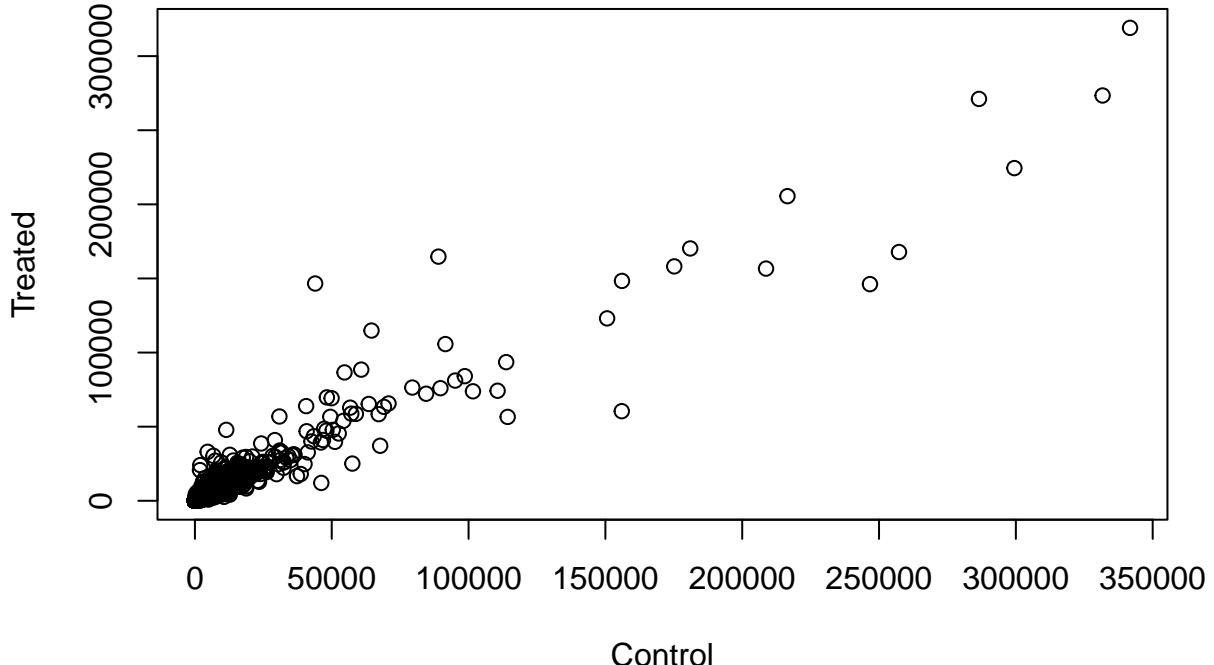
```

```

plot(x = mean_counts$control, y = mean_counts$treated,
      xlab = "Control", ylab = "Treated")

```

[Q5a]: Create a scatter plot showing the mean of the treated samples against the mean of the con-



trol samples.

Control

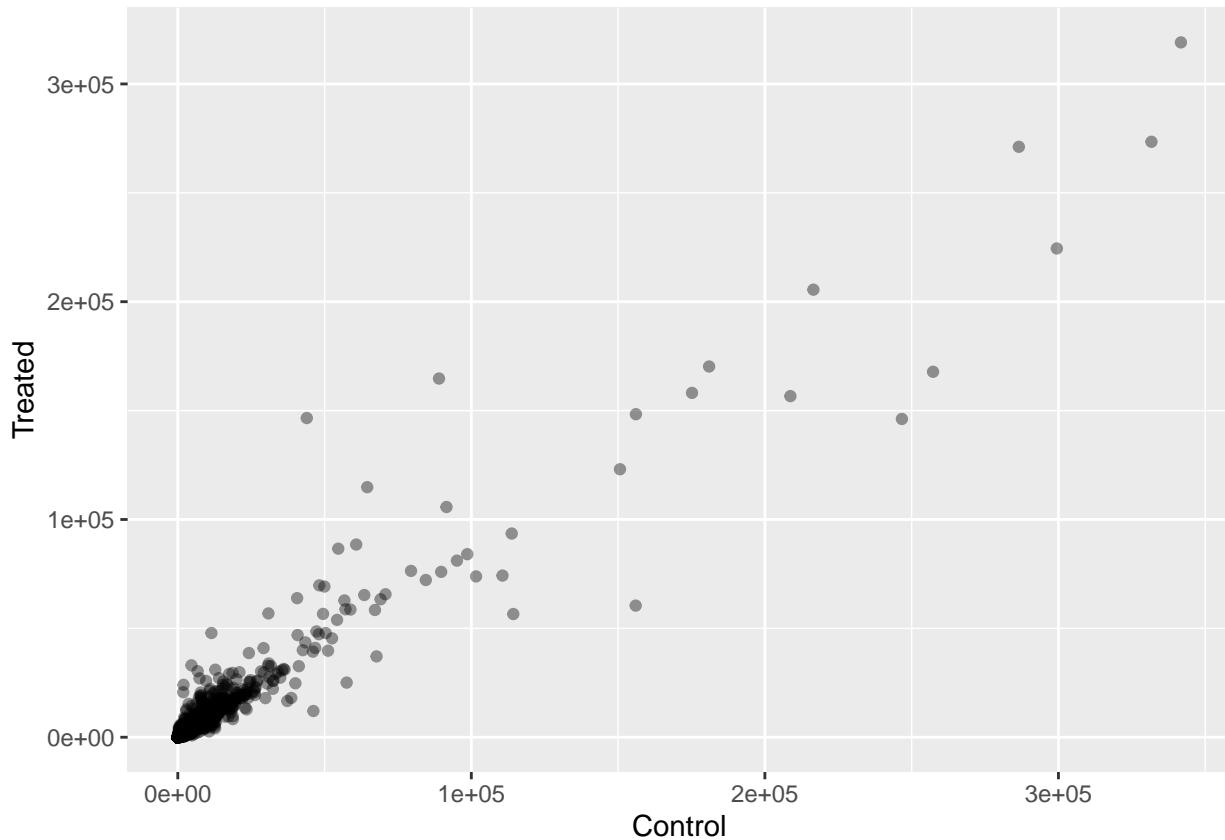
[Q5b]: You could also use the ggplot2 package to make this figure. What geom_?() function would you use for this plot? You would use geom_point().

