Class 13: Transcriptomics and the analysis of RNA-Seq data

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```
library(BiocManager)
```

```
Bioconductor version '3.16' is out-of-date; the current release version '3.18'
  is available with R version '4.3'; 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, aperm, 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
```

##Import countData and colData

```
# Complete the missing code
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
```

Q1. We have 38694 genes.

```
nrow(counts)
```

```
[1] 38694
```

Q2 We have 4 "control" cell lines.

```
View(metadata)
```

##Toy differential gene expression

Let's start by calculating the mean counts per genes in the "control" samples. We can then compare this value for each gene to the mean counts in the "treated" samples (i.e. columns).

-Step 1. Find which columns in `counts` correspond to "control" samples. -Step 2. Calculate the mean value per gene in these columns. -Step 3. Store my answer for late in `control.mean`

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

```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         900.75             0.00           520.50           339.75            97.25
ENSG00000000938
           0.75
```

Q3. We can use `rowSums()` to make the code approach more robust.

```
control.inds=metadata$dex == "control"
```

```
control.inds=metadata$dex=="control"
```

```
metadata[control.inds,]
```

```
         id     dex celltype     geo_id
1 SRR1039508 control   N61311 GSM1275862
3 SRR1039512 control  N052611 GSM1275866
5 SRR1039516 control  N080611 GSM1275870
7 SRR1039520 control  N061011 GSM1275874
```

```
control.counts=counts[,control.inds]
head(control.counts)
```

```
                SRR1039508 SRR1039512 SRR1039516 SRR1039520
ENSG00000000003        723        904       1170        806
ENSG00000000005          0          0          0          0
ENSG00000000419        467        616        582        417
ENSG00000000457        347        364        318        330
ENSG00000000460         96         73        118        102
ENSG00000000938          0          1          2          0
```

```
#apply(control.counts,1,mean)
control.mean=rowMeans(control.counts)
```

Q4. Now do the same steps to get `treated.mean`

```
treated.mean=rowMeans(counts[,metadata$dex == "treated"])
```

To keep us tidy lets put `control.mean` and `treated.mean` vectors together as two columns of a new data.frame.

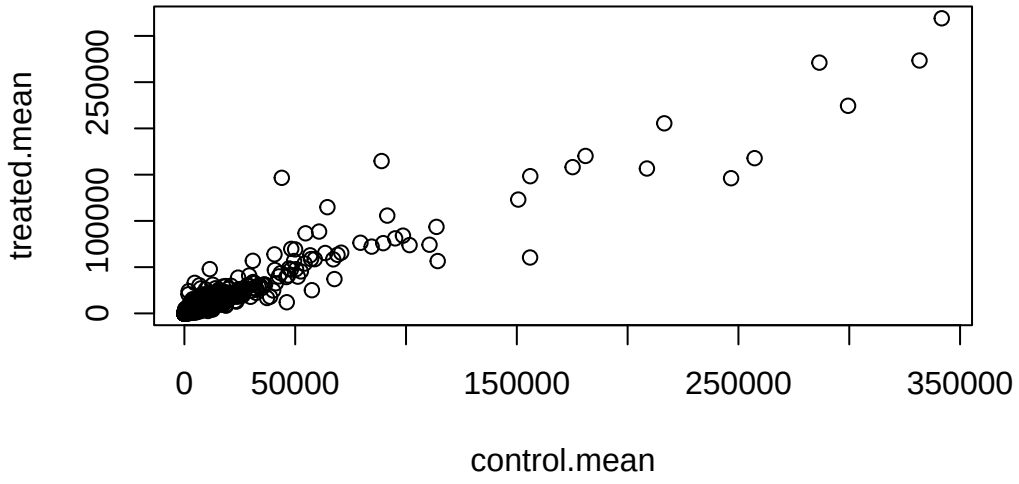
```
meancounts=data.frame(control.mean, treated.mean)
```

