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

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Bioconductor setup

We'll be using Bioconductor in this lab so:

```
# in console - install.packages("BiocManager")
# in console - BiocManager::install()
```

```
# For this class we will need DESeq2:
# in console - BiocManager::install("DESeq2")
```

To check install and enable these packages:

```
library(BiocManager)
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, saveRDS, setdiff,
    table, tapply, union, unique, unsplit, which.max, which.min


Attaching package: 'S4Vectors'

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

    findMatches

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

After downloading and moving the csvs to project directory:

```
# Complete the missing code
counts <- read.csv("airway_scaledcounts.csv", row.names=1)
metadata <-  read.csv("airway_metadata.csv")
```

Data heads:

```
heads(counts)
```

```
   group group_name value
1      1 SRR1039508   723
2      1 SRR1039508     0
3      1 SRR1039508   467
4      1 SRR1039508   347
5      1 SRR1039508    96
6      1 SRR1039508     0
7      2 SRR1039509   486
8      2 SRR1039509     0
9      2 SRR1039509   523
10     2 SRR1039509   258
11     2 SRR1039509    81
12     2 SRR1039509     0
13     3 SRR1039512   904
14     3 SRR1039512     0
15     3 SRR1039512   616
16     3 SRR1039512   364
17     3 SRR1039512    73
18     3 SRR1039512     1
19     4 SRR1039513   445
20     4 SRR1039513     0
21     4 SRR1039513   371
22     4 SRR1039513   237
23     4 SRR1039513    66
24     4 SRR1039513     0
25     5 SRR1039516  1170
26     5 SRR1039516     0
27     5 SRR1039516   582
28     5 SRR1039516   318
29     5 SRR1039516   118
30     5 SRR1039516     2
31     6 SRR1039517  1097
32     6 SRR1039517     0
```

```
33     6 SRR1039517   781
34     6 SRR1039517   447
35     6 SRR1039517    94
36     6 SRR1039517     0
37     7 SRR1039520   806
38     7 SRR1039520     0
39     7 SRR1039520   417
40     7 SRR1039520   330
41     7 SRR1039520   102
42     7 SRR1039520     0
43     8 SRR1039521   604
44     8 SRR1039521     0
45     8 SRR1039521   509
46     8 SRR1039521   324
47     8 SRR1039521    74
48     8 SRR1039521     0
```

```
heads(metadata)
```

```
   group group_name      value
1      1         id SRR1039508
2      1         id SRR1039509
3      1         id SRR1039512
4      1         id SRR1039513
5      1         id SRR1039516
6      1         id SRR1039517
7      2        dex    control
8      2        dex    treated
9      2        dex    control
10     2        dex    treated
11     2        dex    control
12     2        dex    treated
13     3   celltype     N61311
14     3   celltype     N61311
15     3   celltype    N052611
16     3   celltype    N052611
17     3   celltype    N080611
18     3   celltype    N080611
19     4     geo_id GSM1275862
20     4     geo_id GSM1275863
21     4     geo_id GSM1275866
22     4     geo_id GSM1275867
```

```
23     4     geo_id GSM1275870
24     4     geo_id GSM1275871
```

Q1. How many genes are in this dataset?

A1. There are 38694 genes in the dataset.

```
nrow(counts)
```

```
[1] 38694
```

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

A2. There are 4 control cell lines.

```
View(metadata)
# Counted 4 control cell lines.
```

Toy differential gene expression

```
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
```

Alternative to above use dplyr (preferred):

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

Q3. How would you make the above code in either approach more robust? Is there a function that could help here?

A3. Yes, use rowMeans() in place of rowSums(x)/#

```
library(dplyr)
control <- metadata %>% filter(dex=="control")
control.counts <- counts %>% select(control$id)
control.mean <- rowMeans(control.counts)
head(control.mean)
```

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

A4. See code below.