```

ggplot(mean_counts) +
  aes(x = control, y = treated) +

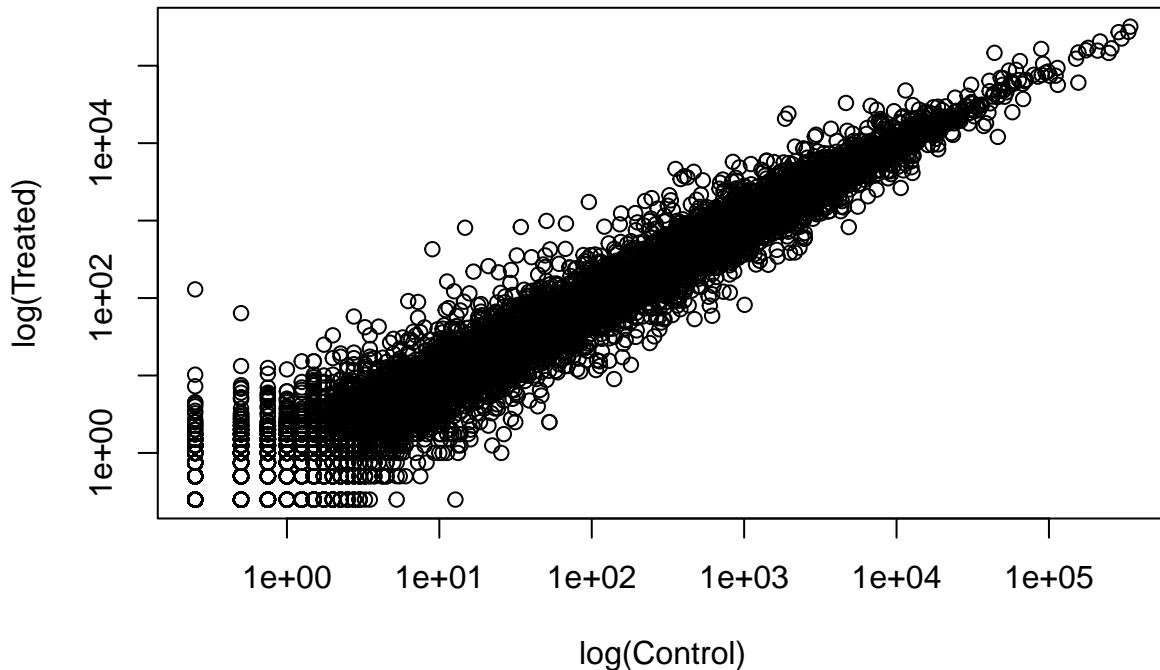
```

```
geom_point(alpha = 0.4) +  
  labs(x = "Control", y = "Treated")
```



[Q6]: Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this? The argument is `log`.

```
plot(x = mean_counts$control, y = mean_counts$treated,  
      xlab = "log(Control)", ylab = "log(Treated)", log = "yx")
```



```
mean_counts$log2fc <- log2(mean_counts$treated / mean_counts$control)
head(mean_counts)
```

```
##           control   treated      log2fc
## ENSG000000000003  900.75  658.00 -0.45303916
## ENSG000000000005    0.00     0.00       NaN
## ENSG00000000419   520.50  546.00  0.06900279
## ENSG00000000457   339.75  316.50 -0.10226805
## ENSG00000000460    97.25   78.75 -0.30441833
## ENSG00000000938     0.75     0.00      -Inf
```

Remove the rows with zero values present.

```
zeros_rows <- which(mean_counts[, c(1, 2)] == 0, arr.ind = TRUE)
remove_rows <- unique(zeros_rows[, 1])
nonzero_counts <- mean_counts[-remove_rows,]
head(nonzero_counts)
```

```
##           control   treated      log2fc
## ENSG000000000003  900.75  658.00 -0.45303916
## ENSG00000000419   520.50  546.00  0.06900279
## ENSG00000000457   339.75  316.50 -0.10226805
## ENSG00000000460    97.25   78.75 -0.30441833
## ENSG00000000971  5219.00 6687.50  0.35769358
## ENSG00000001036 2327.00 1785.75 -0.38194109
```

[Q7]: What is the purpose of the arr.ind argument in the which() function call above? Why would we then take the first column of the output and need to call the unique() function?
The purpose of arr.ind is to return the array indices of for TRUE values, rows and columns. The first column contains the rows which were TRUE for a given column, specified in the second column of this output. Therefore, unique will pull out all rows which have a zero in either or both columns.

```
up_indx <- nonzero_counts$log2fc > 2
down_indx <- nonzero_counts$log2fc < -2
```

```
sum(up_indx)
```

[Q8]: Using the up_indx vector, can you determine how many up regulated genes we have at the greater than 2 fc level?

```
## [1] 250
```

```
sum(down_indx)
```

[Q9]: Using the down_indx vector, can you determine how many down regulated genes we have at the greater than 2 fc level?

```
## [1] 367
```

[Q10]: Do you trust these results? Why or why not? I do not trust these results since there is no accountability for genes with a high variance in gene expression data. Means do not represent the data well enough to rely on solely, statistics would be needed to identify whether the fold change is significant or is observed by chance.

DESeq2 Analysis

```
citation("DESeq2")
```

```
##  
##   Love, M.I., Huber, W., Anders, S. Moderated estimation of fold change  
##   and dispersion for RNA-seq data with DESeq2 Genome Biology 15(12):550  
##   (2014)  
##  
## A BibTeX entry for LaTeX users is  
##  
##   @Article{,  
##     title = {Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2},  
##     author = {Michael I. Love and Wolfgang Huber and Simon Anders},  
##     year = {2014},  
##     journal = {Genome Biology},  
##     doi = {10.1186/s13059-014-0550-8},  
##     volume = {15},  
##     issue = {12},  
##     pages = {550},  
##   }
```

Importing Data

```
Dds <- DESeqDataSetFromMatrix(countData = counts,  
                                colData = metadata,  
                                design = ~dex)
```

```
## converting counts to integer mode  
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in  
## design formula are characters, converting to factors
```

```
Dds

## class: DESeqDataSet
## dim: 38694 8
## metadata(1): version
## assays(1): counts
## rownames(38694): ENSG00000000003 ENSG00000000005 ... ENSG00000283120
##   ENSG00000283123
## rowData names(0):
## colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
## colData names(4): id dex celltype geo_id
```

DESeq Analysis

```
# Must run DESeq first
# results(Dds)

Dds <- DESeq(Dds)

## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## final dispersion estimates
## fitting model and testing
```

Getting Results

```

res <- results(Dds)
res

## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 38694 rows and 6 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue
## <numeric>    <numeric>    <numeric>    <numeric>    <numeric>
## ENSG000000000003  747.1942   -0.3507030  0.168246 -2.084470 0.0371175
## ENSG000000000005  0.0000      NA          NA          NA          NA
## ENSG000000000419  520.1342   0.2061078  0.101059  2.039475 0.0414026
## ENSG000000000457  322.6648   0.0245269  0.145145  0.168982 0.8658106
## ENSG000000000460  87.6826   -0.1471420  0.257007 -0.572521 0.5669691
## ...
## ...          ...
## ENSG0000283115  0.000000      NA          NA          NA          NA
## ENSG0000283116  0.000000      NA          NA          NA          NA
## ENSG0000283119  0.000000      NA          NA          NA          NA
## ENSG0000283120  0.974916   -0.668258  1.69456 -0.394354 0.693319
## ENSG0000283123  0.000000      NA          NA          NA          NA
##           padj
## <numeric>
## ENSG000000000003  0.163035
## ENSG000000000005  NA

```