```
head(meancounts)
```

```
                control.mean treated.mean
ENSG00000000003       900.75       658.00
ENSG00000000005         0.00         0.00
ENSG00000000419       520.50       546.00
ENSG00000000457       339.75       316.50
ENSG00000000460        97.25        78.75
ENSG00000000938         0.75         0.00
```

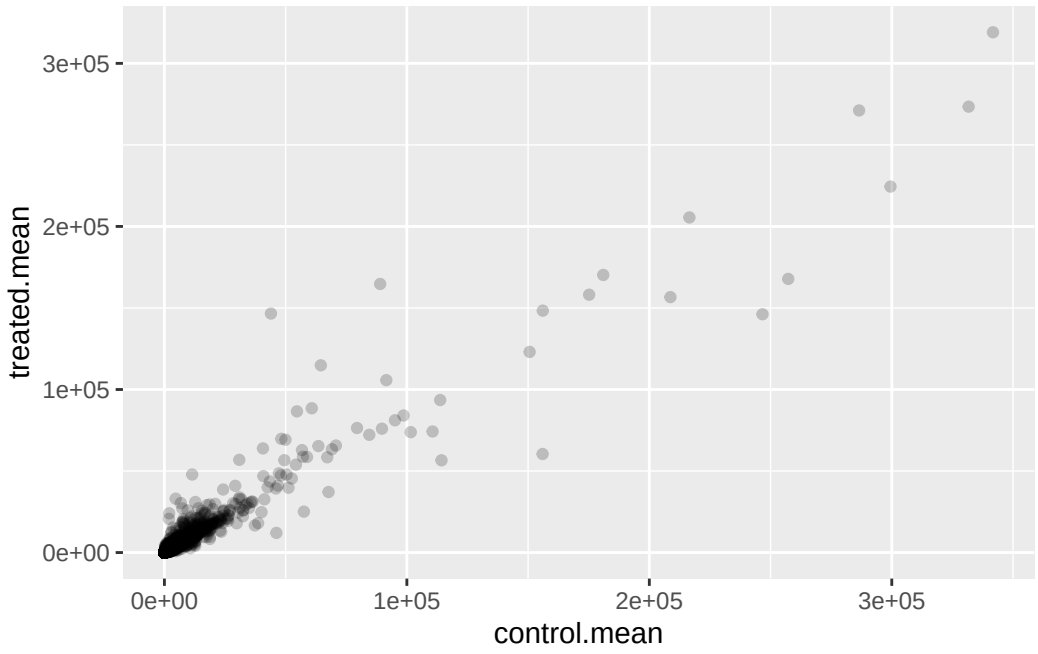
Q5(a).

```
plot(meancounts)
```



And a ggplot version:

```
library(ggplot2)
ggplot(meancounts)+
  aes(control.mean,treated.mean)+
  geom_point(alpha=.2)
```



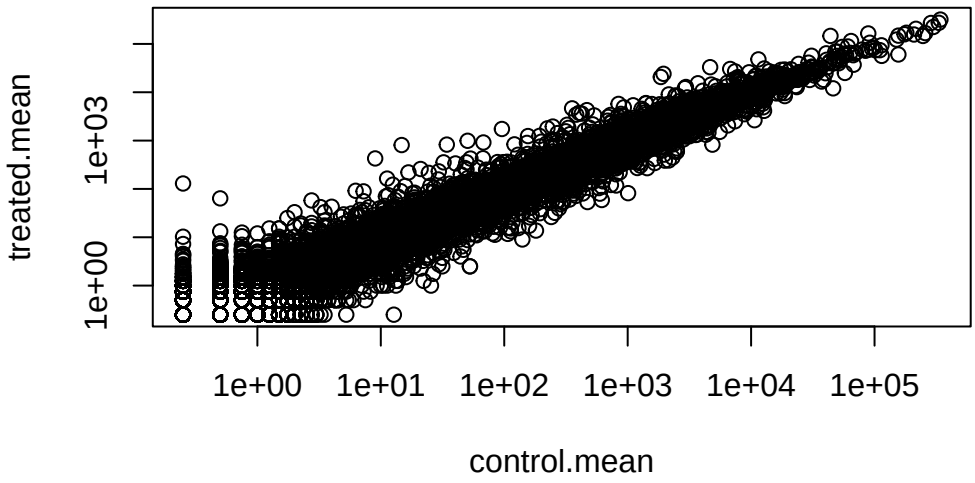
Q5(b). geom_point

Q6.

