

11_04_22_lab

AUTHOR

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```
library(BiocManager)
```

Bioconductor version '3.15' is out-of-date; the current release version '3.16'

is available with R version '4.2'; see <https://bioconductor.org/install>

```
library(DESeq2)
```

Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics

Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

anyDuplicated, append, as.data.frame, basename, cbind,
colnames,
dirname, do.call, duplicated, eval, evalq, Filter, Find,
get, grep,
grepl, intersect, is.unsorted, lapply, Map, mapply, match,
mget,
order, paste, pmax, pmax.int, pmin, pmin.int, Position,
rank,
rbind, Reduce, rownames, sapply, setdiff, sort, table,
tapply,
union, unique, unsplit, which.max, which.min

Attaching package: 'S4Vectors'

The following objects are masked from 'package:base':

expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats

Attaching package: 'MatrixGenerics'

The following objects are masked from 'package:matrixStats':

colAlls, colAnyNAs, colAnys, colAvgsPerRowSet,
colCollapse,
colCounts, colCummaxs, colCummins, colCumprods,
colCumsums,
colDiffs, colIQRDiffs, colIQRs, colLogSumExps,
colMadDiffs,
colMads, colMaxs, colMeans2, colMedians, colMins,
colOrderStats,
colProds, colQuantiles, colRanges, colRanks, colSdDiffs,
colSds,
colSums2, colTabulates, colVarDiffs, colVars,
colWeightedMads,
colWeightedMeans, colWeightedMedians, colWeightedSds,
colWeightedVars, rowAlls, rowAnyNAs, rowAnys,
rowAvgsPerColSet,
rowCollapse, rowCounts, rowCummaxs, rowCummins,
rowCumprods,
rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians,
rowMins,
rowOrderStats, rowProds, rowQuantiles, rowRanges,
rowRanks,

```

    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs,
rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars

```

Loading required package: Biobase

Welcome to Bioconductor

Vignettes contain introductory material; view with
 'browseVignettes()'. To cite Bioconductor, see
 'citation("Biobase")', and for packages
 'citation("pkgname")'.

Attaching package: 'Biobase'

The following object is masked from 'package:MatrixGenerics':

```
rowMedians
```

The following objects are masked from 'package:matrixStats':

```
anyMissing, rowMedians
```

```

counts <- read.csv("airway_scaledcounts.csv", row.names=1)
metadata <- read.csv("airway_metadata.csv")

```

```
head(counts)
```

| | SRR1039508 | SRR1039509 | SRR1039512 | SRR1039513 |
|-------------------|------------|------------|------------|------------|
| SRR1039516 | | | | |
| ENSG000000000003 | 723 | 486 | 904 | 445 |
| 1170 | | | | |
| ENSG000000000005 | 0 | 0 | 0 | 0 |
| 0 | | | | |
| ENSG0000000000419 | 467 | 523 | 616 | 371 |
| 582 | | | | |
| ENSG0000000000457 | 347 | 258 | 364 | 237 |
| 318 | | | | |
| ENSG0000000000460 | 96 | 81 | 73 | 66 |
| 118 | | | | |
| ENSG0000000000938 | 0 | 0 | 1 | 0 |
| 2 | | | | |

| | SRR1039517 | SRR1039520 | SRR1039521 |
|------------------|------------|------------|------------|
| ENSG000000000003 | 1097 | 806 | 604 |
| ENSG000000000005 | 0 | 0 | 0 |
| ENSG000000000419 | 781 | 417 | 509 |
| ENSG000000000457 | 447 | 330 | 324 |
| ENSG000000000460 | 94 | 102 | 74 |
| ENSG000000000938 | 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 |

Q1. How many genes are in this dataset?

```
cat("We have", ncol(counts), "genes in this dataset")
```

We have 8 genes in this dataset

Q2. How many 'control' cell lines do we have?