```
library(dplyr)
treated <- metadata %>% filter(dex=="treated")
treated.counts <- counts %>% select(treated$id)
treated.mean <- rowMeans(treated.counts)
head(treated.mean)
```

```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         658.00            0.00          546.00          316.50           78.75
ENSG00000000938
           0.00
```

Combining the two means:

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

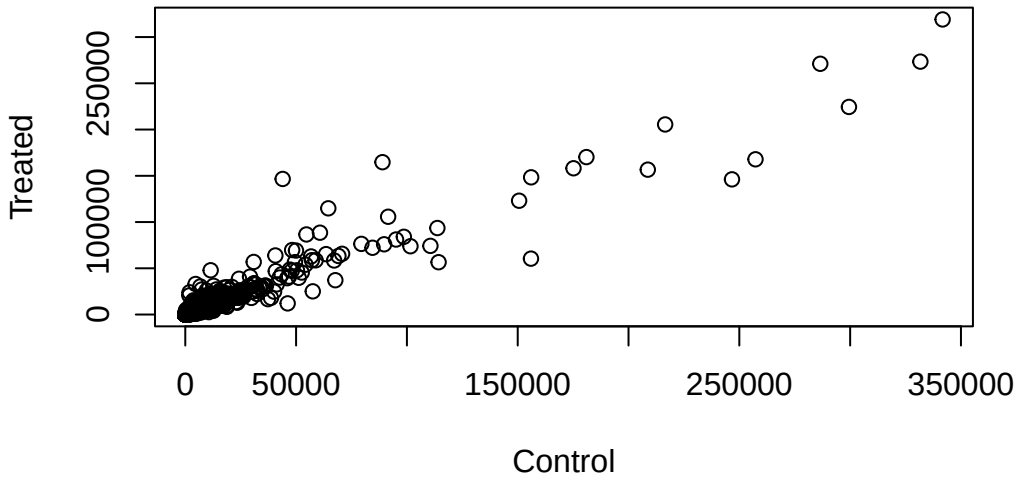
```
control.mean treated.mean
    23005324     22196524
```

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.

A5 (a). See code and result below.

Scatterplot:

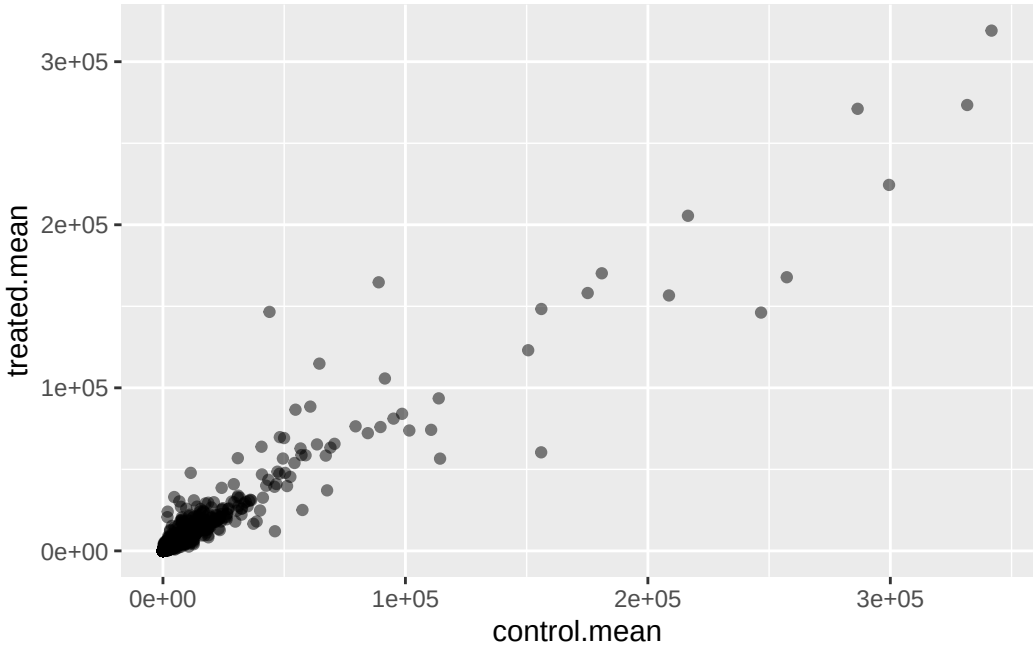
```
plot(meancounts[,1],meancounts[,2], xlab="Control", ylab="Treated")
```



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?

A5 (b). geom_point