```
plot(meancounts,log="xy")
```

```
Warning in xy.coords(x, y, xlabel, ylabel, log): 15032 x values <= 0 omitted
from logarithmic plot
```

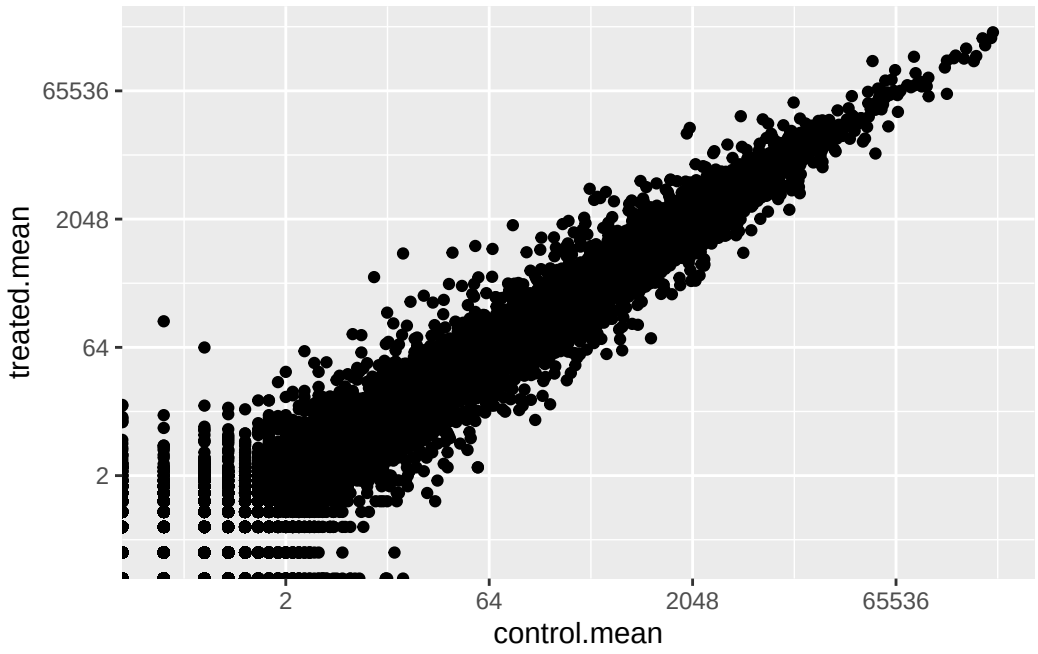
```
Warning in xy.coords(x, y, xlabel, ylabel, log): 15281 y values <= 0 omitted
from logarithmic plot
```




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



Log transformations are super useful when our data is skewed and measured over a wide range like this. We can use different log transformations like base10 or natural logs but we most often prefer log2 units.

```
#Control/Treated
log2(10/10)
```

```
[1] 0
```

What if there was a doubling?

```
#Treated/control
log2(20/10)
```

```
[1] 1
```

Half counts

```
log2(10/20)
```

```
[1] -1
```

```
log2(40/10)
```

```
[1] 2
```

Let's add a log2 fold-change column to our little `meancounts` data.frame:

```
meancounts$log2fc=log2(meancounts$treated.mean/meancounts$control.mean)
head(meancounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000005         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
```

There are a couple of weird results. The `!` flips TRUE values to FALSE and vice versa

```
to.rm.inds=rowSums(meancounts[,1:2] == 0) > 0
mycounts=meancounts[!to.rm.inds,]
```

```
dim(mycounts)
```

```
[1] 21817     3
```

```
head(mycounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       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. The arr.ind = TRUE argument in which() retrieves row and column indices where there are TRUE values. Using unique(zero.vals[, 1]) ensures only unique row indices with zero counts in at least one sample are selected for removal, focusing on rows without zero counts in both specified columns.

```
head(mycounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       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
```

A common threshold used for calling something differentially expressed is a log2(FoldChange) of greater than 2 or less than -2. Let's filter the dataset both ways to see how many genes are up or down-regulated.

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

Q8. 250 up-regulated genes

```
sum(up.ind)
```

```
[1] 250
```

Q9. 367 down-regulated genes

```
sum(down.ind)
```

```
[1] 367
```

Q10. No, we haven't considered the statistical significance of the differences in fold changes.

We will use the DESeq2 package to do this analysis properly.

##Setting up for DESeq We must load it up with a `library()` call.

```
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): ENSG00000000003 ENSG00000000005 ... ENSG00000283120
  ENSG00000283123
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(4): id dex celltype geo_id
```

Now we can run our DESeq analysis

```
dds=DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

Get our results back from the `dds` object.