```

## ENSG00000000419  0.176032
## ENSG00000000457  0.961694
## ENSG00000000460  0.815849
## ...
## ENSG00000283115    NA
## ENSG00000283116    NA
## ENSG00000283119    NA
## ENSG00000283120    NA
## ENSG00000283123    NA

summary(res)

##
## out of 25258 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)      : 1563, 6.2%
## LFC < 0 (down)    : 1188, 4.7%
## outliers [1]       : 142, 0.56%
## low counts [2]     : 9971, 39%
## (mean count < 10)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results

```

Change the alpha cut-off to 0.05 rather than the default 0.1.

```

res_005 <- results(Dds, alpha = 0.05)
summary(res_005)

```

```

##
## out of 25258 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)      : 1236, 4.9%
## LFC < 0 (down)    : 933, 3.7%
## outliers [1]       : 142, 0.56%
## low counts [2]     : 9033, 36%
## (mean count < 6)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results

```

Adding Annotation Data

Available annotation formats.

```
columns(org.Hs.eg.db)
```

```

## [1] "ACCCNUM"        "ALIAS"          "ENSEMBL"         "ENSEMLPROT"      "ENSEMLTRANS"
## [6] "ENTREZID"       "ENZYME"         "EVIDENCE"        "EVIDENCEALL"    "GENENAME"
## [11] "GENETYPE"       "GO"              "GOALL"           "IPI"             "MAP"
## [16] "OMIM"            "ONTOLOGY"        "ONTOLOGYALL"    "PATH"            "PFAM"
## [21] "PMID"           "PROSITE"         "REFSEQ"          "SYMBOL"          "UCSCKG"
## [26] "UNIPROT"

res$symbol <- mapIds(org.Hs.eg.db,
                      keys = row.names(res),
                      keytype = "ENSEMBL",
                      column = "SYMBOL",

```

```

        mutliVals = "first")

## 'select()' returned 1:many mapping between keys and columns
head(res)

## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 6 rows and 7 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue
##           <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG000000000003 747.194195     -0.3507030  0.168246 -2.084470 0.0371175
## ENSG000000000005  0.000000          NA         NA       NA       NA
## ENSG000000000419 520.134160      0.2061078  0.101059  2.039475 0.0414026
## ENSG000000000457 322.664844      0.0245269  0.145145  0.168982 0.8658106
## ENSG000000000460 87.682625      -0.1471420  0.257007 -0.572521 0.5669691
## ENSG000000000938 0.319167      -1.7322890  3.493601 -0.495846 0.6200029
##           padj      symbol
##           <numeric> <character>
## ENSG000000000003 0.163035      TSPAN6
## ENSG000000000005          NA      TNMD
## ENSG000000000419 0.176032      DPM1
## ENSG000000000457 0.961694      SCYL3
## ENSG000000000460 0.815849      C1orf112
## ENSG000000000938          NA      FGR

```

```

res$entrez <- mapIds(org.Hs.eg.db,
                      keys = row.names(res),
                      keytype = "ENSEMBL",
                      column = "ENTREZID",
                      mutliVals = "first")

```

[Q11]: Run the `mapIds()` function [three] more times to add the Entrez ID and UniProt accession and GENENAME as new columns called `res$entrez`, `res$uniprot` and `res$genename`.

```
## 'select()' returned 1:many mapping between keys and columns
```

```

res$uniprot <- mapIds(org.Hs.eg.db,
                      keys = row.names(res),
                      keytype = "ENSEMBL",
                      column = "UNIPROT",
                      mutliVals = "first")

```

```
## 'select()' returned 1:many mapping between keys and columns
```

```

res$gene_name <- mapIds(org.Hs.eg.db,
                        keys = row.names(res),
                        keytype = "ENSEMBL",
                        column = "GENENAME",
                        mutliVals = "first")

```

```
## 'select()' returned 1:many mapping between keys and columns
```

```
head(res)
```

```

## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control

```

```

## DataFrame with 6 rows and 10 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue
##           <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG00000000003 747.194195 -0.3507030  0.168246 -2.084470 0.0371175
## ENSG00000000005  0.000000    NA        NA        NA        NA
## ENSG00000000419 520.134160  0.2061078  0.101059  2.039475 0.0414026
## ENSG00000000457 322.664844  0.0245269  0.145145  0.168982 0.8658106
## ENSG00000000460 87.682625 -0.1471420  0.257007 -0.572521 0.5669691
## ENSG00000000938 0.319167 -1.7322890  3.493601 -0.495846 0.6200029
##           padj      symbol      entrez      uniprot
##           <numeric> <character> <character> <character>
## ENSG00000000003 0.163035   TSPAN6      7105    AOA024RCI0
## ENSG00000000005  NA        TNMD       64102   Q9H2S6
## ENSG00000000419 0.176032   DPM1       8813    060762
## ENSG00000000457 0.961694   SCYL3      57147   Q8IZE3
## ENSG00000000460 0.815849   C1orf112    55732   AOA024R922
## ENSG00000000938 NA        FGR        2268    P09769
##           gene_name
##           <character>
## ENSG00000000003      tetraspanin 6
## ENSG00000000005      tenomodulin
## ENSG00000000419      dolichyl-phosphate m..
## ENSG00000000457      SCY1 like pseudokina..
## ENSG00000000460      chromosome 1 open re..
## ENSG00000000938      FGR proto-oncogene, ..

```

Reorder the results so highly significant differential expression observations are listed first.