```
cat("We have", ncol(metadata[metadata$dex == "control",]), "control cell lines in this dataset")
```

We have 4 control cell lines in this dataset

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

| | | |
|------------------|------------------|------------------|
| ENSG000000000003 | ENSG000000000005 | ENSG000000000419 |
| ENSG000000000457 | ENSG000000000460 | |
| 900.75 | 0.00 | 520.50 |
| 339.75 | 97.25 | |
| ENSG000000000938 | | |
| 0.75 | | |

```
library(dplyr)
```

Attaching package: 'dplyr'

The following object is masked from 'package:Biobase':

combine

The following object is masked from 'package:matrixStats':

count

The following objects are masked from 'package:GenomicRanges':

intersect, setdiff, union

The following object is masked from 'package:GenomeInfoDb':

intersect

The following objects are masked from 'package:IRanges':

collapse, desc, intersect, setdiff, slice, union

The following objects are masked from 'package:S4Vectors':

first, intersect, rename, setdiff, setequal, union

The following objects are masked from 'package:BiocGenerics':

combine, intersect, setdiff, union

The following objects are masked from 'package:stats':

filter, lag

The following objects are masked from 'package:base':

intersect, setdiff, setequal, union

```
control <- metadata %>% filter(dex=="control")
control.counts <- counts %>% select(control$id)
control.mean <- rowSums(control.counts)/4
```

```
head(control.mean)
```

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

Q3. How would you make the above code in either approach more robust? I would change the 4 to `nrow(control)` This would make the process more robust to different sized datasets. Currently it is hard coded to only be able to deal with specifically 4 controls. Outside of that someone would have to find out how many controls there are and manually change it. That changes the code to this for the 1st process:

```
control <- metadata[metadata[,"dex"]=="control",]
control.counts <- counts[ ,control$id]
control.mean <- rowSums( control.counts )/nrow(control)
head(control.mean)
```

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

Q4. Follow the same procedure for the treated samples (i.e. calculate the mean per gene across drug treated samples and assign to a labeled vector called `treated.mean`)

```
treated <- metadata[metadata[,"dex"]=="treated",]
treated.counts <- counts[ ,treated$id]
treated.mean <- rowSums( treated.counts )/nrow(treated)
head(treated.mean)
```

```
ENSG00000000003 ENSG00000000005 ENSG000000000419
ENSG000000000457 ENSG000000000460
          658.00          0.00          546.00
316.50          78.75
ENSG000000000938
```

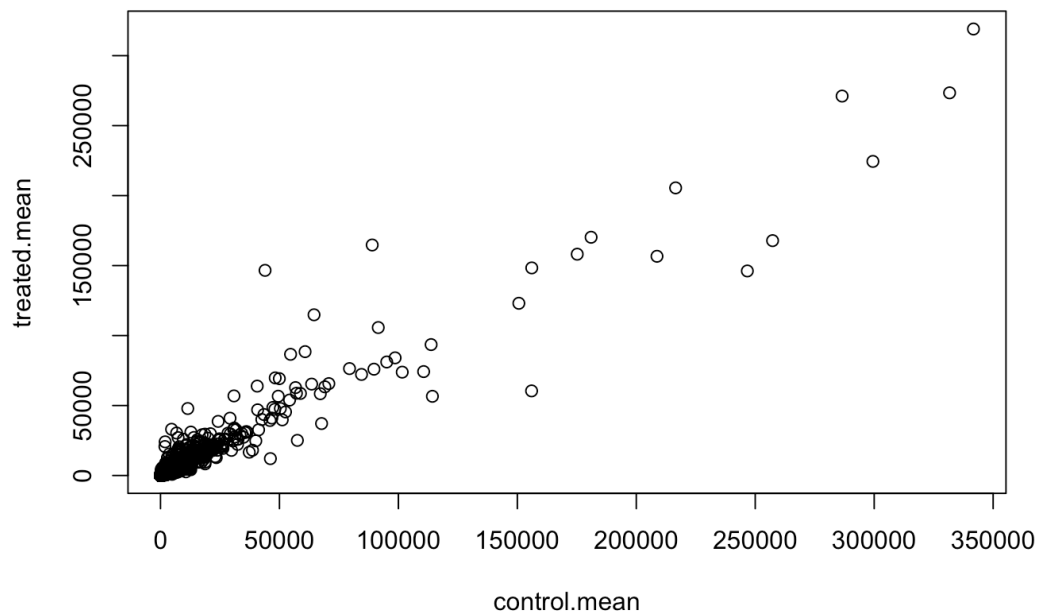
0.00