```
dds=DESeq(dds)
```

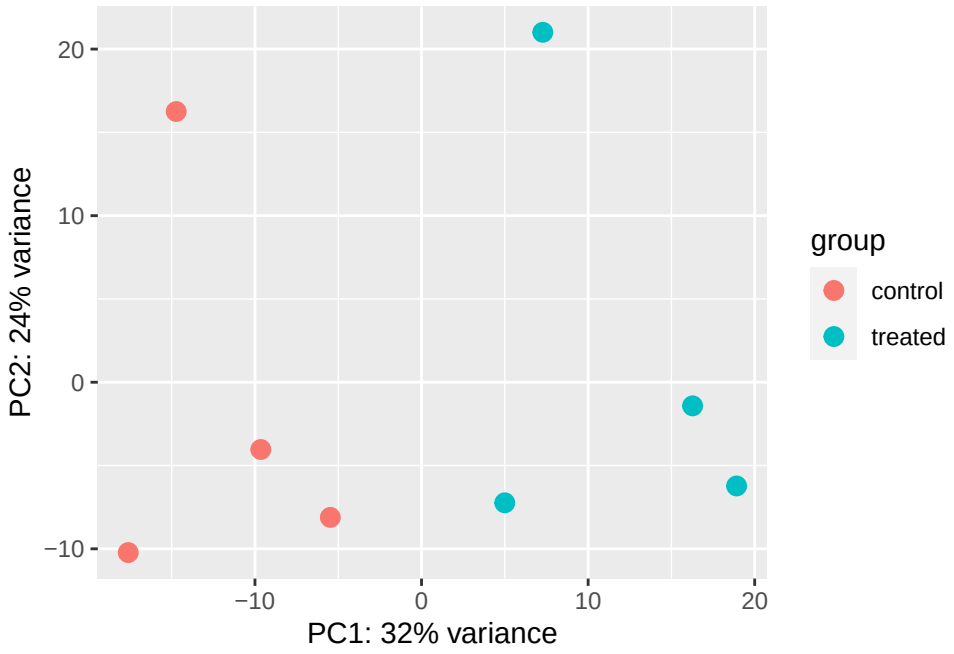
```
using pre-existing size factors

estimating dispersions

found already estimated dispersions, replacing these

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

```
results(dds)
```

```
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>
ENSG00000000003  747.1942     -0.3507030  0.168246 -2.084470 0.0371175
ENSG00000000005    0.0000             NA        NA        NA        NA
ENSG00000000419  520.1342      0.2061078  0.101059  2.039475 0.0414026
ENSG00000000457  322.6648      0.0245269  0.145145  0.168982 0.8658106
ENSG00000000460   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
ENSG00000000419  0.176032
ENSG00000000457  0.961694
ENSG00000000460  0.815849
...                   ...
ENSG00000283115        NA
ENSG00000283116        NA
ENSG00000283119        NA
ENSG00000283120        NA
ENSG00000283123        NA
```