```

p_val_order <- order(res$padj)
head(res[p_val_order,])

```

```

## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 6 rows and 10 columns
##           baseMean log2FoldChange      lfcSE      stat     pvalue
##           <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG00000152583 954.771      4.36836  0.2371268  18.4220 8.74490e-76
## ENSG00000179094 743.253      2.86389  0.1755693  16.3120 8.10784e-60
## ENSG00000116584 2277.913     -1.03470  0.0650984 -15.8944 6.92855e-57
## ENSG00000189221 2383.754      3.34154  0.2124058  15.7319 9.14433e-56
## ENSG00000120129 3440.704      2.96521  0.2036951  14.5571 5.26424e-48
## ENSG00000148175 13493.920     1.42717  0.1003890  14.2164 7.25128e-46
##           padj      symbol      entrez      uniprot
##           <numeric> <character> <character> <character>
## ENSG00000152583 1.32441e-71   SPARCL1     8404   AOA024RDE1
## ENSG00000179094 6.13966e-56    PER1       5187   Q15534
## ENSG00000116584 3.49776e-53   ARHGEF2     9181   Q92974
## ENSG00000189221 3.46227e-52    MAOA      4128    P21397
## ENSG00000120129 1.59454e-44   DUSP1      1843    B4DU40
## ENSG00000148175 1.83034e-42    STOM      2040   F8VSL7
##           gene_name
##           <character>
## ENSG00000152583      SPARC like 1
## ENSG00000179094      period circadian reg..
## ENSG00000116584      Rho/Rac guanine nucl..

```

```
## ENSG00000189221      monoamine oxidase A
## ENSG00000120129 dual specificity pho..
## ENSG00000148175          stomatin
```

Write the DESeq2 results to disc.

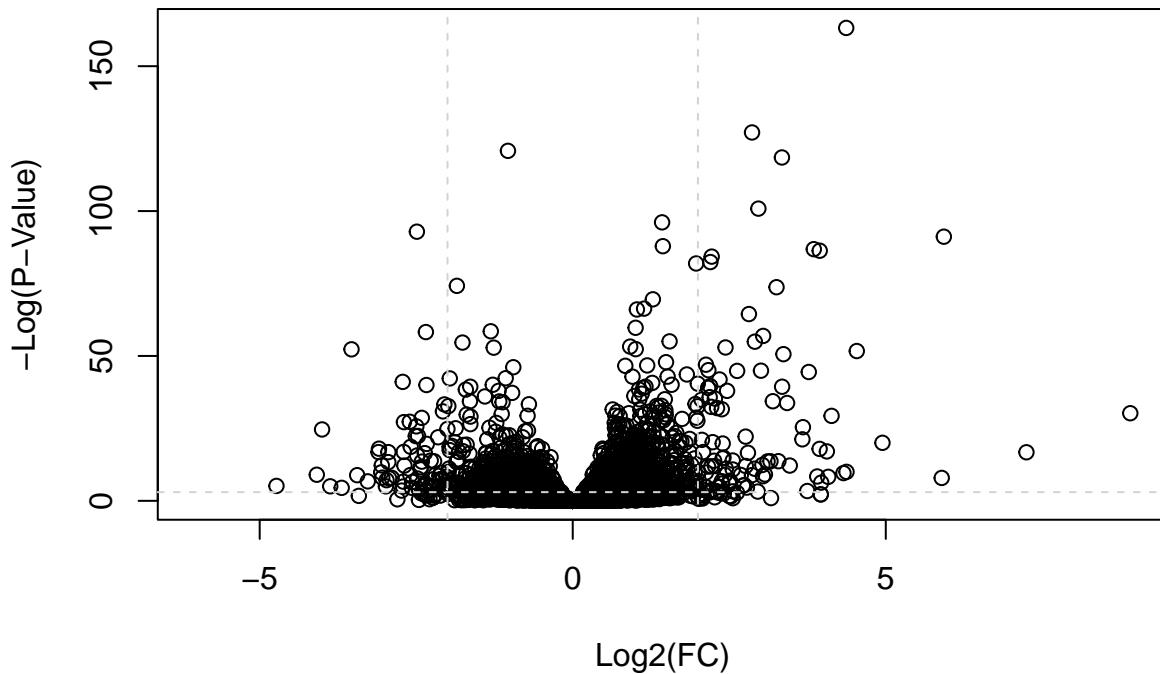
```
write.csv(res[p_val_order,], "deseq_results.csv")
```

Data Visualization

Volcano Plots

```
plot(x = res$log2FoldChange, y = -log(res$padj),
      xlab = "Log2(FC)", ylab = "-Log(P-Value)")

abline(v=c(-2,2), col="lightgray", lty=2)
abline(h=-log(0.05), col="lightgray", lty=2)
```

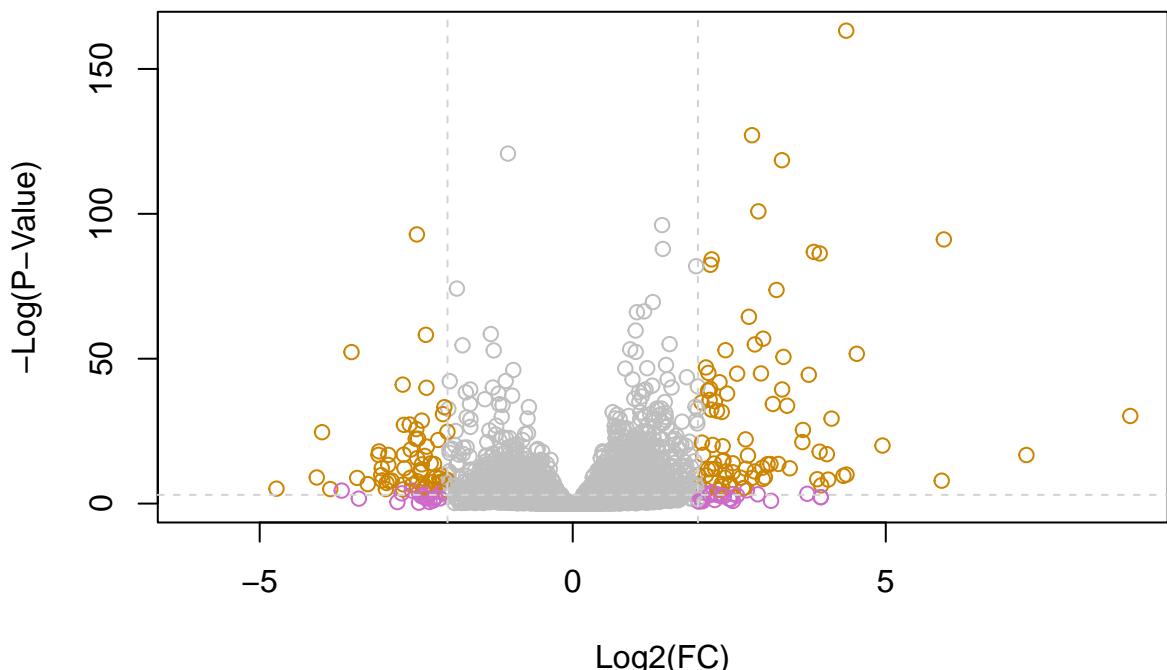


```
my_colors <- rep("gray", nrow(res))
my_colors[abs(res$log2FoldChange) > 2] <- "orchid3"
```

```
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
my_colors[ inds ] <- "orange3"
```

