

Class 12: Transcriptomics and the analysis of RNA-Seq data

Heidi Nam

Bioconductor and DESeq2 setup

code used to set up packages needed for this lab:

```
# install.packages("BiocManager")
# BiocManager::install()
# BiocManager::install("DESeq2")
```

Import countData and colData

importing data used for this lab:

```
counts <- read.csv("airway_scaledcounts.csv", row.names=1)
metadata <- read.csv("airway_metadata.csv")
control_cell_lines <- table(metadata$dex)['control']
```

Q1 answer: There are 38694 genes in the dataset.

Q2 answer: We have 4 ‘control’ cell lines in this dataset.

Toy differential gene expression

let’s look at differential gene expression analysis:

```
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
ENSG0000000000938
          0.75

```

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

- when calculating the mean, instead of putting 4 (which shows the number of control lines) we could use `nrow(control)` which would make the `control.mean` a lot more robust.
- or, just using `rowMeans(control.counts)`

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 )/4
head(treated.mean)

```

```

ENSG000000000003 ENSG000000000005 ENSG000000000419 ENSG000000000457 ENSG000000000460
          658.00           0.00           546.00           316.50           78.75
ENSG0000000000938
          0.00

```

placing `meancount` data into a dataframe:

```

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

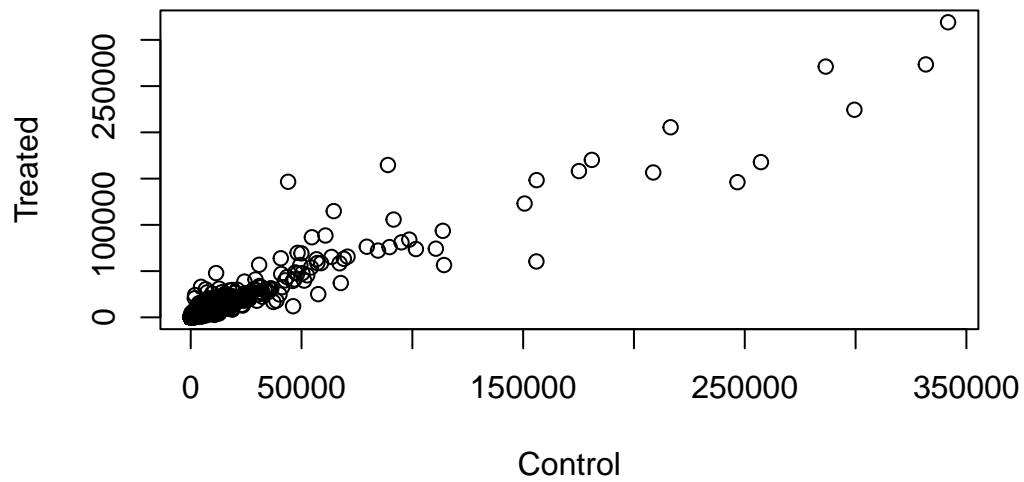
```

Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples.

