Class 12

Arturo Agramont

2. Import countData and colData

We will start by loading the airway data, we will import count and metadata

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

```
head(counts)
```

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

```
head(metadata)
```

```
          id     dex celltype     geo_id
1 SRR1039508 control   N61311 GSM1275862
2 SRR1039509 treated   N61311 GSM1275863
```

```
3 SRR1039512 control  N052611 GSM1275866
4 SRR1039513 treated  N052611 GSM1275867
5 SRR1039516 control  N080611 GSM1275870
6 SRR1039517 treated  N080611 GSM1275871
```

```
nrow(counts)
```

```
[1] 38694
```

```
control_cell_lines = table(metadata$dex)['control']
```

- **Q1.** How many genes are in this dataset? In this code there are 38694genes
- **Q2.** How many 'control' cell lines do we have? We have 4 control cell lines in this data.

3. Toy differential gene expression

Code that was provided

```
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
```

Breaking down the code to understand it

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

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

```
control = metadata[metadata[,"dex"]=="control",]
control$id
```

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

```r
control.counts = counts[,control$id]
control.mean <- rowMeans( control.counts )
```

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

```r
 control <- metadata[metadata[,"dex"]=="control",]
control.counts <- counts[ ,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

```r
treated = metadata[metadata[,"dex"]=="treated",]
treated.counts = counts[ ,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
```

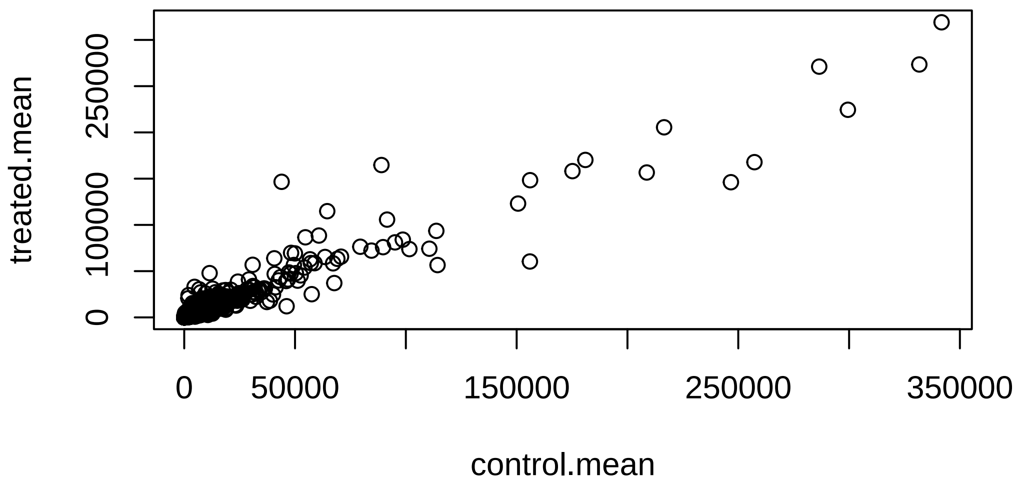
Combining both means

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

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

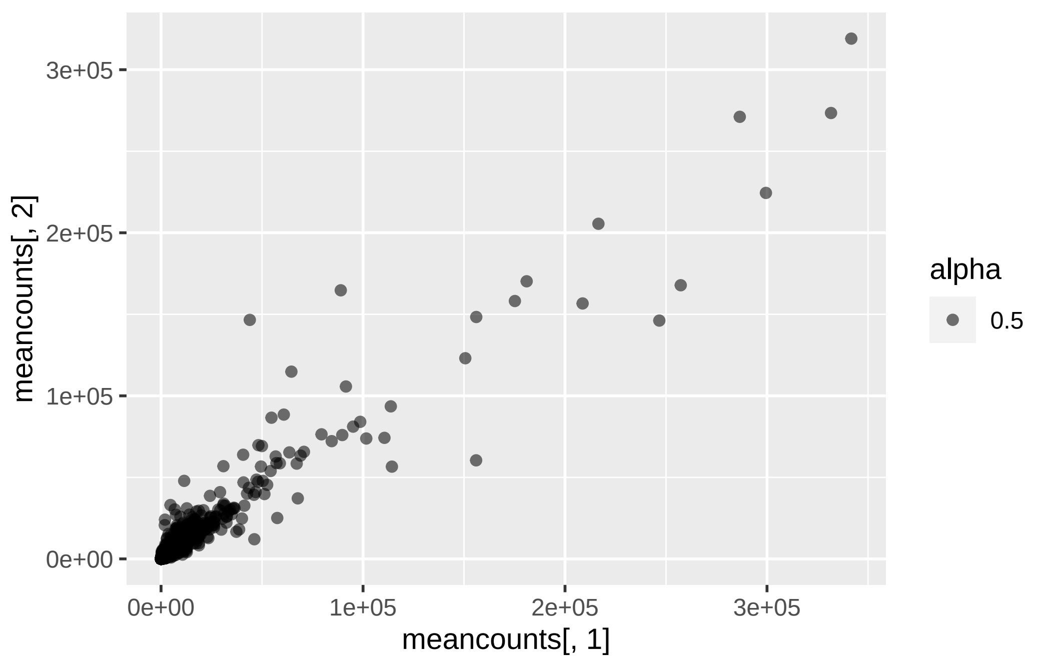
- **Q5 (a).** Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