```
plot(x = res$log2FoldChange, y = -log(res$padj),
      xlab = "Log2(FC)", ylab = "-Log(P-Value)",
      col = my_colors)
```

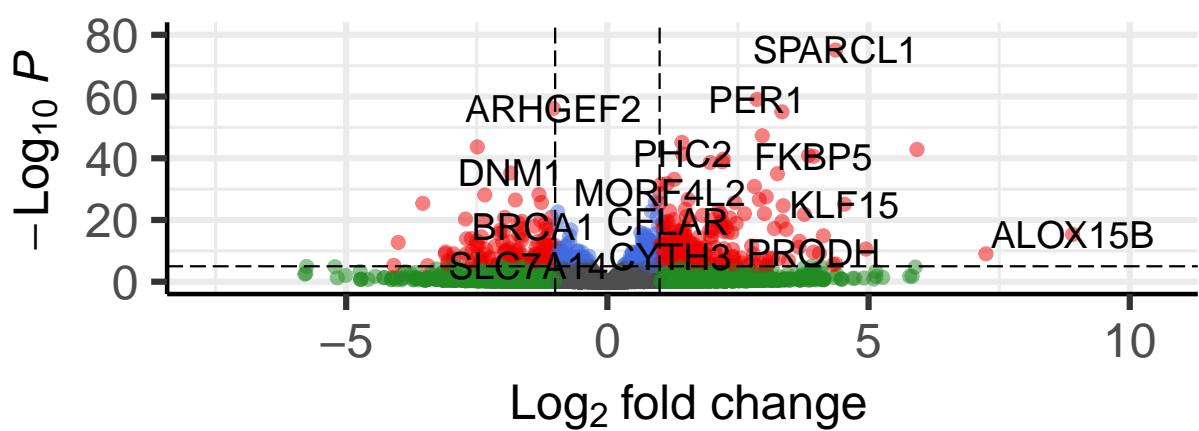
```
abline(v=c(-2,2), col="lightgray", lty=2)
abline(h=-log(0.05), col="lightgray", lty=2)
```



Volcano plot

EnhancedVolcano

● NS ● Log₂ FC ● p-value ● p – value and log₂ FC



Pathway Analysis

```
data(kegg.sets.hs)

head(kegg.sets.hs, 2)

## $`hsa00232 Caffeine metabolism`
## [1] "10"    "1544"   "1548"   "1549"   "1553"   "7498"   "9"
##
## $`hsa00983 Drug metabolism - other enzymes`
## [1] "10"    "1066"   "10720"  "10941"  "151531"  "1548"   "1549"   "1551"
## [9] "1553"  "1576"   "1577"   "1806"   "1807"   "1890"   "221223"  "2990"
## [17] "3251"  "3614"   "3615"   "3704"   "51733"   "54490"  "54575"   "54576"
## [25] "54577" "54578"  "54579"  "54600"  "54657"   "54658"  "54659"   "54963"
## [33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"
## [41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549"
## [49] "8824"   "8833"   "9"      "978"

foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)

##          7105        64102        8813        57147        55732        2268
## -0.35070302           NA  0.20610777  0.02452695 -0.14714205 -1.73228897

kegg_res = gage(foldchanges, gsets=kegg.sets.hs)

attributes(kegg_res)

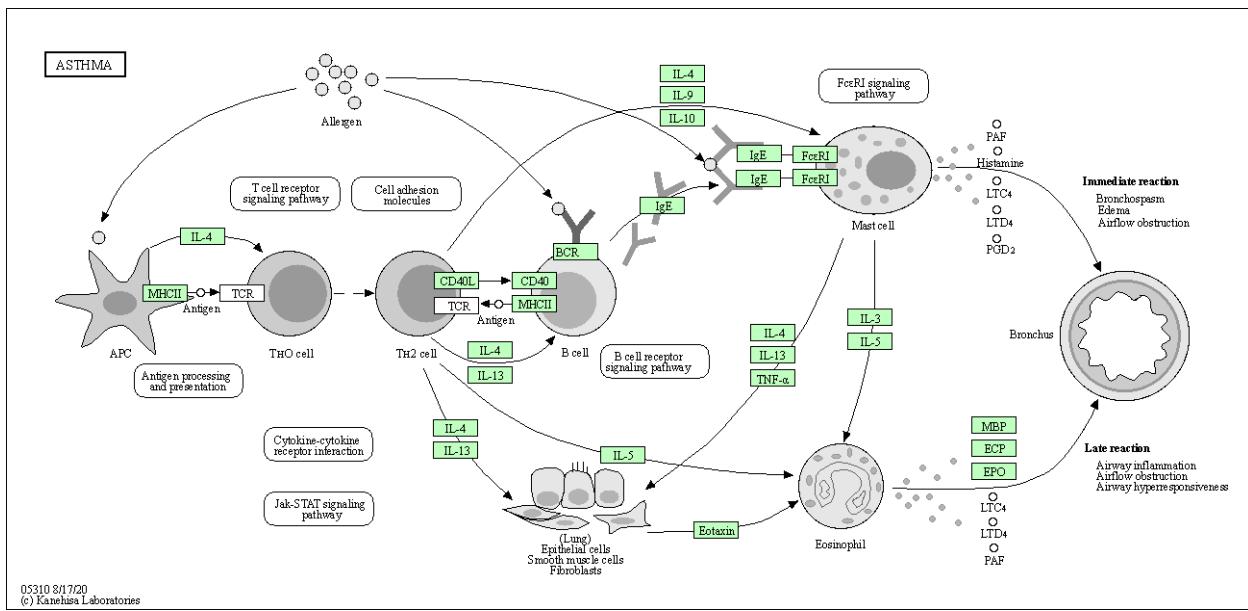
## $names
## [1] "greater" "less"     "stats"
head(kegg_res$less, 3)

##                               p.geomean stat.mean      p.val
## hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
## hsa04940 Type I diabetes mellitus 0.0017820293 -3.002352 0.0017820293
## hsa05310 Asthma                  0.0020045888 -3.009050 0.0020045888
##                               q.val set.size      exp1
## hsa05332 Graft-versus-host disease 0.09053483      40 0.0004250461
## hsa04940 Type I diabetes mellitus 0.14232581      42 0.0017820293
## hsa05310 Asthma                  0.14232581      29 0.0020045888

pathview(gene.data=foldchanges, pathway.id="hsa05310")

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/jack/UCSD_BioSci/Classes/AY2021/Fall21/BGGN_213/bggn_213-git_repo/c
## Info: Writing image file hsa05310.pathview.png
pathview(gene.data=foldchanges, pathway.id="hsa05310", kegg.native=FALSE)

