

Class 15 Transcriptomics and the analysis of RNA-Sequence data

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```
library(BiocManager)

## Bioconductor version '3.13' is out-of-date; the current release version '3.14'
##   is available with R version '4.1'; see https://bioconductor.org/install

library(DESeq2)

## Loading required package: S4Vectors

## Loading required package: stats4

## Loading required package: BiocGenerics

## Loading required package: parallel

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:parallel':
## 
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##   clusterExport, clusterMap, parApply, parCapply, parLapply,
##   parLapplyLB, parRapply, parSapply, parSapplyLB

## The following objects are masked from 'package:stats':
## 
##   IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
## 
##   anyDuplicated, 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, uname

## 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

```

Importing, reading, and storing the CSV files

```

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

```

```
head(counts)
```

| | SRR1039508 | SRR1039509 | SRR1039512 | SRR1039513 | SRR1039516 |
|---------------------|------------|------------|------------|------------|------------|
| ## ENSG000000000003 | 723 | 486 | 904 | 445 | 1170 |
| ## ENSG000000000005 | 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 | | |
| ## ENSG000000000003 | 1097 | 806 | 604 | | |
| ## ENSG000000000005 | 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
```

Q1 How many genes are in this data set?

There are 38694 genes in this data set

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

```
## [1] 4
```

Q2. How many ‘control’ cell lines do we have?

We have 4 control cell lines

First I need to extract all the “control” columns. Then I will take the row-wise mean to get the average count values for all genes in these four experiments

```
control inds <- metadata$dex == "control"  
control counts <- counts[, control inds]  
head(control counts)
```

```
##          SRR1039508 SRR1039512 SRR1039516 SRR1039520  
## ENSG000000000003    723      904     1170      806  
## ENSG000000000005      0        0        0        0  
## ENSG00000000419     467      616      582      417  
## ENSG00000000457     347      364      318      330  
## ENSG00000000460      96       73      118      102  
## ENSG00000000938      0        1        2        0
```

```
control mean <- rowMeans(control counts)  
head(control mean)
```

```
## ENSG000000000003 ENSG000000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460  
##         900.75           0.00         520.50         339.75         97.25  
## ENSG00000000938  
##         0.75
```

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

In order to make it more robust, we can just use the equation given above so we can get the average the rows even when we add more control or treated group.

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 inds <- metadata$dex == "treated"  
treated counts <- counts[, treated inds]  
head(treated counts)
```

```
##          SRR1039509 SRR1039513 SRR1039517 SRR1039521  
## ENSG000000000003    486      445     1097      604  
## ENSG000000000005      0        0        0        0  
## ENSG00000000419     523      371      781      509  
## ENSG00000000457     258      237      447      324  
## ENSG00000000460      81       66      94       74  
## ENSG00000000938      0        0        0        0
```

```
treated.mean <- rowMeans(treated.counts)
head(treated.mean)