##PCA

```
vsd <- vst(dds, blind = FALSE)
plotPCA(vsd, intgroup = c("dex"))
```

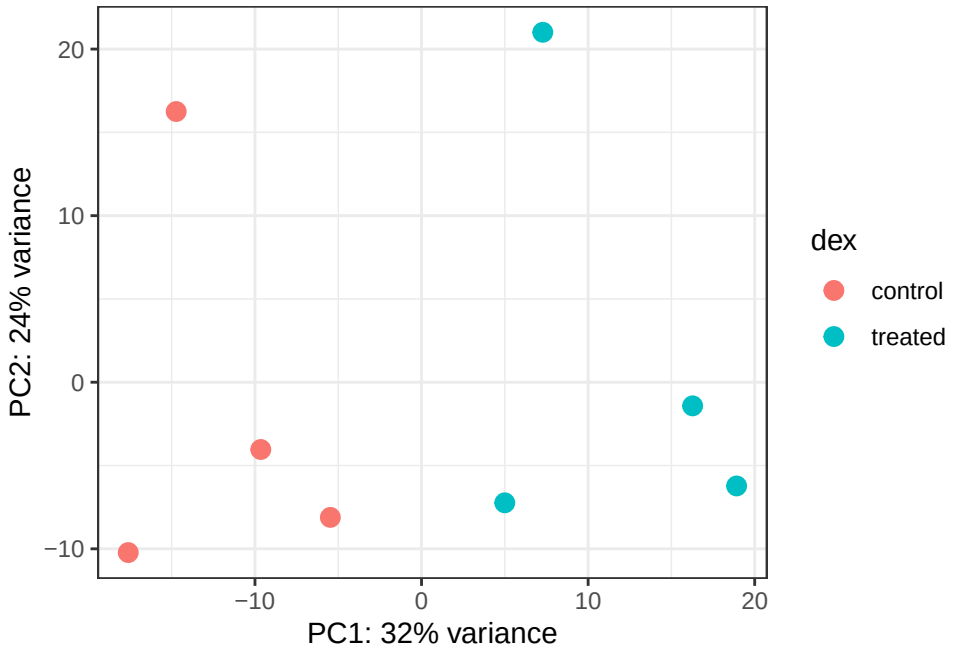



```
pcaData <- plotPCA(vsd, intgroup=c("dex"), returnData=TRUE)
head(pcaData)
```

```
                 PC1        PC2   group     dex       name
SRR1039508 -17.607922 -10.225252 control control SRR1039508
SRR1039509   4.996738  -7.238117 treated treated SRR1039509
SRR1039512  -5.474456  -8.113993 control control SRR1039512
SRR1039513  18.912974  -6.226041 treated treated SRR1039513
SRR1039516 -14.729173  16.252000 control control SRR1039516
SRR1039517   7.279863  21.008034 treated treated SRR1039517
```

```
# Calculate percent variance per PC for the plot axis labels
percentVar <- round(100 * attr(pcaData, "percentVar"))
```

```
ggplot(pcaData) +
  aes(x = PC1, y = PC2, color = dex) +
  geom_point(size =3) +
  xlab(paste0("PC1: ", percentVar[1], "% variance")) +
  ylab(paste0("PC2: ", percentVar[2], "% variance")) +
  coord_fixed() +
  theme_bw()
```



##DESeq Analysis

```
results(dds)
```

```
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>
ENSG00000000003  747.1942     -0.3507030  0.168246 -2.084470 0.0371175
ENSG00000000005    0.0000             NA        NA        NA        NA
ENSG00000000419  520.1342      0.2061078  0.101059  2.039475 0.0414026
ENSG00000000457  322.6648      0.0245269  0.145145  0.168982 0.8658106
ENSG00000000460   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
ENSG00000000419  0.176032
ENSG00000000457  0.961694
ENSG00000000460  0.815849
...                   ...
ENSG00000283115        NA
ENSG00000283116        NA
ENSG00000283119        NA
ENSG00000283120        NA
ENSG00000283123        NA
```

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

```
using pre-existing size factors
```

```
estimating dispersions
```

```
found already estimated dispersions, replacing these
```

```
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>
ENSG00000000003  747.1942     -0.3507030  0.168246 -2.084470 0.0371175
```

```
ENSG00000000005    0.0000             NA        NA        NA        NA
ENSG00000000419  520.1342      0.2061078  0.101059  2.039475 0.0414026
ENSG00000000457  322.6648      0.0245269  0.145145  0.168982 0.8658106
ENSG00000000460   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
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
```

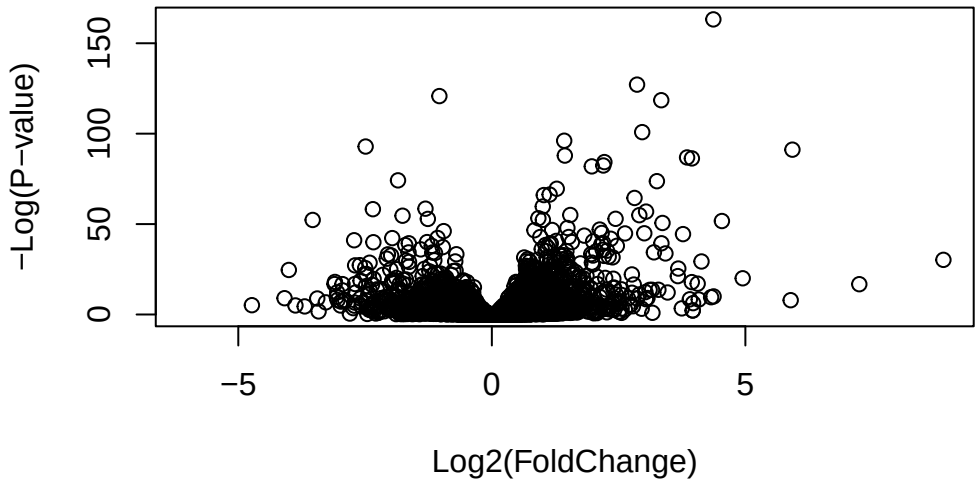
```
res05 <- results(dds, alpha=0.05)
summary(res05)
```

```
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
```