```
meancounts <- data.frame(control.mean, treated.mean)
```

Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

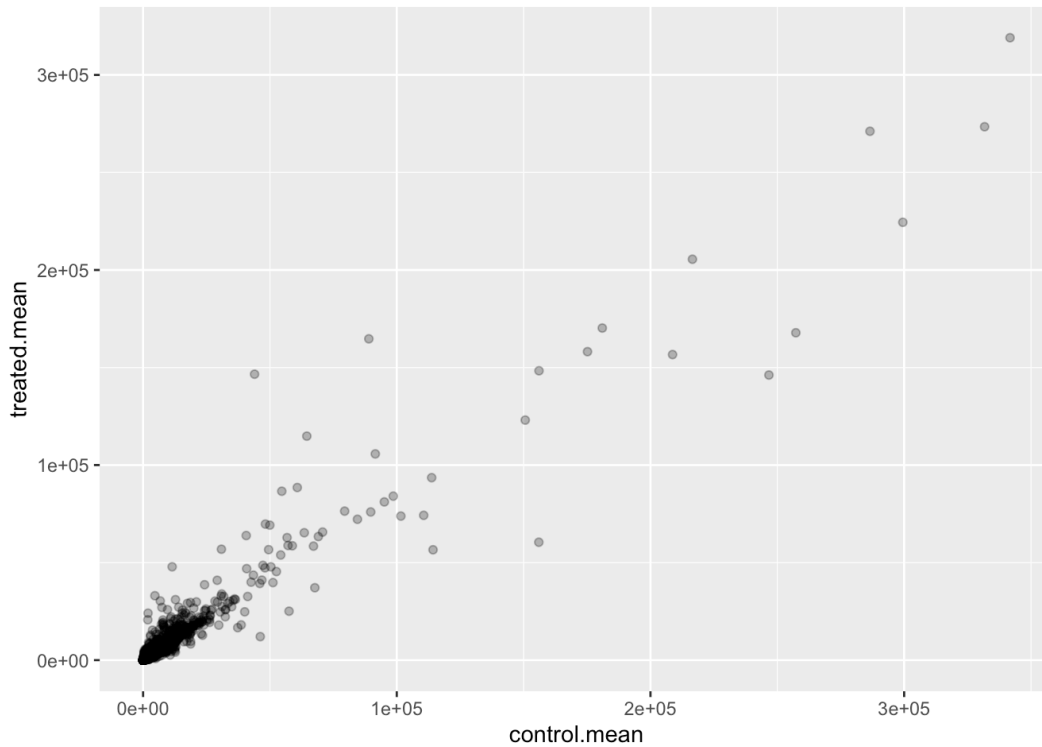
```
library(ggplot2)
```

```
plot(control.mean, treated.mean)
```



Q5 (b). You could also use the ggplot2 package to make this figure producing the plot below. What geom_?() function would you use for this plot? geom_point()

```
ggplot(meancounts, aes(control.mean, treated.mean))+  
  geom_point(alpha = 0.3)
```

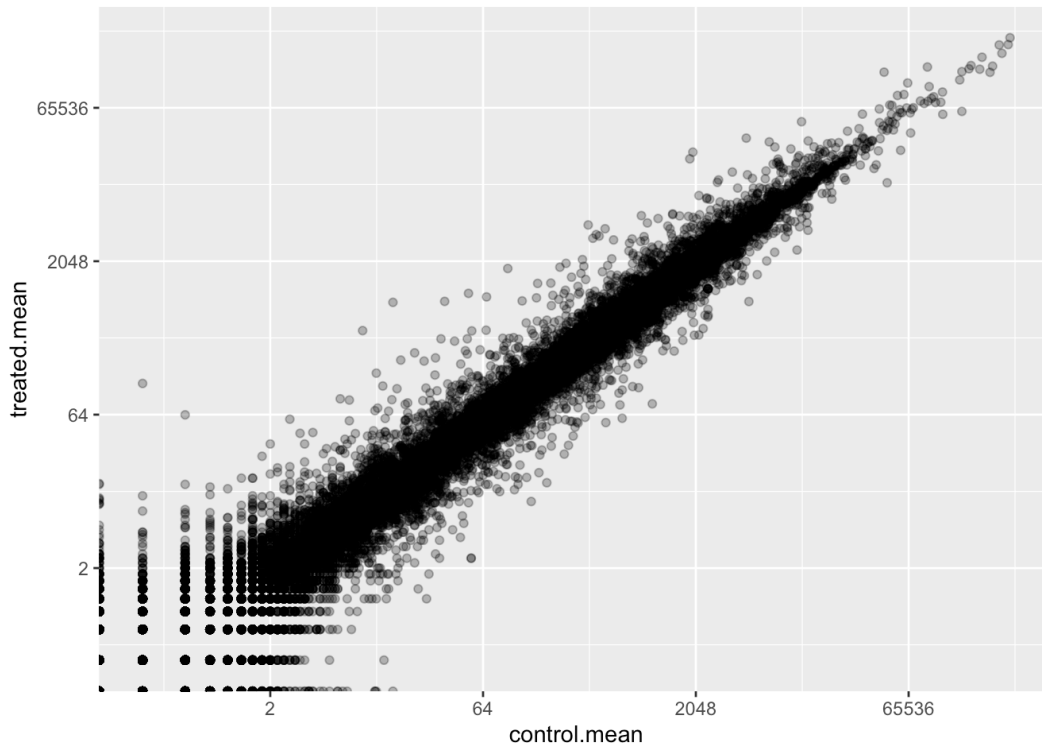


Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this? `scale_x_continuous` and `scale_y_continuous` are the additional functions needed for ggplot