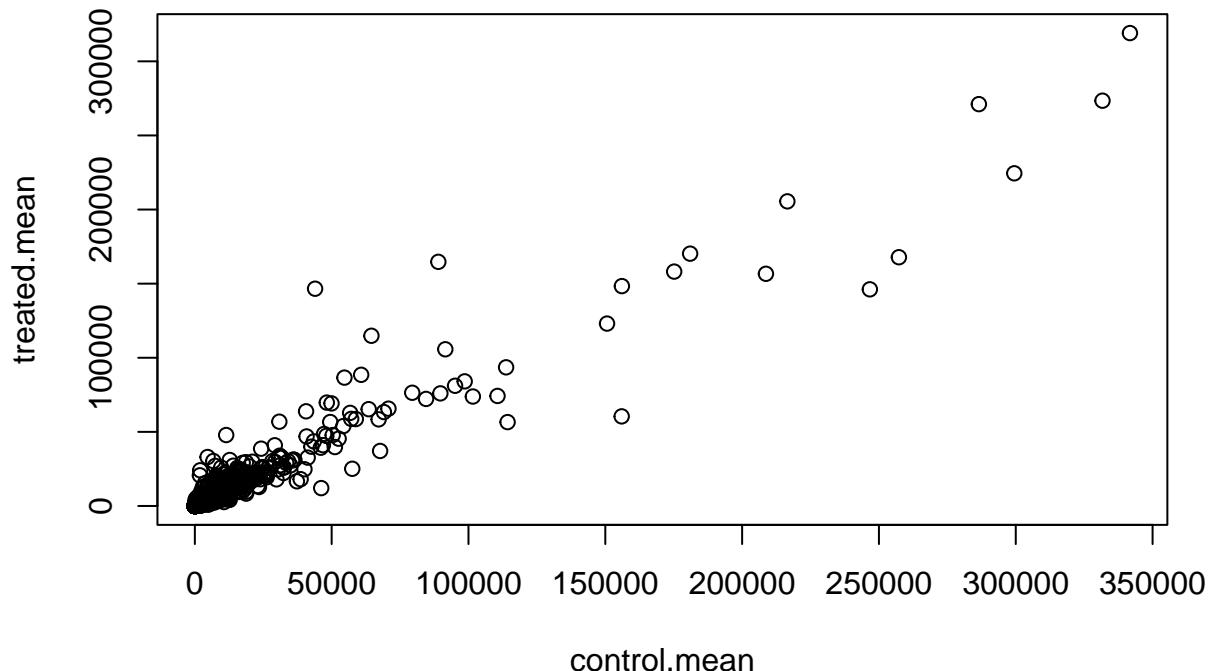
## ENSG00000000003 ENSG00000000005 ENSG000000000419 ENSG000000000457 ENSG000000000460
##          658.00           0.00          546.00          316.50          78.75
## ENSG000000000938
##          0.00
```

We will combine our meancount data for bookkeeping purposes.

```
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.