```
# Install/update - install.packages(ggplot2)
library(ggplot2)
ggplot(meancounts, aes(control.mean, treated.mean))+
  geom_point(alpha=0.5)
```



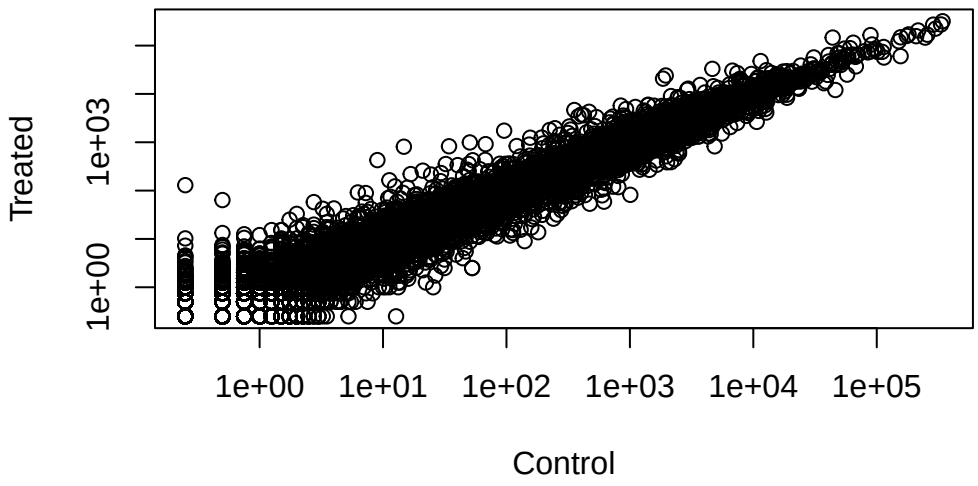
Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

A6. The argument to plot() is `log="xy"`.

```
plot(meancounts[,1],meancounts[,2], xlab="Control", ylab="Treated", 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
```

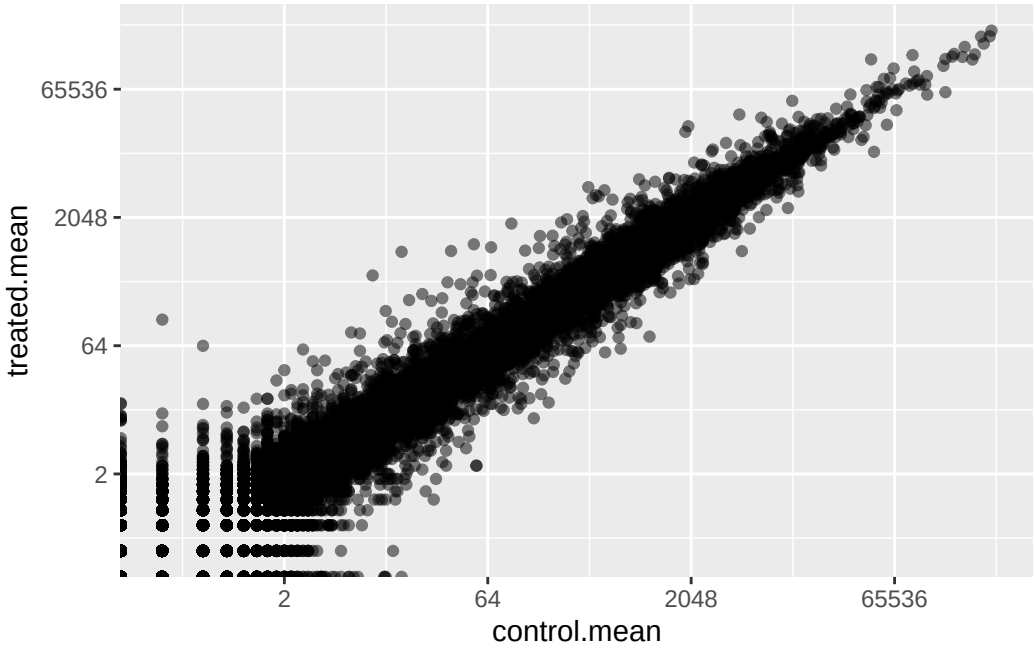


For ggplot() it's scale_x_continuous(trans="log2") and scale_y_continuous(trans="log2")