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/jack/UCSD_BioSci/Classes/AY2021/Fall21/BGGN_213/bggn_213-git_repo/c
## Info: Writing image file hsa05310.pathview.pdf
ASTHMA Pathview for RNA-Seq Data (hsa05310)
```



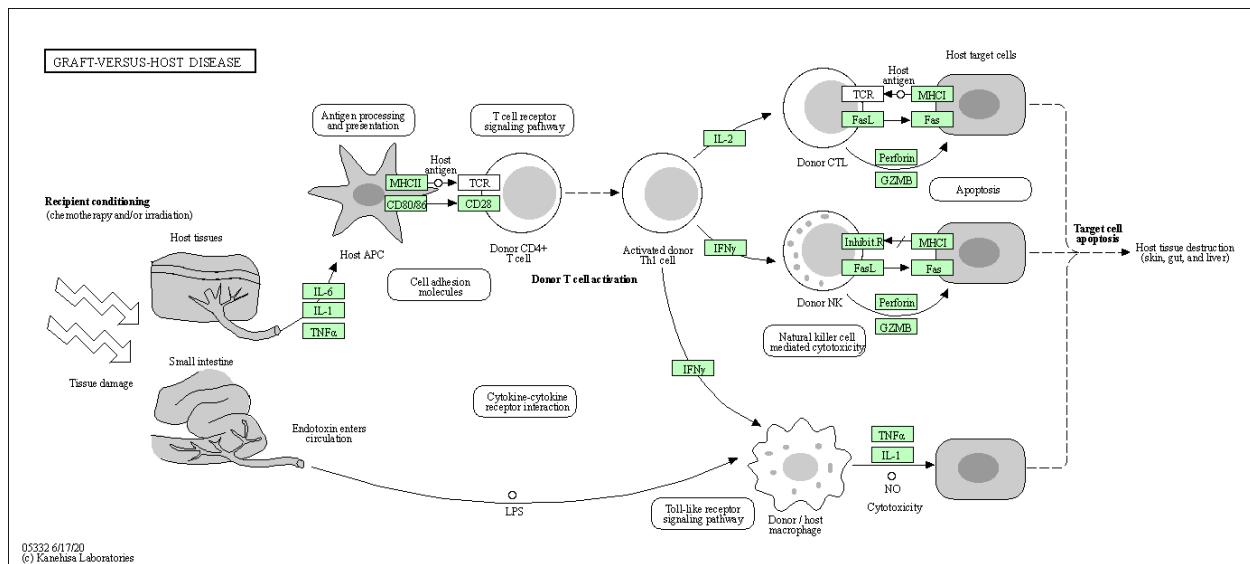
[Q12]: Can you do the same procedure as above to plot the pathview figures for the top 2 down-regulated pathways? Yes.

```
pathview(gene.data=foldchanges, pathway.id=substr(rownames(kegg_res$less)[1], start = 0, stop = 8))

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/jack/UCSD_BioSci/Classes/AY2021/Fall21/BGGN_213/bggn_213-git_repo/c
## Info: Writing image file hsa05332.pathview.png
pathview(gene.data=foldchanges, pathway.id=substr(rownames(kegg_res$less)[2], start = 0, stop = 8))

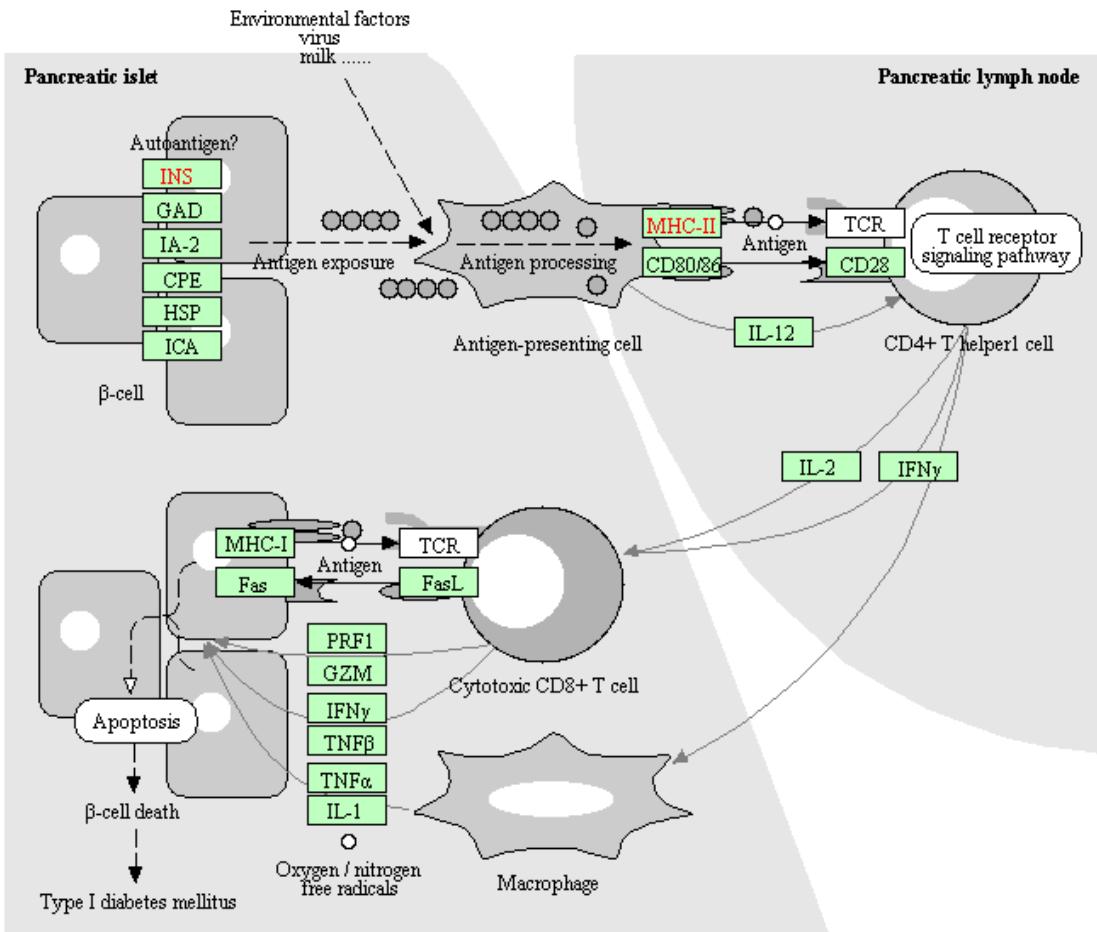
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /home/jack/UCSD_BioSci/Classes/AY2021/Fall21/BGGN_213/bggn_213-git_repo/c
## Info: Writing image file hsa04940.pathview.png