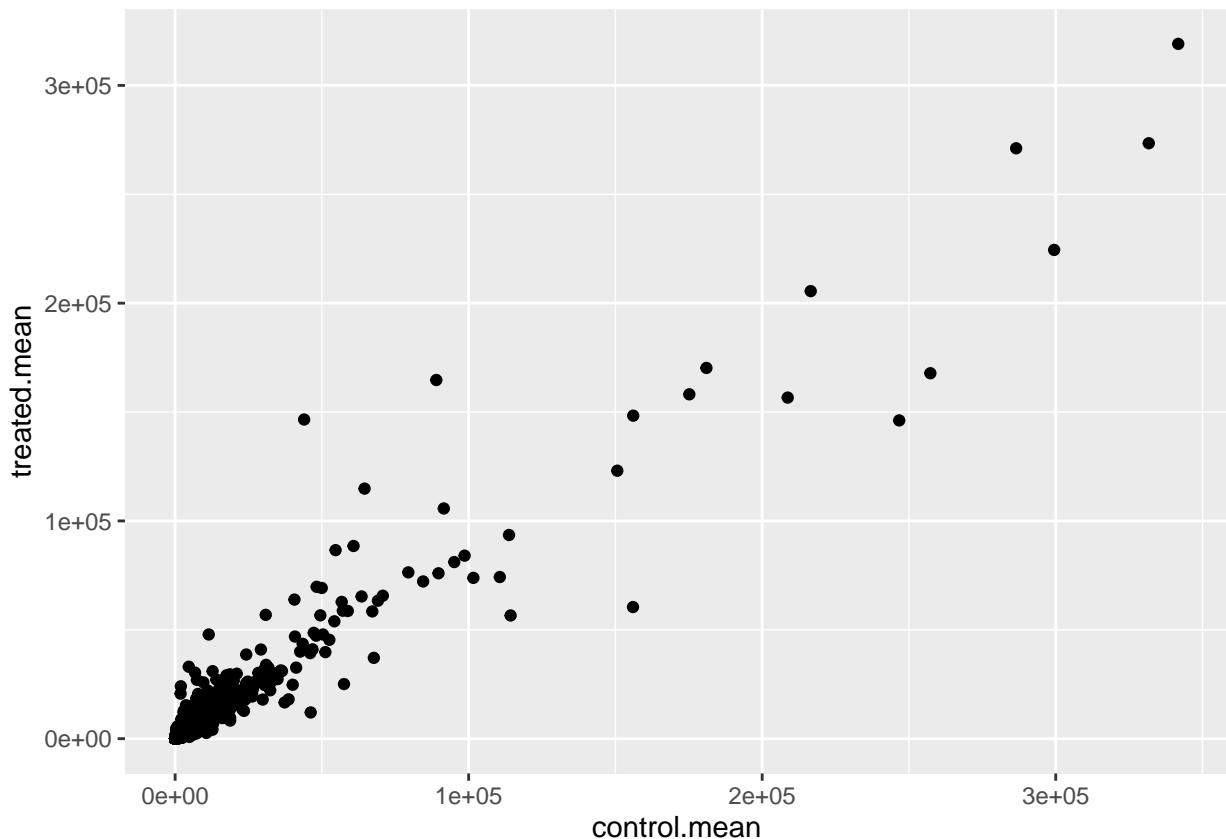
```
plot(meancounts)
```



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?

I would use the geom_point() function for this plot

```
library(ggplot2)
ggplot(data = meancounts, aes(x = control.mean, y = treated.mean)) +
  geom_point()
```



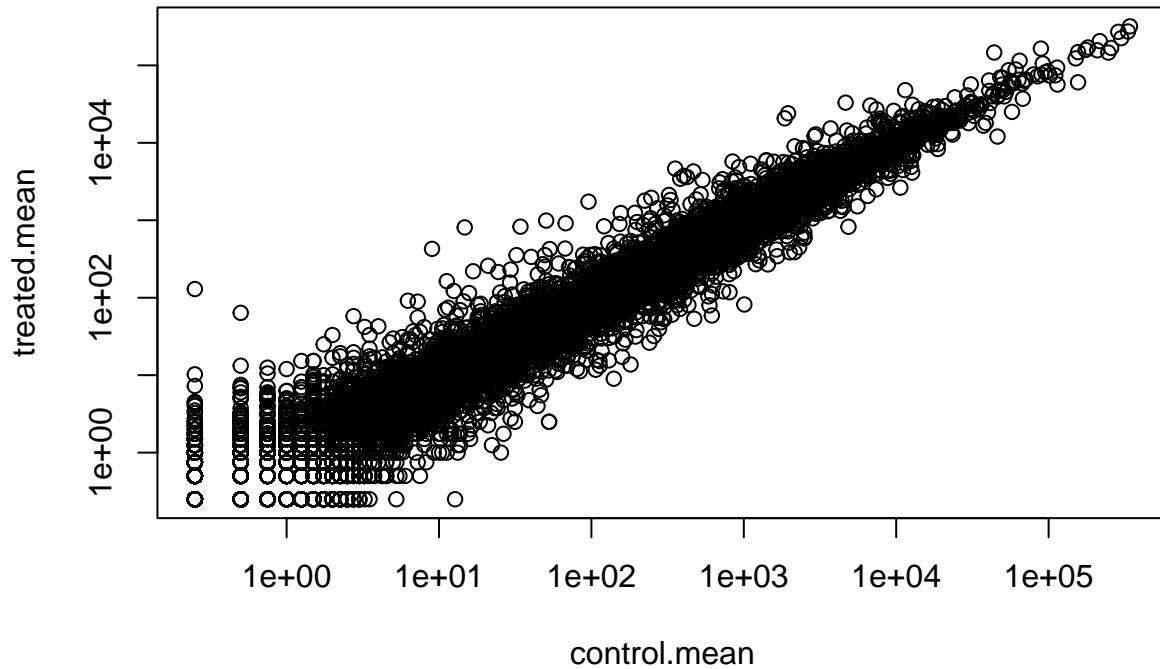
Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