```
ggplot(meancounts, aes(control.mean, treated.mean))+
  geom_point(alpha=0.5)+
  scale_x_continuous(trans="log2")+
  scale_y_continuous(trans="log2")
```

```
Warning in scale_x_continuous(trans = "log2"): log-2 transformation introduced
infinite values.
```

```
Warning in scale_y_continuous(trans = "log2"): log-2 transformation introduced
infinite values.
```




```
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
```

```
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
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
```

Now our data has been filtered >:)

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?

> A7. arr.ind functions in the which() function call to return the indices (row & column) with true values with the previous ==0, so row indices and column indices that have zero values. Then taking the first column instead of both so we can count a row twice in case there were any with zero values in both control.mean and treated.mean columns

```
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?

> A8. Yes, we can by counting how many TRUE values from it using sum, as FALSE is 0 and TRUE is 1.

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

```
[1] 250
```

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

> A9. Yes, same logic.

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

```
[1] 367
```

Q10. Do you trust these results? Why or why not?

> A10. Not quite, log2FoldChange seems rather arbitrary as a definition of up-/down-regulation, especially only by 2. But if it's a common threshold, maybe it's not so bad?

Setting up for DESeq

```
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
```

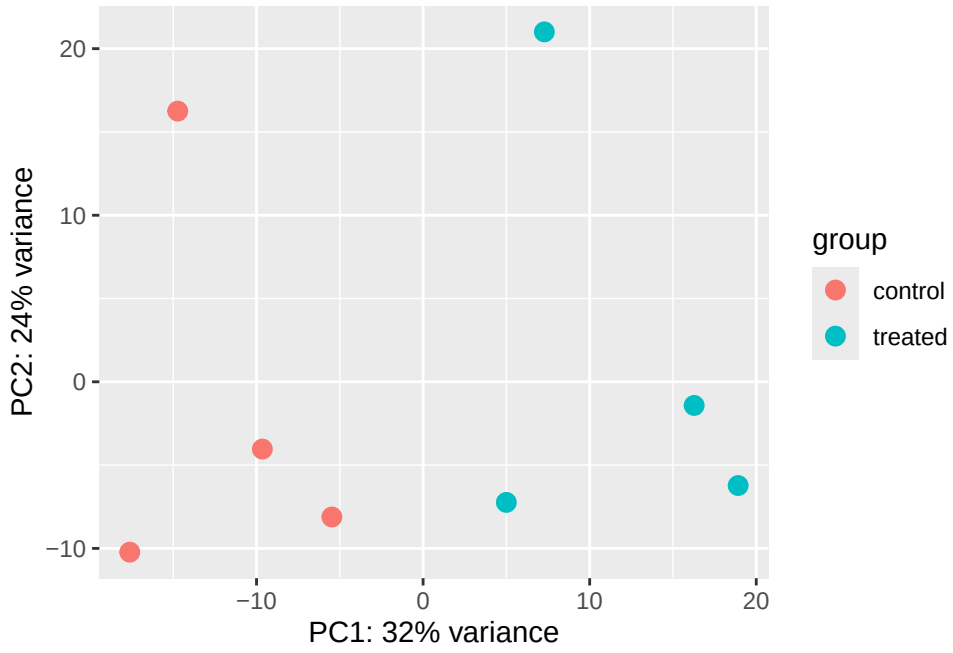
```
  ENSG00000283123
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(4): id dex celltype geo_id
```

Principal Component Analysis (PCA)

Our old friend...