GRAFT VS HOST DISEASE Pathview (hsa05332)
```



TYPE 1 DIABETES MELLITUS

TYPE I DIABETES MELLITUS



04940 2/24/16
(c) Kanehisa Laboratories

Plotting Counts for Genes of Interest

```

indx <- grep("CRISPLD2", res$symbol)
res[indx,]

## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 1 row and 10 columns
##           baseMean log2FoldChange      lfcSE       stat      pvalue
##             <numeric>      <numeric> <numeric> <numeric>   <numeric>
## ENSG00000103196    3096.16        2.62603  0.267444  9.81899 9.32747e-23
##                  padj     symbol      entrez      uniprot
##                  <numeric> <character> <character> <character>
## ENSG00000103196 3.36344e-20    CRISPLD2      83716  A0A140VK80
##                   gene_name
##                   <character>

```

```

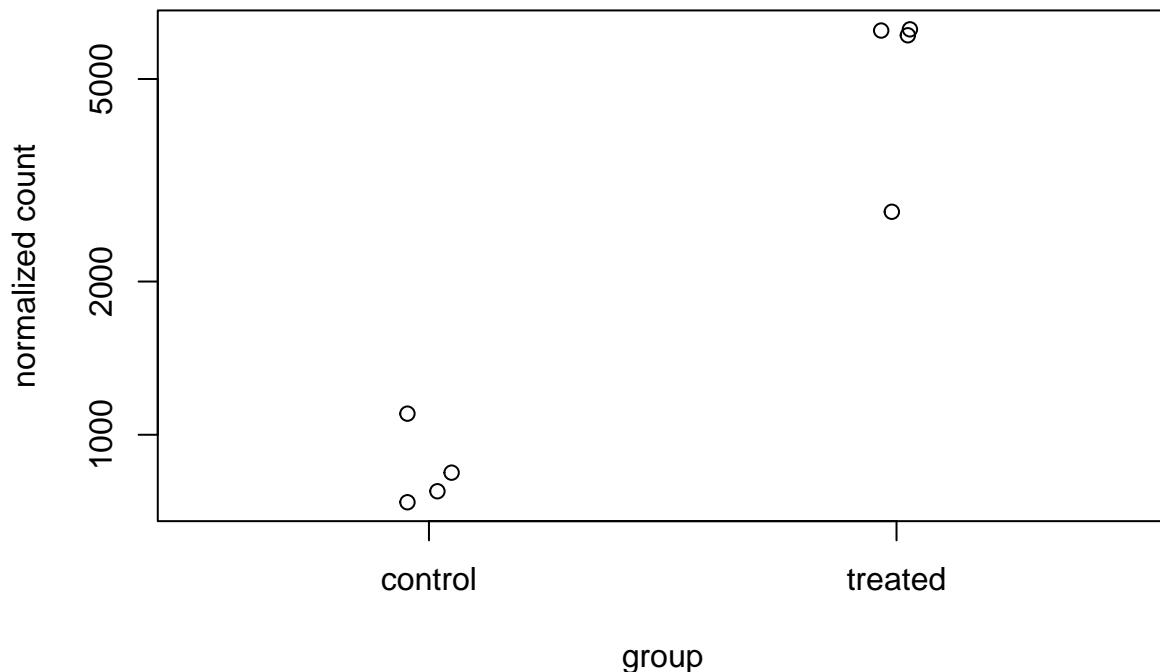
## ENSG00000103196 cysteine rich secret..
rownames(res[indx,])

## [1] "ENSG00000103196"

plotCounts(dds = Dds,
           gene = rownames(res[indx,]),
           intgroup = "dex")

```

ENSG00000103196



```

pc <- plotCounts(dds = Dds,
                  gene = rownames(res[indx,]),
                  intgroup = "dex",
                  returnData = TRUE)
head(pc)

```