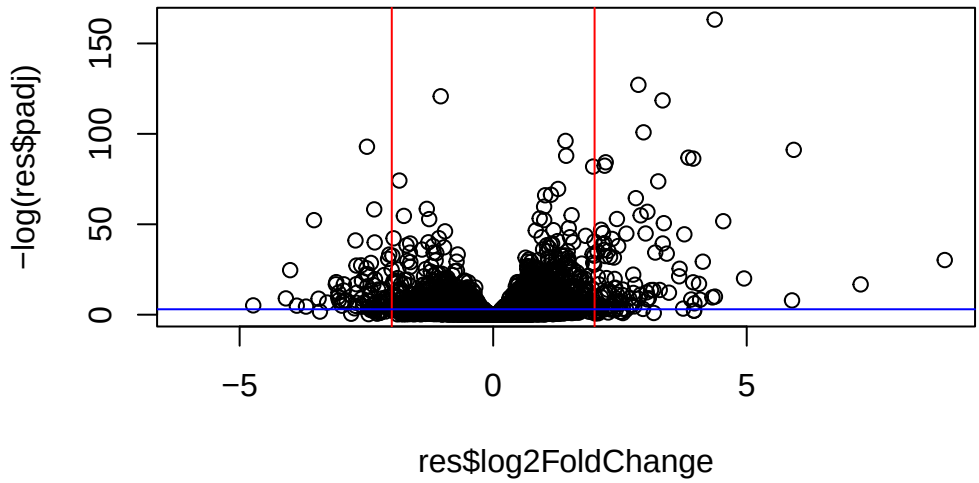
##Data Visualization

Volcano plot. This is a common type of summary figure that keep both our inner biologist and inner stats nerd happy because it shows both P-values and Log2(Fold-Changes).

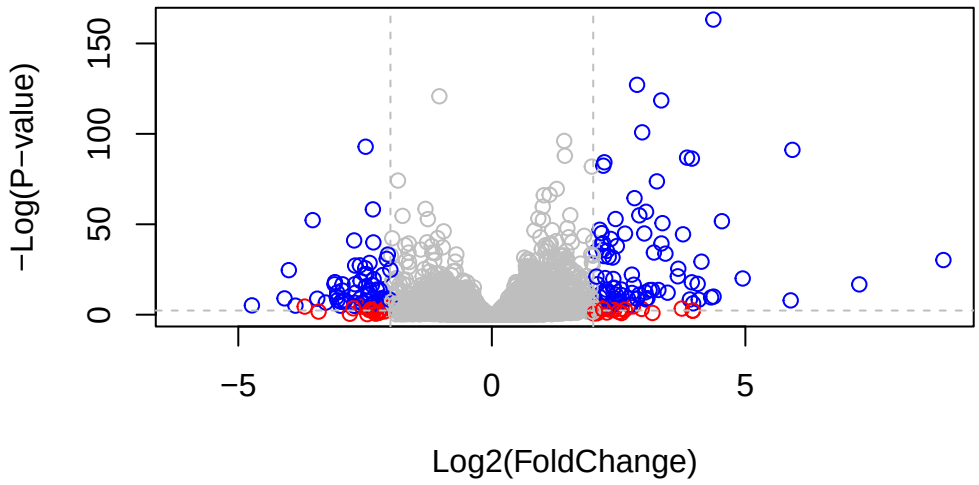
```
plot( res$log2FoldChange,  -log(res$padj),
      xlab="Log2(FoldChange)",
      ylab="-Log(P-value)")
```




```
plot(res$log2FoldChange, -log(res$padj))
abline(v=2,col="red")
abline(v=-2,col="red")
abline(h=-log(0.05),col="blue")
```




```
# 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
```

```
res=results(dds)
```