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

```
using ntop=500 top features by variance
```



This was made possible by the DESeq package. Now for ggplot2.

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

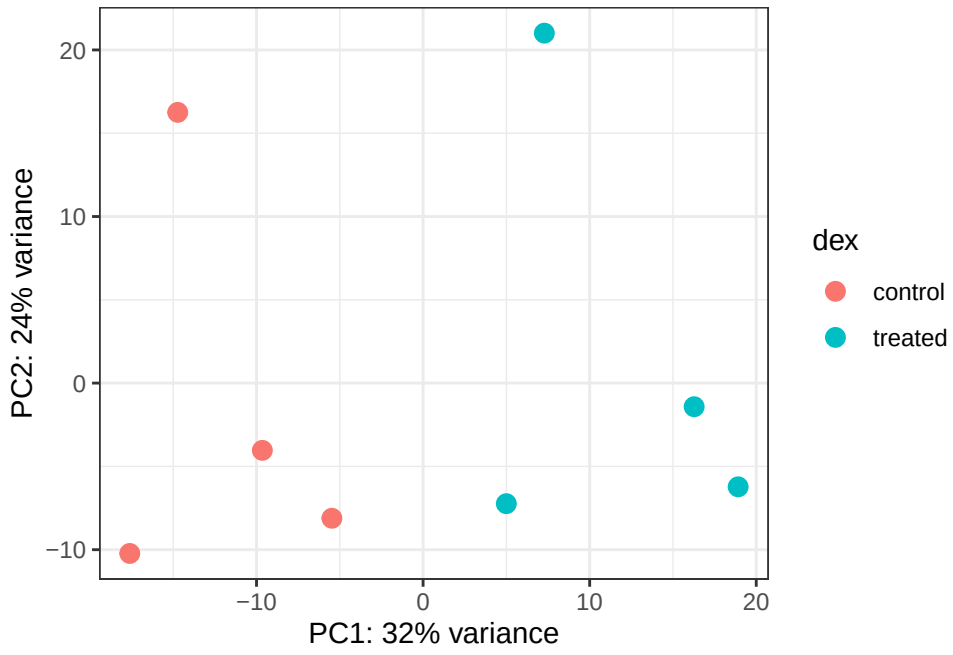
```
using ntop=500 top features by variance
```

```
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()
```



Clearly more code to write...

DESeq analysis

```
# results(dds) pulls an error
dds <- DESeq(dds)
```

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```
# To get 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>
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
```

```
# as.data.frame(res)
# View(res)
```

```
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
```

Adding annotation data

```
# Install and enable:
# BiocManager::install("AnnotationDbi")
# BiocManager::install("org.Hs.eg.db")
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 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       FIRRM
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.

A11. See code below.

For Entrez ID

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

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

For Uniprot accession

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

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

For gene name

```
res$genename <- mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     column="GENENAME", # New format
                     keytype="ENSEMBL",
                     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 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  A0A087WYV6
ENSG00000000005        NA        TNMD       64102      Q9H2S6
ENSG00000000419  0.176032        DPM1        8813      H0Y368
ENSG00000000457  0.961694       SCYL3       57147      X6RHX1
ENSG00000000460  0.815849       FIRRM       55732      A6NFP1
ENSG00000000938        NA         FGR        2268      B7Z6W7
                           genename
                        <character>
ENSG00000000003          tetraspanin 6
ENSG00000000005            tenomodulin
ENSG00000000419 dolichyl-phosphate m..
ENSG00000000457 SCY1 like pseudokina..
ENSG00000000460 FIGNL1 interacting r..
ENSG00000000938 FGR proto-oncogene, ..
```