```

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

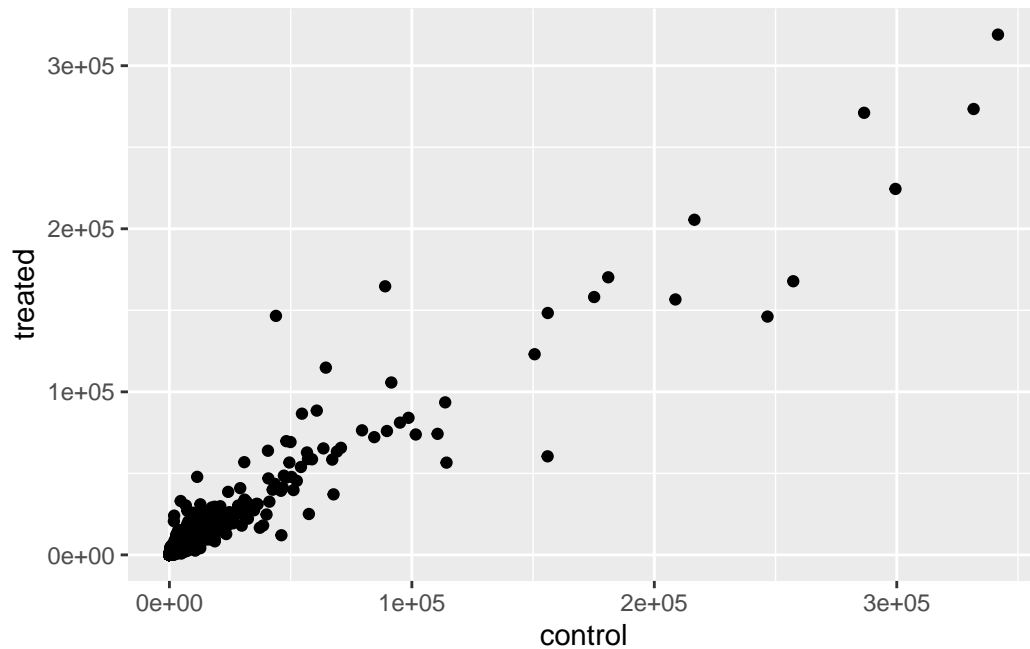
```



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?

- `geom_point()` would be used for this plot.

```
library(ggplot2)
ggplot(meancounts) +
  aes(x=meancounts[,1], y=meancounts[,2]) +
  labs(x = "control", y = "treated") +
  geom_point()
```



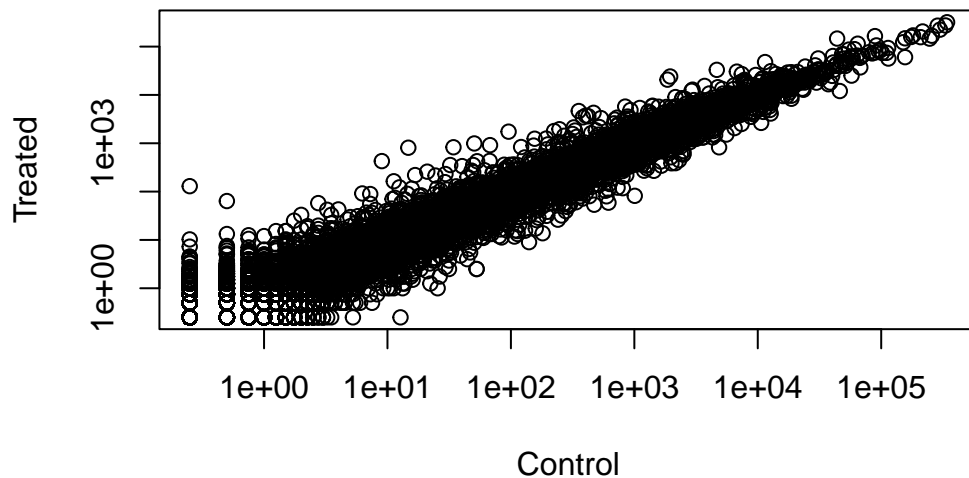
Q6. Try plotting both axes on a log scale. What is the argument to **plot()** that allows you to do this?

- `log = "xy"`

```
plot(meancounts[,1], meancounts[,2], 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



looking at log2 of the fold change between and treated and control:

```
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

filtering weird results by removing zero values:

```
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
ENSG000000001036	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?

- The `arr.ind` calls anything that shows anything where they have zero counts for both control mean and treated mean; the first column was taken in order to ignore genes that have zero counts in any sample; the `unique()` function ensured we don't count genes twice if both of their entries are zero.

filtering for over and under-expressed 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?

- there are 250 genes that are up regulated.

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

- there are 367 genes that are down regulated.

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

- These results are not reliable as we have not brought in statistical analysis to it; we do not know if the results are statistically significant in order to trust these results.

DESeq2 Analysis

Loading the library:

```
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

Attaching package: 'IRanges'

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

windows

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")
```

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

```
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)
```

```
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

Changing p-value to 0.05:

```
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 plots:

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

