class13

Wanning Cui

```
library(BiocManager)
Warning: package 'BiocManager' was built under R version 4.3.2
  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

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

Warning: package 'matrixStats' was built under R version 4.3.2

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, rowWeightedMedians, rowWeightedMedians, rowWeightedMedians, 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

##Data import

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

Q1. How many genes are in this dataset?

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		

nrow(counts)

[1] 38694

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

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
```

sum(metadata\$dex=="control")

```
[1] 4
```

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

control treated 4 4

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
7 SRR1039520 control N061011 GSM1275874
8 SRR1039521 treated N061011 GSM1275875
```

I want to compare the control to the treated columns. To do this, I will

-Step 1. Identify and extract the "control" columns. -Step 2. Calculate the mean value per gene for all these "control" columns and save as control.mean -Step 3. Do the same for treated -Step 4. Compare the control.mean and treated.mean values

Step1:

```
SRR1039508 SRR1039512 SRR1039516 SRR1039520
ENSG0000000003
                       723
                                   904
                                             1170
                                                         806
                                    0
ENSG0000000005
                         0
                                                0
                                                           0
ENSG00000000419
                       467
                                   616
                                              582
                                                         417
ENSG00000000457
                       347
                                   364
                                              318
                                                         330
ENSG0000000460
                        96
                                    73
                                                         102
                                              118
ENSG00000000938
                         0
                                    1
                                                2
                                                           0
```

```
control.mean <- rowMeans(counts[,control.inds])
head(control.mean)</pre>
```

```
ENSG0000000003 ENSG0000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
900.75 0.00 520.50 339.75 97.25
ENSG00000000938
0.75
```

```
treated.mean <- rowMeans(counts[,metadata$dex=="treated"])
head(treated.mean)</pre>
```

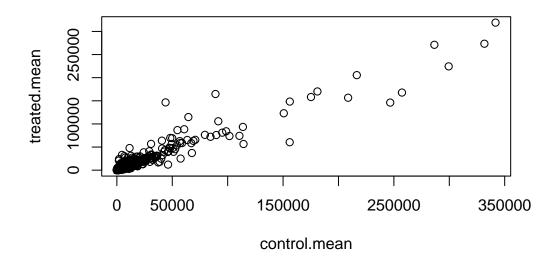
```
ENSG00000000003 ENSG0000000005 ENSG000000000419 ENSG00000000457 ENSG00000000460 658.00 0.00 546.00 316.50 78.75 ENSG00000000938 0.00
```

We will combine our mean data counts

```
meancounts <- data.frame(control.mean, treated.mean)</pre>
```

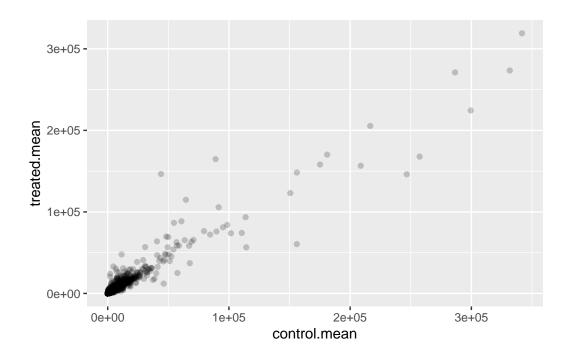
Let's see what these count values look like

```
plot(meancounts)
```



```
library(ggplot2)

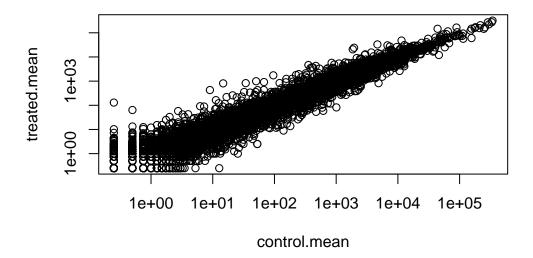
ggplot(meancounts)+
  aes(control.mean,treated.mean)+
  geom_point(alpha=0.2)
```



plot(meancounts,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



Logs are super useful when we have such skewed data