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

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

Save our results to date

```
#write.csv(res,fule="deseq-results.csv")
```

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 6 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
                <numeric>
ENSG00000000003  0.163035
ENSG00000000005        NA
ENSG00000000419  0.176032
ENSG00000000457  0.961694
ENSG00000000460  0.815849
ENSG00000000938        NA
```

##Adding annotation data

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

```
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"
```

The main function we will use here is called `mapIDs()`

Our current IDs are here:

```
#mapIDs()
head(rownames(res))
```

```
[1] "ENSG00000000003" "ENSG00000000005" "ENSG00000000419" "ENSG00000000457"
[5] "ENSG00000000460" "ENSG00000000938"
```

Q11. These are in ENSEMBLE format. I want "SYMBOL" ids.

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     keytype="ENSEMBL",   # The format of our genenames
                     column="SYMBOL",     # The new format we want to add
                     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>
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
                <numeric> <character>
ENSG00000000003  0.163035      TSPAN6
ENSG00000000005        NA        TNMD
ENSG00000000419  0.176032        DPM1
ENSG00000000457  0.961694       SCYL3
ENSG00000000460  0.815849    C1orf112
ENSG00000000938        NA         FGR
```

Let's add GENENAME

```
res$genename = mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
```

```
                    keytype="ENSEMBL",    # The format of our genenames
                    column="SYMBOL",      # The new format we want to add
                    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 8 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    genename
                <numeric> <character> <character>
ENSG00000000003  0.163035      TSPAN6      TSPAN6
ENSG00000000005        NA        TNMD        TNMD
ENSG00000000419  0.176032        DPM1        DPM1
ENSG00000000457  0.961694       SCYL3       SCYL3
ENSG00000000460  0.815849    C1orf112    C1orf112
ENSG00000000938        NA         FGR         FGR
```

```
  res$entrez=mapIds(org.Hs.eg.db,
                    keys=row.names(res), # Our genenames
                    keytype="ENSEMBL",    # The format of our genenames
                    column="SYMBOL",      # The new format we want to add
                    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 9 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    genename      entrez
                <numeric> <character> <character> <character>
ENSG00000000003  0.163035      TSPAN6      TSPAN6      TSPAN6
ENSG00000000005        NA        TNMD        TNMD        TNMD
ENSG00000000419  0.176032        DPM1        DPM1        DPM1
ENSG00000000457  0.961694       SCYL3       SCYL3       SCYL3
ENSG00000000460  0.815849    C1orf112    C1orf112    C1orf112
ENSG00000000938        NA         FGR         FGR         FGR
```

##Pathway analysis

We will use the **gage** package along with **pathview** here to do geneset enrichment (aka pathway analysis) and figure generation respectively.

```
library(pathview)
library(gage)
library(gageData)
```

Let's have a peak at the first two pathways in KEGG.

```
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"
```

What we need for `gage()` is our genes in ENTREZ id format with a measure of their importance.

It wants a vector of e.g. fold-changes.

```
foldchanges = res$log2FoldChange
head(foldchanges)
```

```
[1] -0.35070302          NA  0.20610777  0.02452695 -0.14714205 -1.73228897
```

Add ENTREZ ids as `names()` to my `foldchanges` vector.

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

```
     TSPAN6        TNMD        DPM1       SCYL3    C1orf112         FGR
-0.35070302          NA  0.20610777  0.02452695 -0.14714205 -1.73228897
```

Now we can run `gage()` with this input vector and the genset we want to examine for overlap/enrichment.

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

Look at the results.

```
attributes(keggres)
```

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

```
head(keggres$less,3)
```

```
                                      p.geomean stat.mean p.val q.val
hsa00232 Caffeine metabolism                 NA       NaN    NA    NA
hsa00983 Drug metabolism - other enzymes     NA       NaN    NA    NA
hsa01100 Metabolic pathways                  NA       NaN    NA    NA
                                      set.size exp1
hsa00232 Caffeine metabolism                 0   NA
hsa00983 Drug metabolism - other enzymes     0   NA
hsa01100 Metabolic pathways                  0   NA
```

We can view these pathways with our geneset genes highlighted using the `pathview()` function. E.g. for "Asthma" I will use the pathway.id hs05310 as seen above.

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

```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory /Users/lhmel/Desktop/BIMM 143/class13

Info: Writing image file hsa05310.pathview.png
```