Class 12: Transcriptomics and the Analysis of RNA-Seq Data

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5/12/23

First, we installed the DESeq package before moving on to the second portion of the lab.

2. Import countData and colData

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

Now, we will have a brief look at the data

```r
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
```

```r
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?

```r
nrow(counts)
```

```
[1] 38694
```

There are 38694 genes in the dataset

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

There are 4 control cell lines.

3. Toy Differential Gene Expression

First, we will get a better understanding of differential gene expression analysis.

```r
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
```

We will separate this code out to understand it better:

```r
metadata[,"dex"] == "control"
```

```
[1]  TRUE FALSE  TRUE FALSE  TRUE FALSE  TRUE FALSE
```

This table shows just the control samples

```
control <- metadata[metadata[,"dex"] == "control", ]

control
```

```
          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
```

Now, we can separate out the id's

```
control$id
```

```
[1] "SRR1039508" "SRR1039512" "SRR1039516" "SRR1039520"
```

Now, we can use this as a subset in the counts data set and summarize the rows using the mean of the rows.

```
control.counts <- counts[,control$id]
control.means <- rowMeans(control.counts)
```

Q3. How would you make the above code in either approach more robust?

We can use the rowMeans function. This allows the function to be applied to other datasets without having to alter the value that you are dividing by. In this particular data set, we divide by 4 since there are 4 genes, but this is not the case for all datasets. The number of values in each row is going to be different.

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

```
treated <- metadata[metadata[,"dex"] == "treated",]
treated$id
```

```
[1] "SRR1039509" "SRR1039513" "SRR1039517" "SRR1039521"
```

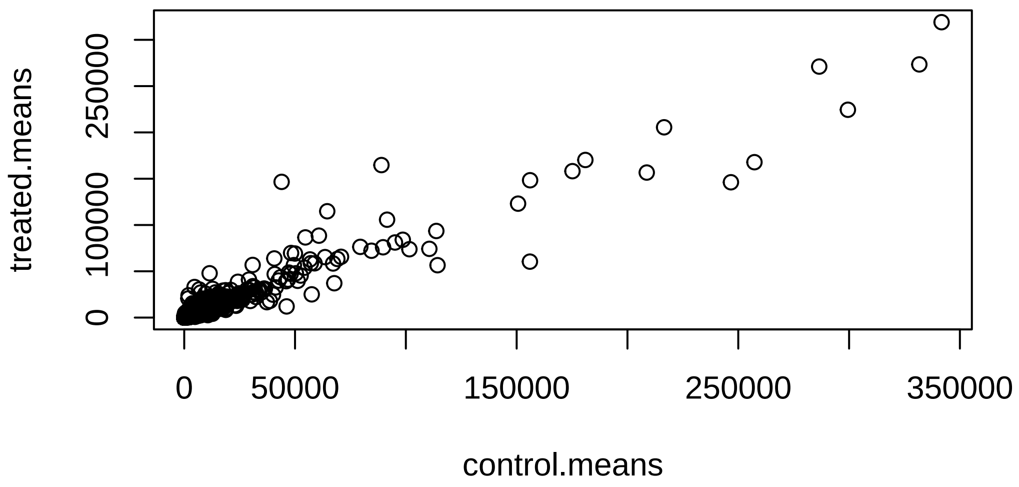
```
treated.counts <- counts[,treated$id]
treated.means <- rowMeans(treated.counts)

#View(as.data.frame(treated.means))
```

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

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

```
plot(meancounts)
```

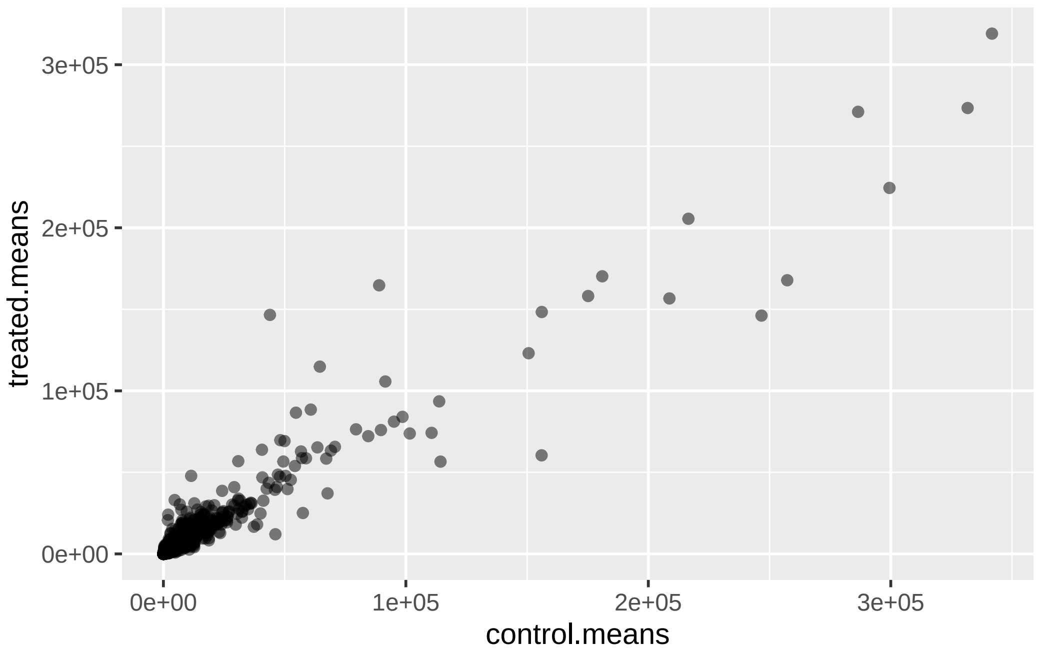


Now, we can make the plot a little fancier by using ggplot2.

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?

You would use the geom_point function.