```r
plot(meancounts)
```



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 geom_point()

```r
library(ggplot2)
ggplot(meancounts) + geom_point(aes(meancounts[,1], meancounts[,2], alpha = 0.5))
```

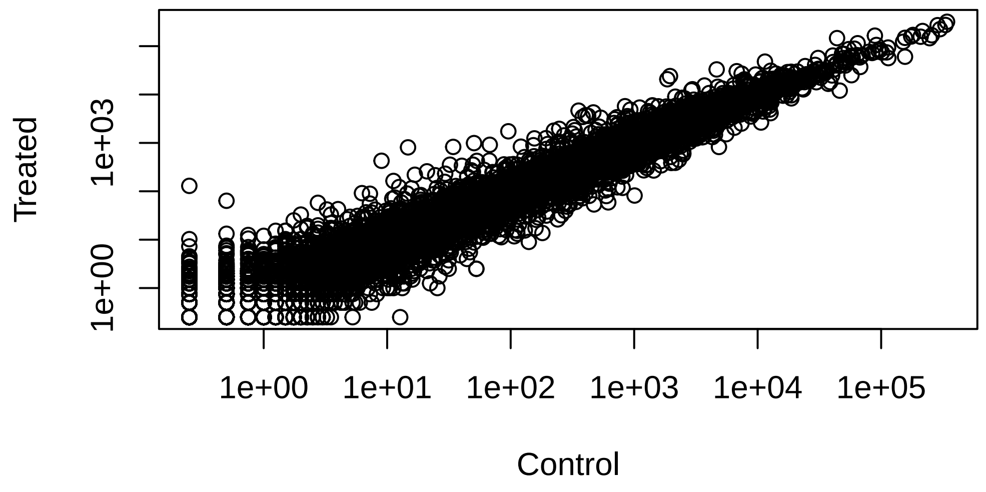


Q6. Try plotting both axes on a log scale. What is the argument to **plot()** that allows you to do this?

```
plot(meancounts, log = "xy", xlab = "Control", ylab = "Treated")
```

```
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
```



We will begin to take log2 of the data in order to find larger mathematical differences between control and treated. Log2 of the fold change between both.

```
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
```

We will try to remove and filter out zeros so that we do not get the same errors

```
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
```

- **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?

Overexpressed and underexpressed genes

```
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?

```
table(up.ind)
```

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

There are 250 up regulated genes

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

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

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

There are 367 down regulated genes

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

  No there are many other factors that need to be included into having statistical significance. We need to bring the statistics that represent why the difference matters.

4. DESeq2 analysis

first step, loading the library

```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
```

```
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 that DESeq2 needs:

```
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
DESeq(dds)
```

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```
class: DESeqDataSet
dim: 38694 8
metadata(1): version
assays(4): counts mu H cooks
rownames(38694): ENSG00000000003 ENSG00000000005 ... ENSG00000283120
  ENSG00000283123
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(5): id dex celltype geo_id sizeFactor
```