Class 13: Transcriptomics

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```r
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, setdiff, sort,
    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

DESeq2 Required Inputs: As input, the DESeq2 package expects (1) a data.frame of count data (as obtained from RNA-seq or another high-throughput sequencing experiment) and (2) a second data.frame with information about the samples - often called sample metadata (or colData in DESeq2-speak because it supplies metadata/information about the columns of the countData matrix) (Figure 2).

```
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
```

```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
```

```r
# View(counts)
# View(metadata)
```

> Q1. How many genes are in this dataset?

```r
nrow(counts)
```

```
[1] 38694
```

> Q. How many cell lines are there?

```r
ncol(counts)
```

```
[1] 8
```

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

There are 4 control cell lines.

```r
table(metadata$dex)
```

```

control treated 
      4       4 
```

```
# or
sum(metadata$dex == "control")
```

```
[1] 4
```

Lets verify that the metadata names match the counts name. (The cell line names are the same)

```
colnames(counts) == metadata$id #verify that the columns are in the same order
```

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

Can also use the `all()` function, a common flow-control function to verify if your statements are true.

```
all(colnames(counts) == metadata$id) #verify that the columns are in the same order
```

```
[1] TRUE
```

What do we want to compare? We would like to first start with the mean of gene expression between the control groups and the treatmetn group. So we must subset all the control data and treatment data into separate groups.

```
control_inds <- metadata$dex == "control" #gives us the indexes (column position) of every
control_counts <- counts[,control_inds] #applies that indexes to the data and puts into a
control_means <- apply(control_counts, 1, mean) #apply mean function across the rows of th
```

Lets do the same for the treated group.

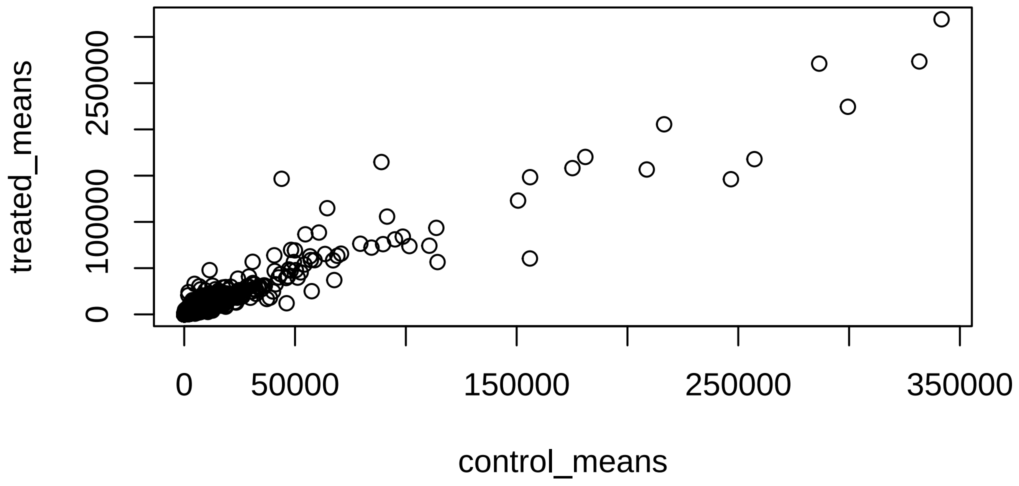
```
treated_inds <- metadata$dex == "treated"
treated_counts <- counts[,treated_inds]
treated_means <- apply(treated_counts, 1, mean)
```

Lets combine them back together for data saving purposes.

```
mean_counts <- 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 something like the following.

```r
plot(mean_counts)
```



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

```r
head(mean_counts)
```

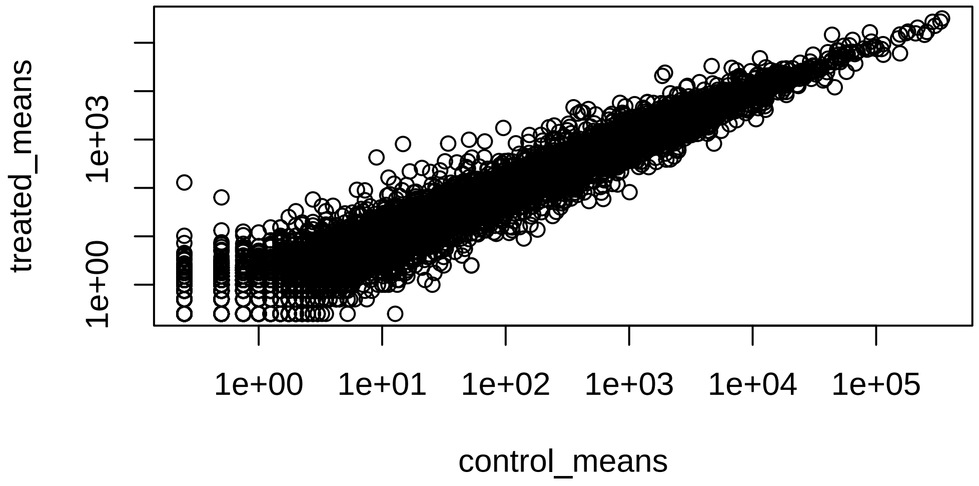
```
                control_means treated_means
ENSG00000000003        900.75        658.00
ENSG00000000005          0.00          0.00
ENSG00000000419        520.50        546.00
ENSG00000000457        339.75        316.50
ENSG00000000460         97.25         78.75
ENSG00000000938          0.75          0.00
```

We have such skewed data that we need to adjust the axis to see more of the data.