```
ggplot(meancounts, aes(control.mean, treated.mean))+  
  geom_point(alpha = 0.3)+  
  scale_x_continuous(trans="log2")+  
  scale_y_continuous(trans="log2")
```

Warning: Transformation introduced infinite values in continuous x-axis

Warning: Transformation introduced infinite values in continuous y-axis



```
meancounts$log2fc <- log2(meancounts[, "treated.mean"] / meancounts[, "control.mean"])
head(meancounts)
```

| | control.mean | treated.mean | log2fc |
|-------------------|--------------|--------------|-------------|
| ENSG000000000003 | 900.75 | 658.00 | -0.45303916 |
| ENSG000000000005 | 0.00 | 0.00 | NaN |
| ENSG0000000000419 | 520.50 | 546.00 | 0.06900279 |
| ENSG0000000000457 | 339.75 | 316.50 | -0.10226805 |
| ENSG0000000000460 | 97.25 | 78.75 | -0.30441833 |
| ENSG0000000000938 | 0.75 | 0.00 | -Inf |

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? First we are looking at which values are 0 and then everytime one is found, `arr.ind` allows us to know which rowname each row is associated with. Then `unique` allows us to make sure nothing is double counted if there are 0s in both columns.

```
zero.vals <- which(meancounts[, 1:2] == 0, arr.ind = TRUE)

to.rm <- unique(zero.vals[, 1])
mycounts <- meancounts[-to.rm, ]
head(mycounts)
```

| | control.mean | treated.mean | log2fc |
|------------------|--------------|--------------|-------------|
| ENSG000000000003 | 900.75 | 658.00 | -0.45303916 |
| ENSG000000000419 | 520.50 | 546.00 | 0.06900279 |
| ENSG000000000457 | 339.75 | 316.50 | -0.10226805 |
| ENSG000000000460 | 97.25 | 78.75 | -0.30441833 |
| ENSG000000000971 | 5219.00 | 6687.50 | 0.35769358 |
| ENSG00000001036 | 2327.00 | 1785.75 | -0.38194109 |

```
up.ind <- mycounts$log2fc > 2
down.ind <- mycounts$log2fc < (-2)
```

Q8. Using the up.ind vector above can you determine how many up regulated genes we have at the greater than 2 fc level?

```
cat("There are", sum(up.ind), "upregulated genes at a greater t
```

There are 250 upregulated genes at a greater than 2x fold change level

Q9. Using the down.ind vector above can you determine how many down regulated genes we have at the greater than 2 fc level?

```
cat("There are", sum(down.ind), "upregulated genes at a greater
```

There are 367 upregulated genes at a greater than 2x fold change level

Q10. Do you trust these results? Why or why not? I do trust that all the results are true to the fact that there is a 2x fold change, but in the greater context, I don't have enough information to draw conclusions yet.

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

To cite package 'DESeq2' in publications use:

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},
}
```

```
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): ENSG000000000003 ENSG000000000005 ...
ENSG00000283120
ENSG00000283123
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(4): id dex celltype geo_id
```

```
dds <- DESeq(dds)
```