I would use the log argument to plot in order to

```
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
```



We often use log2 in this field because it has nice mathproperties that make interpretation easier

```
log2(10/10)
```

```
## [1] 0
```

```
log2(40/10)
```

```
## [1] 2
```

```
log2(5/10)
```

```
## [1] -1
```

We see 0 values for no change and positive values for increases and negative values for decreases. Thsi nice property leads us to work with **log2(fold-chage)** all the time in the genomics and proteomics filled.

Let's add the **log2(fold-change)** to our “mean-counts” dataframe

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

| | control.mean | treated.mean | log2fc |
|---------------------|--------------|--------------|-------------|
| ## ENSG000000000003 | 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

```

I need to exclude the genes (i.e. rows) with zero counts because we can't say anything about these as we have no data for them

```
head(meancounts[, 1:2])
```

```

##           control.mean treated.mean
## 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

```

```
head(meancounts[, 1:2] == 0)
```

```

##           control.mean treated.mean
## ENSG00000000003    FALSE     FALSE
## ENSG00000000005     TRUE      TRUE
## ENSG00000000419    FALSE     FALSE
## ENSG00000000457    FALSE     FALSE
## ENSG00000000460    FALSE     FALSE
## ENSG00000000938    FALSE      TRUE

```

```
which( c(F,F,T,T))
```

```
## [1] 3 4
```

I can use the `which()` function with the 'arr.ind=TRUE' argument to get the columns and rows where the TRUE values are (i.e the zero counts in our case)

```
zero.vals <- which(meancounts[, 1:2] == 0, arr.ind=TRUE)
head(zero.vals)
```

```

##           row col
## ENSG00000000005    2   1
## ENSG00000004848   65   1
## ENSG00000004948   70   1
## ENSG00000005001   73   1
## ENSG00000006059  121   1
## ENSG00000006071  123   1

```