```
library(ggplot2)
ggplot(counts, aes(control.means, treated.means)) + geom_point(alpha = 0.5)
```

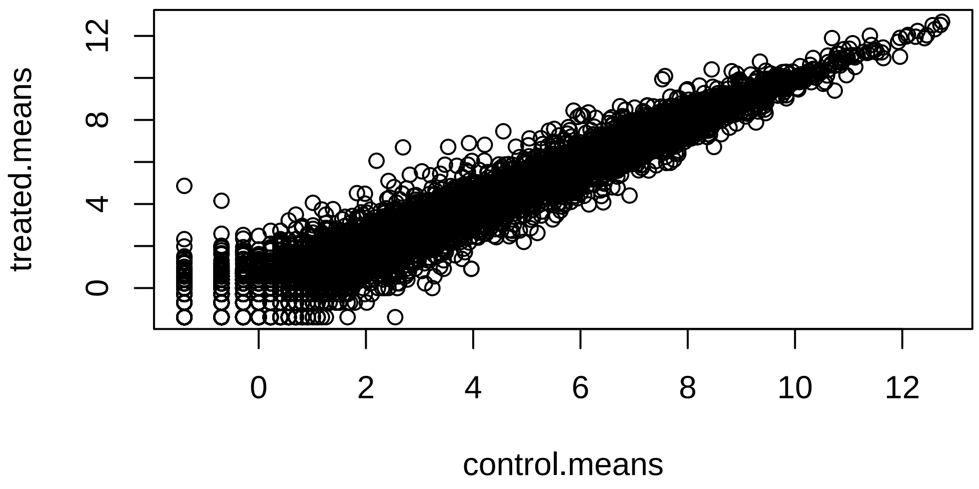


Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

The argument we can use is `log()`

Note: this emits the 0s

```
plot(log(meancounts))
```



To calculate the log2 of the fold change between treated and control...

We need to add a log2 column to the data frame

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

```
                control.means treated.means       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
```

To remove zero values...

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

This gives us the row and the column where we have a 0.

```
#which(meancounts[,1:2] == 0, arr.ind = TRUE)
```

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 unique() function?

The arr.ind argument will tell us the row and column numbers for where we have 0s in the data frame instead of just gettting a matrix of TRUE and FALSE. We then need to use the unique() function to avoid repetitions.

Overexpressed and underexpressed genes

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

```
up.ind
FALSE  TRUE
21567   250
```

```
table(down.ind)
```

```
down.ind
FALSE  TRUE
21450   367
```

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

We have 250 genes.

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

We have 367 genes.

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

No. We would need to see the p-values first since we need some quantitative analysis to see if our results are significant. When we actually run the DESeq analysis, we find that the boundaries for the fold-change are much smaller.

4. DESeq2 Analysis

First, we need to load in the package

```r
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
```

```r
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},
  }
```

Let's generate the specific object DESeq2 needs:

```r
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
```

```r
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

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

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

```r
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
```

```
#always use the padj column for analysis
```

With the summary command, we can have a quick overview of the results

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

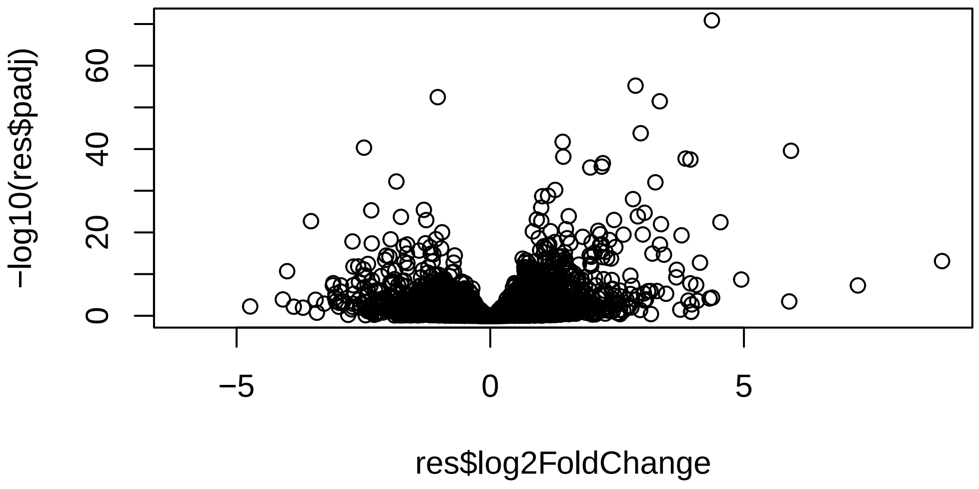
```

out of 25258 with nonzero total read count
adjusted p-value < 0.05
LFC > 0 (up)       : 1242, 4.9%
LFC < 0 (down)     : 939, 3.7%
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
```

6. Data Visualization

Volcano Plots

```
plot(res$log2FoldChange, -log10(res$padj))
```


−log10(res$padj)
res$log2FoldChange