```
#Treated/control log2(10/10)
```

[1] 0

log2(20/10)

[1] 1

Add log2(Fold-change) values to our wee results table.

control.mean treated.mean log2fc ENSG0000000003 900.75 658.00 -0.45303916

NaN	0.00	0.00	ENSG00000000005
0.06900279	546.00	520.50	ENSG00000000419
-0.10226805	316.50	339.75	ENSG00000000457
-0.30441833	78.75	97.25	ENSG00000000460
-Inf	0.00	0.75	ENSG00000000938

I need to exclude any genes with zero counts as we can't say anything about them anyways from this experiment and it causes me math pain.

```
# What values in the first two cols are zero
to.rm.inds <- rowSums(meancounts[,1:2]==0) > 0
mycounts <- meancounts[!to.rm.inds,]</pre>
```

Q. How many genes do I have left

```
nrow(mycounts)
```

[1] 21817

Q.How many genes are "up regulated" i.e. have a log2(fold-change) greater than +2?

```
sum(mycounts$log2fc > +2)
```

[1] 250

Q. How many genes are "down regulated"?

```
sum(mycounts$log2fc < -2)</pre>
```

[1] 367

Q. We don't trust the data results because there are a lot of missing information ##Running DESeq

Like many bioconductor analysis packages DESeq wants its input in a very particular way.

```
design=~dex)
```

converting counts to integer mode

Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in design formula are characters, converting to factors

To run DESeq analysis, we call the main function from the package called DESeq()

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

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

To get the results out of this dds object, we can use the DESeq results() function.

```
res <- results(dds)
head(res)</pre>
```

log2 fold change (MLE): dex treated vs control

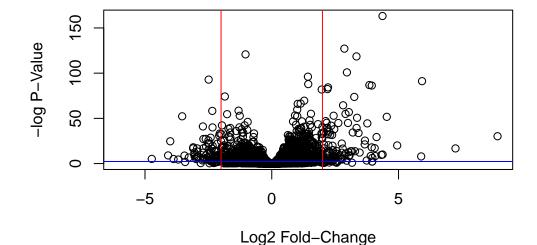
Wald test p-value: dex treated vs control

DataFrame with 6 rows and 6 columns

pvalue	stat	lfcSE	log2FoldChange	baseMean	
<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	
0.0371175	-2.084470	0.168246	-0.3507030	747.194195	ENSG00000000003
NA	NA	NA	NA	0.000000	ENSG00000000005
0.0414026	2.039475	0.101059	0.2061078	520.134160	ENSG00000000419
0.8658106	0.168982	0.145145	0.0245269	322.664844	ENSG00000000457

```
-0.1471420 0.257007 -0.572521 0.5669691
ENSG00000000460
                 87.682625
ENSG00000000938
                  0.319167
                               -1.7322890 3.493601 -0.495846 0.6200029
                     padj
                <numeric>
ENSG00000000003
                0.163035
ENSG0000000005
ENSG00000000419
                 0.176032
ENSG00000000457
                 0.961694
ENSG00000000460
                 0.815849
ENSG00000000938
                       NA
```

A common summary visualization is called a Volcano plot.