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

```
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 | | | | |
| ... | ... | ... | ... | ... |
| ... | | | | |
| ENSG00000283115 | 0.000000 | NA | NA | NA |
| NA | | | | |
| ENSG00000283116 | 0.000000 | NA | NA | NA |
| NA | | | | |
| ENSG00000283119 | 0.000000 | NA | NA | NA |
| NA | | | | |
| ENSG00000283120 | 0.974916 | -0.668258 | 1.69456 | -0.394354 |
| 0.693319 | | | | |
| ENSG00000283123 | 0.000000 | NA | NA | NA |
| NA | | | | |
| | padj | | | |
| | <numeric> | | | |

```

ENSG00000000003  0.163035
ENSG00000000005           NA
ENSG000000000419  0.176032
ENSG000000000457  0.961694
ENSG000000000460  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

```

```

res05 <- results(dds, alpha=0.05)
summary(res05)

```

```

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

```

```
library("AnnotationDbi")
```

Attaching package: 'AnnotationDbi'

The following object is masked from 'package:dplyr':

select

```
library("org.Hs.eg.db")
```

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

```
[1] "ACCNUM"      "ALIAS"      "ENSEMBL"
"ENSEMBLPROT" "ENSEMBLTRANS"
[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), # Our genenames
  keytype="ENSEMBL",   # The format of
  column="SYMBOL",     # The new format
  multiVals="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 | | | | |

| | | | | |
|-----------------|------------|------------|----------|-----------|
| 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 |
|-----------------|-----------|-------------|
| | <numeric> | <character> |
| ENSG00000000003 | 0.163035 | TSPAN6 |
| ENSG00000000005 | NA | TNMD |
| ENSG00000000419 | 0.176032 | DPM1 |
| ENSG00000000457 | 0.961694 | SCYL3 |
| ENSG00000000460 | 0.815849 | C1orf112 |
| ENSG00000000938 | NA | FGR |

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

```
res$entrez <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     keytype="ENSEMBL",   # The format of
                     column="ENTREZID",    # The new form
                     multiVals="first")
```

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

```
res$uniprot <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     keytype="ENSEMBL",   # The format of
                     column="UNIPROT",     # The new form
                     multiVals="first")
```

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

```
res$genename <- mapIds(org.Hs.eg.db,
                      keys=row.names(res), # Our genenames
                      keytype="ENSEMBL",   # The format of
                      column="GENENAME",    # The new form
                      multiVals="first")
```

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

```
ord <- order( res$padj )
#View(res[ord,])
head(res[ord,])
```

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 | |
| A0A024RDE1 | | | | |
| ENSG00000179094 | 6.13966e-56 | PER1 | 5187 | |
| 015534 | | | | |
| 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 | | | | |
| | | genename | | |
| | | <character> | | |
| ENSG00000152583 | | SPARC like 1 | | |


```

ENSG00000179094 period circadian reg..
ENSG00000116584 Rho/Rac guanine nucl..
ENSG00000189221 monoamine oxidase A
ENSG00000120129 dual specificity pho..
ENSG00000148175 stomatin

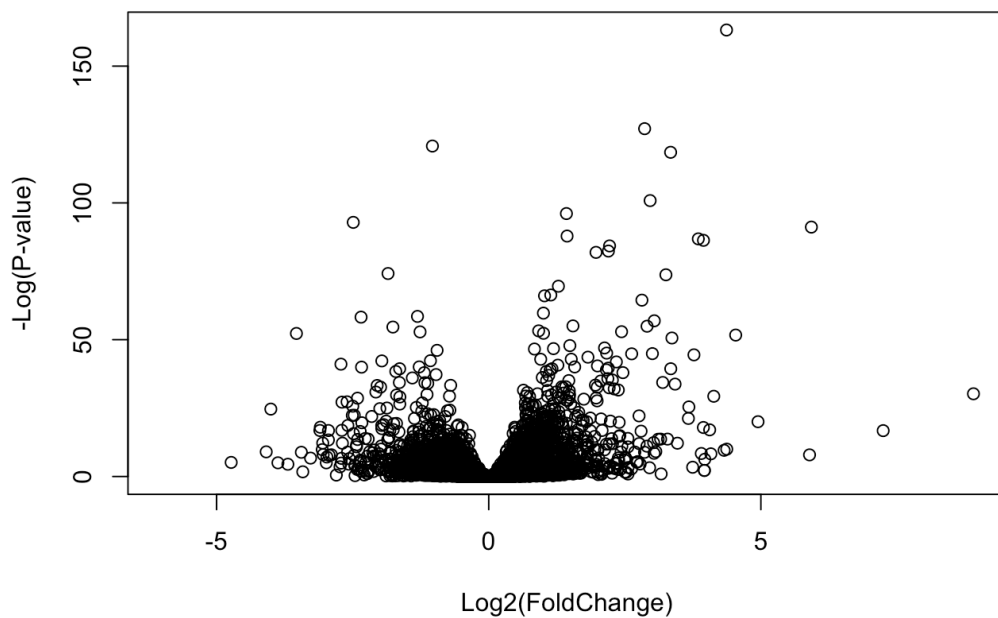
```