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

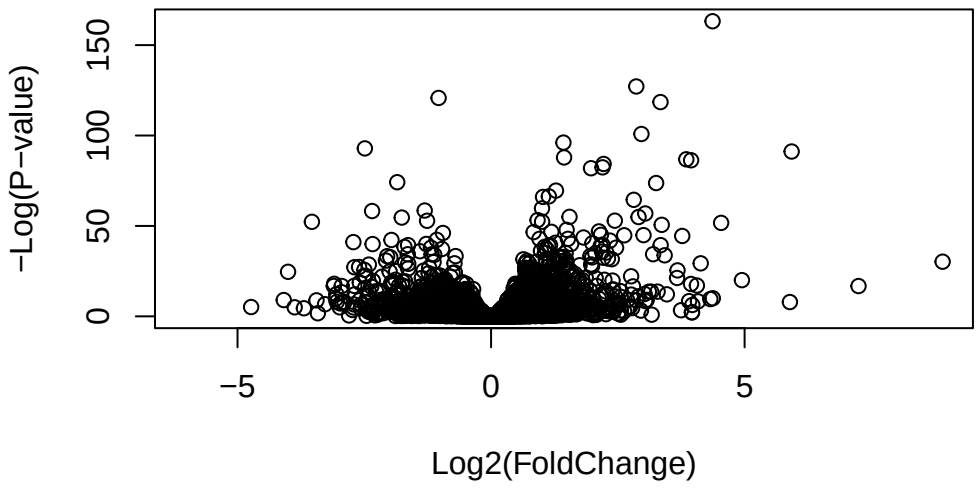
```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 10 columns
                 baseMean log2FoldChange     lfcSE      stat      pvalue
                <numeric>      <numeric> <numeric> <numeric>   <numeric>
ENSG00000152583   954.771        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      B4E2Z0
ENSG00000179094 6.13966e-56        PER1        5187      A2I2P6
ENSG00000116584 3.49776e-53     ARHGEF2        9181  A0A8Q3SIN5
ENSG00000189221 3.46227e-52        MAOA        4128      B4DF46
ENSG00000120129 1.59454e-44       DUSP1        1843      B4DRR4
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")
# Woah we just wrote our own csv :0
```

Data Visualization

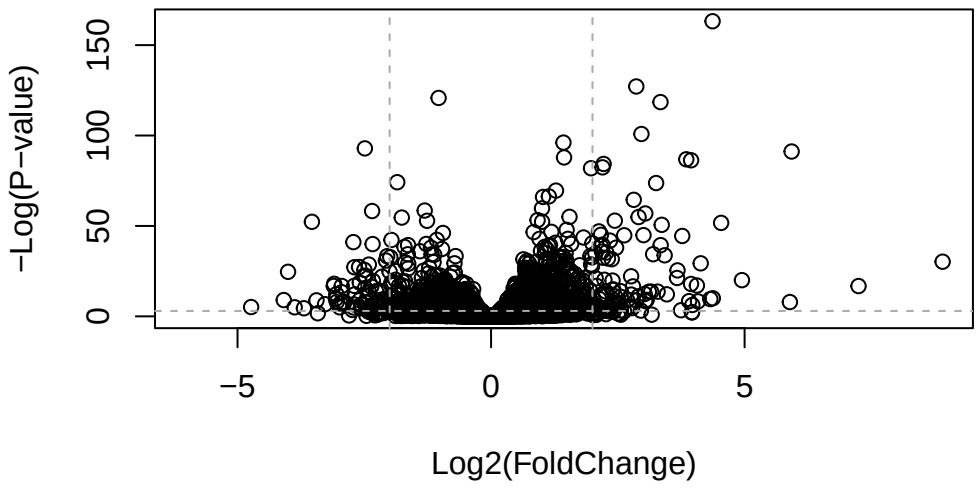
Volcano Plots

```
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)
```