```r
plot(mean_counts, 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
```



We can look at differences here via a fraction between conditions in the data to give us log-fold changes in expression.

```
mean_counts$log_change <- log2(treated_means/control_means)
```

We have data that has no values, thus giving us weird log values. So lets filter out that data to only use the data that makes sense.

```
zero.sums <- (rowSums(mean_counts[,1:2] == 0)) #turn into a logical finding everything in
to.rm.ind <- zero.sums > 0 #gives us opposite of what we want
my_counts<- mean_counts[!to.rm.ind,] #exclamation point switches the logicals
```

A common threshold for calling something “differentially expressed” is a log2 fold-change value of +2 or -2.

How many of our remaining genes are “up” regulated?

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

```r
sum(my_counts$log_change >= +2)
```

```
[1] 314
```

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

```r
sum(my_counts$log_change <= -2)
```

```
[1] 485
```

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

No I do not since we havent accounted for significance and this was taken across the mean of the samples.

#DESeq2 Analysis

Lets use the DESeq2 package to do this anaylsis properly. DESeq requires a specific format of your data to be compatible.

```r
dds<- DESeqDataSetFromMatrix(counts, metadata, ~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<- DESeq(dds)
```

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

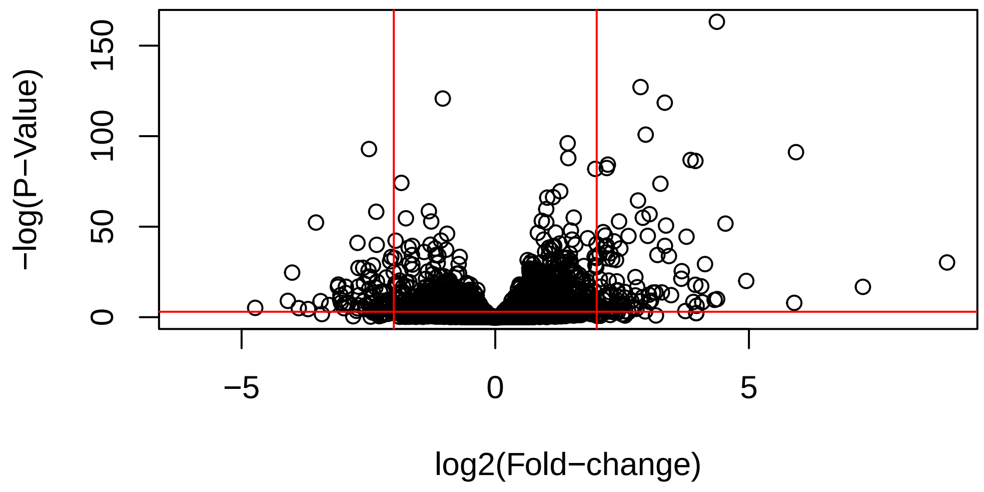
```
res <- results(dds)
head(res)
```

```
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
```

Volcano plot

A common visualization for this type of data is called a volcano plot.

```
plot(res$log2FoldChange, -log(res$padj), ylab = "-log(P-Value)", xlab = "log2(Fold-change)
abline(v=-2, col = "red")
abline(v=+2, col ="red")
abline(h = -log(0.05), col = "red")
```



Save our results thus far. Thus far it has not been filtered or annotated, so not very useful as is.

```r
write.csv(res, file = "myresults.csv")
```

Adding annotations

Lets add the proper gene names to the dataset that we can evaluate and reference known pathways later.

Our table thus far contains the Ensembl gene IDs, but are not the actual gene names. So we can use some packages to aid us in translating these IDs into their gene names.

```r
library(AnnotationDbi)
library(org.Hs.eg.db)
```

```r
columns(org.Hs.eg.db) #These are all the databases useable to reference
```

```
 [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 from the AnnotationDbi package is called `mapIds()`.

We can use the `mapIds()` function to add individual columns to our results table. We provide the row names of our results table as a key, and specify that keytype=ENSEMBL. The column argument tells the `mapIds()` function which information we want, and the multiVals argument tells the function what to do if there are multiple possible values for a single input value. Here we ask to just give us back the first one that occurs in the database.

```
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") #default is first but can be changed
```

```
'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
```

Run it again and add the GENENAME, ENTREZ and UNIPROT names

```r
res$entrez <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     column="ENTREZID", # The new format we want to add
                     keytype="ENSEMBL", # The format of our genenames
                     multiVals="first")
```

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

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

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

```r
res$genename <- mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     column="GENENAME",
                     keytype="ENSEMBL",
                     multiVals="first")
```

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

Finally, lets resave our data with all the proper tags.

```r
write.csv(res, file = "myresults.csv")
```