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

```

plot( res$log2FoldChange, -log(res$padj),
      xlab="Log2(FoldChange)",
      ylab="-Log(P-value)")

```



```

plot( res$log2FoldChange, -log(res$padj),
      ylab="-Log(P-value)", xlab="Log2(FoldChange)")

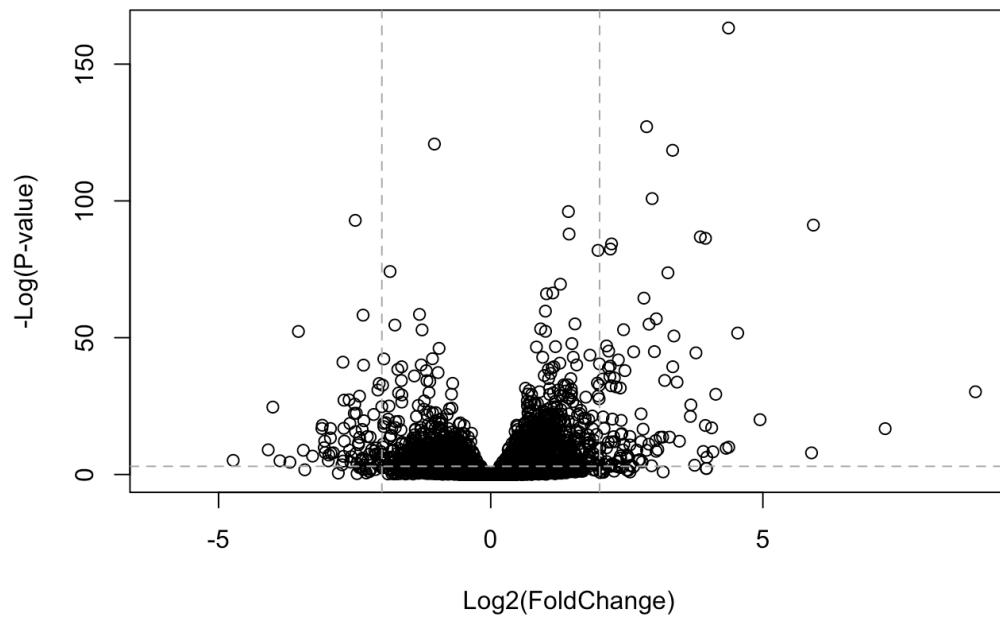
```

```
# Add some cut-off lines
```

```

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

```

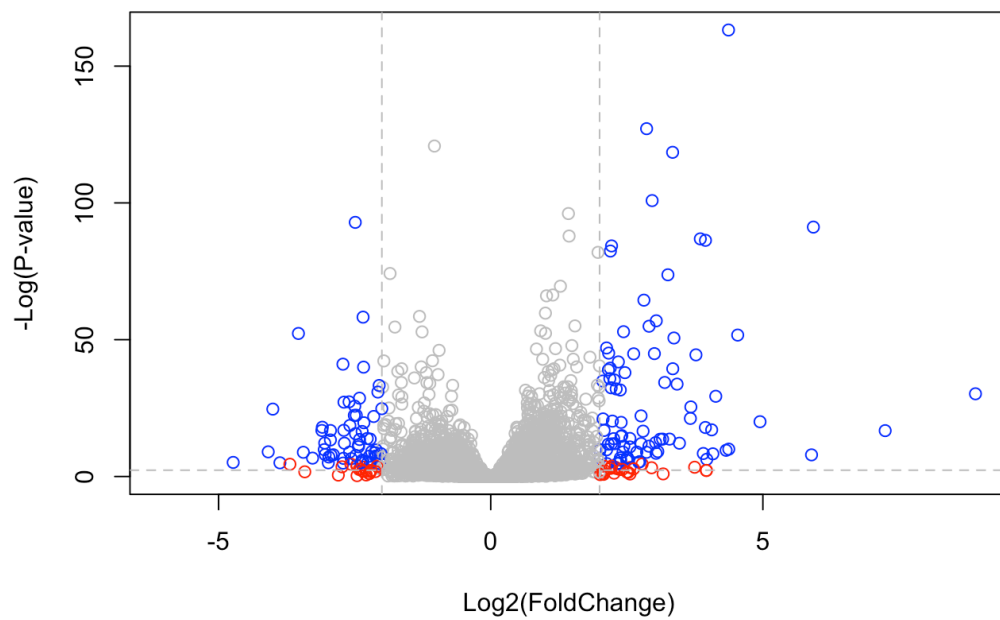


```
# Setup our custom point color vector
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

# Volcano plot with custom colors
plot( res$log2FoldChange, -log(res$padj),
      col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )

# Cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", lty=2)
```



```
library(EnhancedVolcano)
```