```

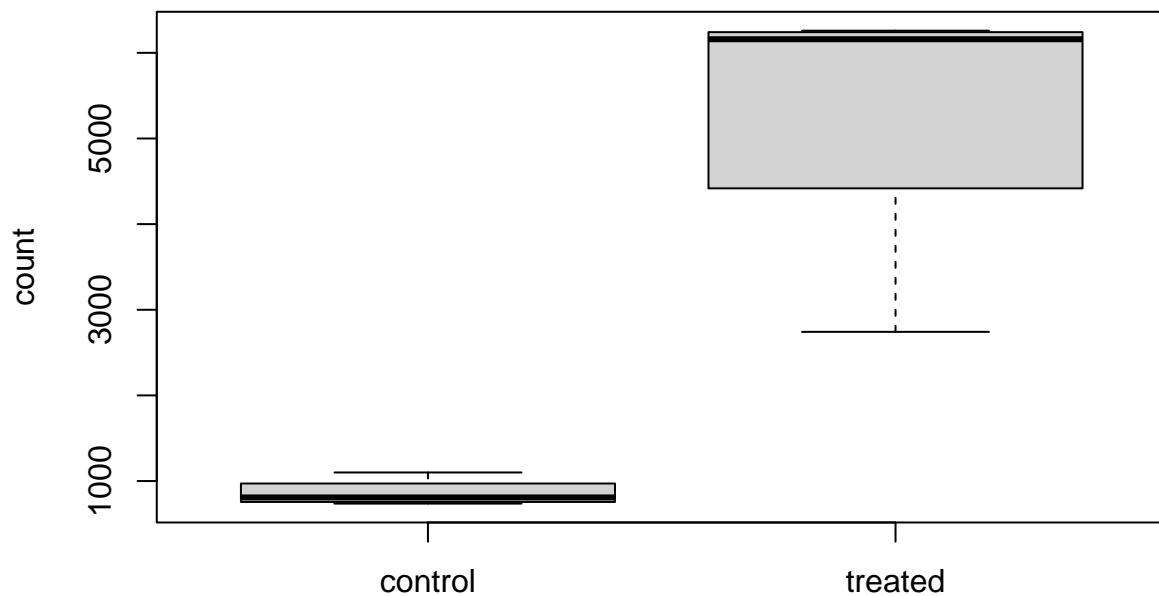
##          count     dex
## SRR1039508  774.5002 control
## SRR1039509  6258.7915 treated
## SRR1039512 1100.2741 control
## SRR1039513 6093.0324 treated
## SRR1039516  736.9483 control
## SRR1039517 2742.1908 treated

```

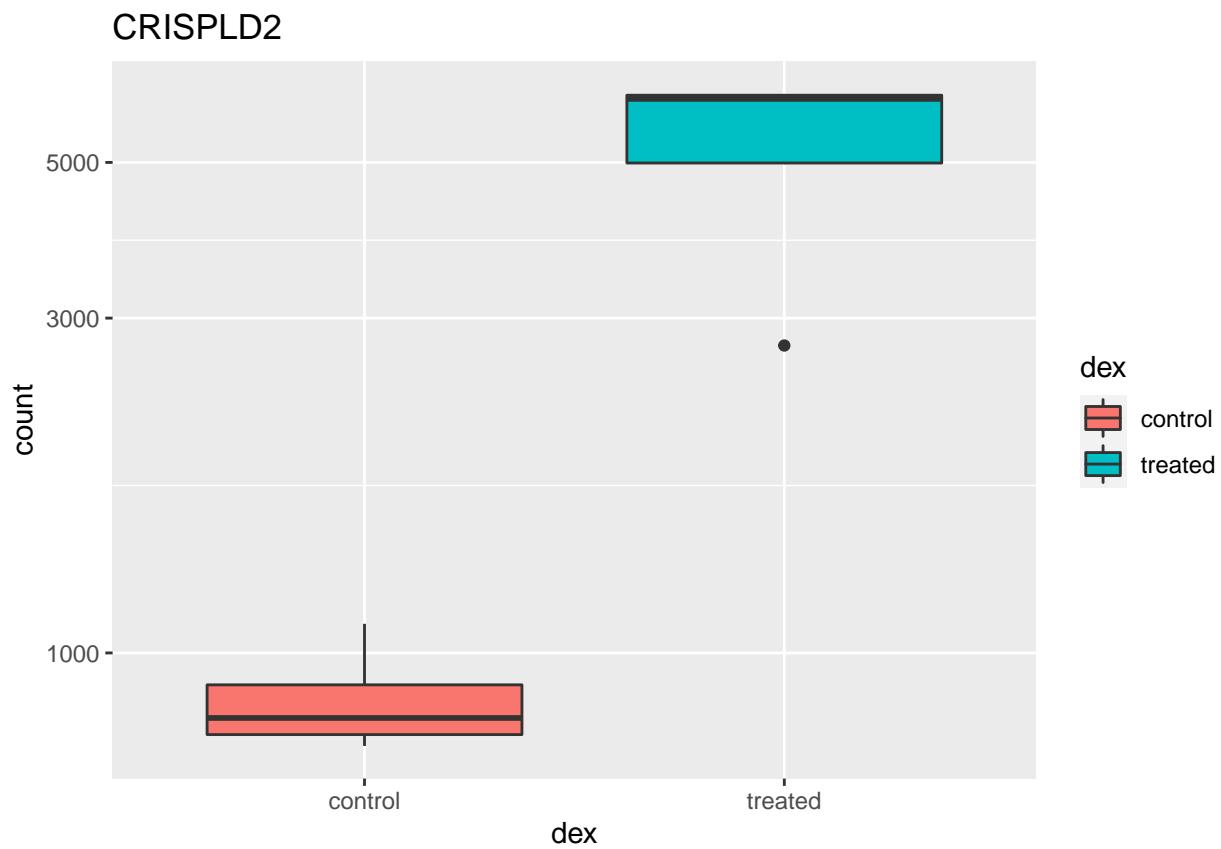
```

boxplot(count ~ dex,
        data = pc)

```



```
ggplot(data = pc) +
  aes(x = dex, y = count, fill = dex) +
  geom_boxplot() +
  scale_y_log10() +
  labs(title = "CRISPLD2")
```



Session Information

```
sessionInfo()

## R version 4.1.2 (2021-11-01)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Arch Linux
##
## Matrix products: default
## BLAS:    /usr/lib/libblas.so.3.10.0
## LAPACK:  /usr/lib/liblapack.so.3.10.0
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
## [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=en_US.UTF-8   LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
## [9] LC_ADDRESS=C              LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel  stats4   stats    graphics  grDevices utils    datasets
## [8] methods   base
##
## other attached packages:
## [1] gageData_2.30.0          gage_2.42.0
## [3] pathview_1.32.0          EnhancedVolcano_1.13.2
## [5] ggrepel_0.9.1            org.Hs.eg.db_3.13.0
## [7] AnnotationDbi_1.54.1    ggplot2_3.3.5
## [9] dplyr_1.0.7               DESeq2_1.32.0
## [11] SummarizedExperiment_1.22.0 Biobase_2.52.0
## [13] MatrixGenerics_1.4.3     matrixStats_0.61.0
## [15] GenomicRanges_1.44.0     GenomeInfoDb_1.28.4
## [17] IRanges_2.26.0           S4Vectors_0.30.2
## [19] BiocGenerics_0.38.0     BiocManager_1.30.16
##
## loaded via a namespace (and not attached):
## [1] httr_1.4.2                bit64_4.0.5      splines_4.1.2
## [4] assertthat_0.2.1          highr_0.9        blob_1.2.2
## [7] GenomeInfoDbData_1.2.6    yaml_2.2.1       pillar_1.6.4
## [10] RSQLite_2.2.8             lattice_0.20-45 glue_1.5.0
## [13] digest_0.6.28            RColorBrewer_1.1-2 XVector_0.32.0
## [16] colorspace_2.0-2          htmltools_0.5.2  Matrix_1.3-4
## [19] XML_3.99-0.8             pkgconfig_2.0.3  genefilter_1.74.1
## [22] zlibbioc_1.38.0          GO.db_3.13.0    purrr_0.3.4
## [25] xtable_1.8-4             scales_1.1.1    BiocParallel_1.26.2
## [28] tibble_3.1.6              annotate_1.70.0 KEGGREST_1.32.0
## [31] farver_2.1.0              generics_0.1.1  ellipsis_0.3.2
## [34] withr_2.4.2               cachem_1.0.6    survival_3.2-13
## [37] magrittr_2.0.1            crayon_1.4.2    KEGGgraph_1.52.0
## [40] memoise_2.0.0             evaluate_0.14   fansi_0.5.0
## [43] graph_1.70.0              tools_4.1.2     lifecycle_1.0.1
## [46] stringr_1.4.0             locfit_1.5-9.4  munsell_0.5.0
## [49] DelayedArray_0.18.0       Biostrings_2.60.2 compiler_4.1.2
```

```
## [52] rlang_0.4.12           grid_4.1.2            RCurl_1.98-1.5
## [55] labeling_0.4.2          bitops_1.0-7          rmarkdown_2.11
## [58] gtable_0.3.0            DBI_1.1.1             R6_2.5.1
## [61] knitr_1.36              fastmap_1.1.0         bit_4.0.4
## [64] utf8_1.2.2              Rgraphviz_2.36.0      stringi_1.7.5
## [67] Rcpp_1.0.7               vctrs_0.3.8           geneplotter_1.70.0
## [70] png_0.1-7               tidyselect_1.1.1      xfun_0.28
```