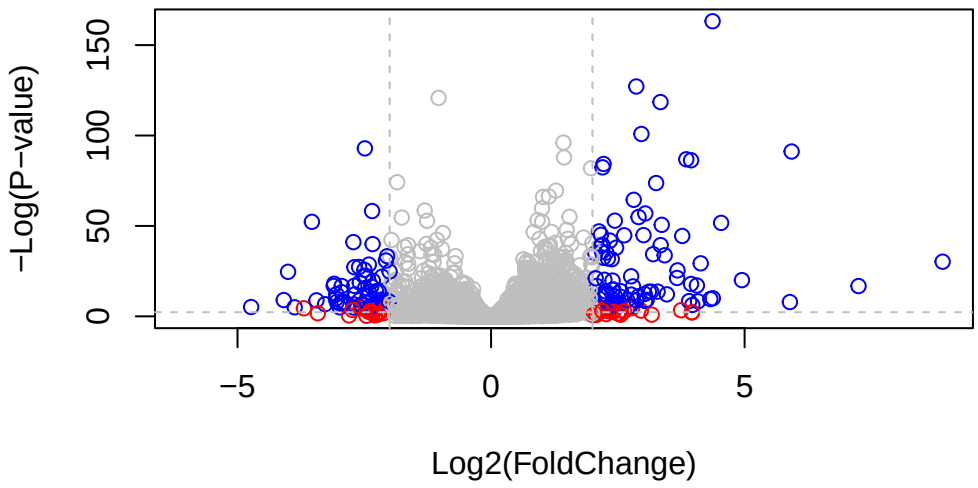



```
# Setup our custom point color vector
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "red"

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)
```



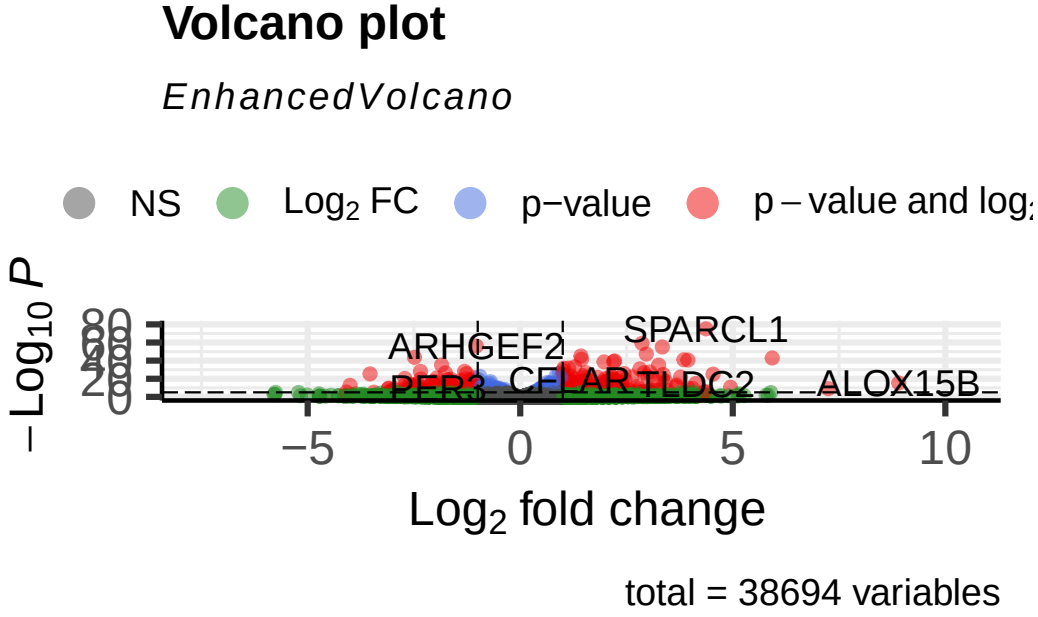

```
# in console - BiocManager::install("EnhancedVolcano")
```

```
library(EnhancedVolcano)
```

```
Loading required package: ggrepel
```

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

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


Volcano plot
EnhancedVolcano
NS
Log2 FC
p−value
p – value and log2
−Log10 P
80
60
40
20
0
ARHGEF2
SPARCL1
PFR3
CFLAR
TLDC2
ALOX15B
−5
0
5
10
Log2 fold change
total = 38694 variables