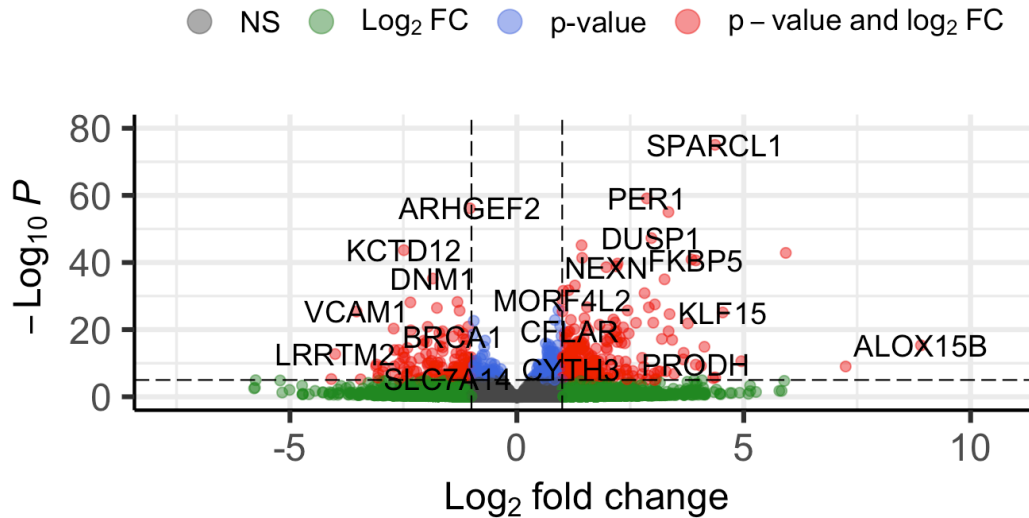
Loading required package: ggrepel

```
x <- as.data.frame(res)

EnhancedVolcano(x,
  lab = x$symbol,
  x = 'log2FoldChange',
  y = 'pvalue')
```

Volcano plot

EnhancedVolcano



```
library(pathview)
```

```
#####
#####
```

Pathview is an open source software package distributed under GNU General

Public License version 3 (GPLv3). Details of GPLv3 is available at

<http://www.gnu.org/licenses/gpl-3.0.html>. Particullary, users are required to

formally cite the original Pathview paper (not just mention it) in publications

or products. For details, do `citation("pathview")` within R.

The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG

license agreement (details at

<http://www.kegg.jp/kegg/legal.html>).

```
#####
#####
```

```
library(gage)
```

```
library(gageData)

data(kegg.sets.hs)

# Examine the first 2 pathways in this kegg set for humans
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
```

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
attributes(keggres)
```

```
$names
[1] "greater" "less" "stats"
```

```
head(keggres$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
/Users/andytong/Downloads/Bioinformatics Work/11_04_22

Info: Writing image file hsa05310.pathview.png

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

Yes I can do the same for both of these.

```
head(keggres$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 | | |

Type I diabetes mellitus

```
pathview(gene.data=foldchanges, pathway.id="hsa04940")
```

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

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/Users/andytong/Downloads/Bioinformatics Work/11_04_22

Info: Writing image file hsa04940.pathview.png

Graft-versus-host disease

```
pathview(gene.data=foldchanges, pathway.id="hsa05332")
```

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_04_22

Info: Writing image file hsa05332.pathview.png