```
[1] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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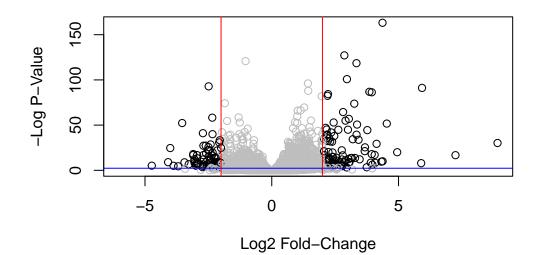
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[16] "OMIM"
                     "ONTOLOGY"
                                     "ONTOLOGYALL" "PATH"
                                                                     "PFAM"
                                                                     "UCSCKG"
[21] "PMID"
                     "PROSITE"
                                     "REFSEQ"
                                                     "SYMBOL"
[26] "UNIPROT"
  res$symbol <- mapIds(org.Hs.eg.db,</pre>
                         keys=row.names(res), #Our genenames
                         keytype="ENSEMBL",
                         column="SYMBOL",
                         multiVals="first")
'select()' returned 1:many mapping between keys and columns
  head(res)
```

Wald test p-value: dex treated vs control DataFrame with 6 rows and 7 columns baseMean log2FoldChange lfcSE pvalue stat <numeric> <numeric> <numeric> <numeric> <numeric> ENSG00000000003 747.194195 -0.3507030 0.168246 -2.084470 0.0371175 ENSG0000000005 0.000000 NAENSG00000000419 520.134160 0.2061078 0.101059 2.039475 0.0414026 ENSG00000000457 322.664844 0.0245269 0.145145 0.168982 0.8658106 -0.1471420 0.257007 -0.572521 0.5669691 ENSG00000000460 87.682625 ENSG00000000938 -1.7322890 3.493601 -0.495846 0.6200029 0.319167 padj symbol <numeric> <character> ENSG0000000000 0.163035 TSPAN6 ENSG00000000005 NATNMD ENSG00000000419 0.176032 DPM1 ENSG00000000457 0.961694 SCYL3 ENSG00000000460 0.815849 FIRRM ENSG00000000938 NA FGR res\$entrez <- mapIds(org.Hs.eg.db, keys=row.names(res), keytype="ENSEMBL", column="ENTREZID", 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 8 columns baseMean log2FoldChange lfcSE stat pvalue <numeric> <numeric> <numeric> <numeric> <numeric> ENSG00000000003 747.194195 -0.3507030 0.168246 -2.084470 0.0371175 ENSG0000000005 0.000000 NANANAENSG00000000419 520.134160 ENSG00000000457 322.664844 0.0245269 0.145145 0.168982 0.8658106 -0.1471420 0.257007 -0.572521 0.5669691 ENSG00000000460 87.682625

log2 fold change (MLE): dex treated vs control

```
ENSG00000000938
                 0.319167
                              -1.7322890 3.493601 -0.495846 0.6200029
                    padj
                              symbol
                                          entrez
               <numeric> <character> <character>
ENSG0000000000 0.163035
                              TSPAN6
                                           7105
ENSG00000000005
                      NA
                                TNMD
                                           64102
ENSG00000000419 0.176032
                                DPM1
                                           8813
ENSG00000000457 0.961694
                               SCYL3
                                           57147
ENSG00000000460 0.815849
                               FIRRM
                                           55732
ENSG00000000938
                               FGR
                                          2268
                      NA
  res$genename <- mapIds(org.Hs.eg.db,</pre>
                       keys=row.names(res),
                       keytype="ENSEMBL",
                       column="GENENAME",
                       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 9 columns

	baseMean	log2FoldChange		lfcS	E stat	pvalue
	<numeric></numeric>	<numer< td=""><td>ic></td><td><numeric< td=""><td><pre>> <numeric></numeric></pre></td><td><numeric></numeric></td></numeric<></td></numer<>	ic>	<numeric< td=""><td><pre>> <numeric></numeric></pre></td><td><numeric></numeric></td></numeric<>	<pre>> <numeric></numeric></pre>	<numeric></numeric>
ENSG00000000003	747.194195	-0.3507	030	0.16824	6 -2.084470	0.0371175
ENSG00000000005	0.000000		NA	N	A NA	NA
ENSG00000000419	520.134160	0.2061	078	0.10105	9 2.039475	0.0414026
ENSG00000000457	322.664844	0.0245	269	0.14514	5 0.168982	0.8658106
ENSG00000000460	87.682625	-0.1471	420	0.25700	7 -0.572521	0.5669691
ENSG00000000938	0.319167	-1.7322	890	3.49360	1 -0.495846	0.6200029
	padj	symbol		entrez		genename
	<numeric> <</numeric>	<pre><character></character></pre>	<cha< td=""><td>aracter></td><td></td><td><character></character></td></cha<>	aracter>		<character></character>
ENSG0000000003	0.163035	TSPAN6		7105	te	traspanin 6
ENSG00000000005	NA	TNMD		64102		tenomodulin
ENSG00000000419	0.176032	DPM1		8813	dolichyl-ph	osphate m
ENSG00000000457	0.961694	SCYL3		57147	SCY1 like p	seudokina
ENSG00000000460	0.815849	FIRRM		55732	FIGNL1 inte	racting r
ENSG00000000938	NA	FGR		2268	FGR proto-o	ncogene,

##Pathway analysis

library(pathview)

Pathview is an open source software package distributed under GNU General Public License version 3 (GPLv3). Details of GPLv3 is available at http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to formally cite the original Pathview paper (not just mention it) in publications or products. For details, do citation("pathview") within R.

The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG license agreement (details at http://www.kegg.jp/kegg/legal.html).

```
library(gage)
```

```
library(gageData)
  data(kegg.sets.hs)
  # Examine the first 2 pathways in this kegg set for humans
  head(kegg.sets.hs, 2)
$`hsa00232 Caffeine metabolism`
           "1544" "1548" "1549" "1553" "7498" "9"
[1] "10"
$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"
             "1066"
                      "10720" "10941" "151531" "1548"
                                                                    "1551"
                                                           "1549"
 [9] "1553"
             "1576"
                      "1577"
                                "1806"
                                         "1807"
                                                           "221223" "2990"
                                                  "1890"
[17] "3251"
             "3614"
                      "3615"
                                "3704"
                                        "51733"
                                                 "54490"
                                                           "54575"
                                                                    "54576"
[25] "54577"
             "54578" "54579" "54600"
                                        "54657"
                                                 "54658"
                                                           "54659"
                                                                    "54963"
[33] "574537" "64816" "7083"
                                "7084"
                                        "7172"
                                                  "7363"
                                                           "7364"
                                                                    "7365"
                                        "7378"
[41] "7366"
             "7367"
                      "7371"
                               "7372"
                                                  "7498"
                                                           "79799"
                                                                    "83549"
[49] "8824"
                       "9"
                                "978"
             "8833"
  foldchanges = res$log2FoldChange
  names(foldchanges) = res$entrez
```

head(foldchanges)

```
7105
                  64102
                                          57147
                                                      55732
                                                                    2268
                               8813
-0.35070302
                     NA 0.20610777 0.02452695 -0.14714205 -1.73228897
Run gage:
  # Get the results
  keggres = gage(foldchanges, gsets=kegg.sets.hs)
  attributes(keggres)
$names
[1] "greater" "less"
                        "stats"
  # Look at the first three down (less) pathways
  head(keggres$less, 3)
                                      p.geomean stat.mean
                                                                  p.val
hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
hsa04940 Type I diabetes mellitus 0.0017820293 -3.002352 0.0017820293
hsa05310 Asthma
                                   0.0020045888 -3.009050 0.0020045888
                                        q.val set.size
hsa05332 Graft-versus-host disease 0.09053483
                                                    40 0.0004250461
hsa04940 Type I diabetes mellitus
                                   0.14232581
                                                   42 0.0017820293
hsa05310 Asthma
                                   0.14232581
                                                    29 0.0020045888
Let's have a look at one of these pathways
  pathview(gene.data=foldchanges, pathway.id="hsa05310")
'select()' returned 1:1 mapping between keys and columns
Info: Working in directory D:/UCSD/R/class13
Info: Writing image file hsa05310.pathview.png
```