```
to.rm <- unique(zero.vals[,1])
sort(to.rm)
```

```

## [1] 2   6   65  70  73  81  96 121 123 133 145 168
## [13] 173 177 181 192 206 236 254 318 320 367 371 382
## [25] 389 399 414 417 524 530 535 591 597 598 601 673
## [37] 686 718 735 736 757 761 762 769 812 815 816 829
## [49] 862 873 877 901 1062 1064 1068 1127 1138 1149 1163 1238
## [61] 1239 1245 1246 1276 1285 1304 1312 1313 1320 1329 1358 1364
## [73] 1367 1382 1403 1422 1424 1430 1443 1471 1473 1489 1503 1515
## [85] 1533 1690 1697 1724 1784 1798 1805 1817 1865 1875 1880 1916
## [97] 1926 1939 1952 1953 1954 1970 2001 2003 2034 2037 2040 2044
## [109] 2045 2048 2049 2054 2060 2102 2103 2108 2150 2171 2175 2185
## [121] 2189 2211 2246 2257 2258 2277 2282 2284 2285 2287 2314 2315
## [133] 2316 2338 2354 2366 2368 2397 2417 2454 2458 2467 2469 2489
## [145] 2492 2496 2497 2498 2507 2517 2524 2525 2528 2530 2533 2578
## [157] 2585 2590 2596 2603 2609 2620 2622 2629 2635 2642 2648 2650
## [169] 2659 2702 2707 2717 2727 2733 2743 2758 2778 2806 2818 2836
## [181] 2839 2854 2866 2892 2910 2911 2917 2929 2955 2957 2967 2972
## [193] 3002 3003 3006 3016 3018 3048 3051 3061 3065 3080 3086 3090
## [205] 3106 3112 3117 3120 3121 3130 3131 3141 3163 3167 3168 3203
## [217] 3231 3248 3264 3283 3286 3288 3291 3305 3323 3345 3351 3369
## [229] 3379 3380 3393 3397 3418 3450 3482 3501 3523 3528 3531 3542
## [241] 3575 3601 3605 3613 3621 3632 3638 3644 3645 3649 3650 3652
## [253] 3663 3669 3710 3743 3760 3761 3766 3781 3783 3815 3822 3835
## [265] 3840 3862 3863 3865 3896 3903 3904 3933 3934 3996 4002 4003
## [277] 4012 4023 4025 4028 4033 4053 4066 4067 4068 4073 4079 4112
## [289] 4115 4117 4140 4142 4151 4159 4173 4186 4191 4232 4248 4267
## [301] 4275 4279 4297 4335 4356 4364 4395 4407 4424 4441 4459 4461
## [313] 4465 4473 4497 4519 4523 4545 4555 4609 4614 4616 4639 4650
## [325] 4656 4666 4667 4679 4720 4729 4768 4788 4790 4795 4809 4810
## [337] 4825 4839 4843 4855 4858 4866 4905 4924 4933 4978 4981 5001
## [349] 5002 5007 5009 5016 5022 5033 5041 5048 5051 5055 5065 5072
## [361] 5117 5121 5150 5157 5159 5160 5179 5188 5192 5197 5198 5200
## [373] 5203 5218 5223 5232 5235 5237 5239 5244 5249 5253 5280 5281
## [385] 5299 5312 5313 5331 5351 5352 5357 5364 5373 5376 5379 5385
## [397] 5397 5399 5403 5404 5405 5443 5457 5461 5472 5475 5476 5489
## [409] 5491 5492 5493 5504 5516 5527 5529 5530 5542 5543 5554 5562
## [421] 5577 5588 5590 5591 5596 5598 5599 5603 5613 5614 5621 5631
## [433] 5635 5656 5662 5664 5668 5686 5688 5704 5706 5707 5710 5712
## [445] 5713 5716 5721 5730 5732 5742 5743 5745 5748 5750 5751 5765
## [457] 5769 5792 5797 5799 5803 5822 5823 5838 5864 5865 5871 5880
## [469] 5883 5884 5893 5905 5918 5922 5923 5933 5942 5973 5988 5995
## [481] 6002 6004 6005 6021 6042 6046 6052 6056 6057 6058 6059 6098
## [493] 6102 6129 6131 6147 6156 6165 6170 6171 6172 6196 6211 6222
## [505] 6231 6250 6251 6274 6287 6295 6297 6301 6302 6307 6346 6350
## [517] 6358 6374 6379 6380 6383 6396 6405 6415 6419 6432 6468 6473
## [529] 6474 6487 6498 6511 6573 6574 6588 6610 6619 6628 6630 6635
## [541] 6658 6663 6677 6680 6697 6704 6708 6712 6745 6750 6757 6832
## [553] 6834 6837 6841 6847 6852 6868 6882 6885 6887 6904 6910 6919
## [565] 6921 6938 6940 6947 6949 6952 6989 7007 7010 7015 7016 7017
## [577] 7042 7056 7057 7066 7094 7104 7109 7148 7151 7152 7161 7172
## [589] 7196 7200 7212 7233 7244 7247 7248 7267 7285 7294 7295 7296
## [601] 7297 7298 7299 7300 7311 7328 7329 7354 7359 7366 7387 7389
## [613] 7390 7410 7414 7421 7422 7438 7439 7444 7446 7447 7449 7450
## [625] 7458 7459 7482 7497 7516 7554 7585 7609 7630 7667 7746 7750
## [637] 7751 7762 7783 7785 7801 7806 7818 7823 7830 7832 7833 7850

```

| | | | | | | | | | | | | | |
|----|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| ## | [649] | 7871 | 7872 | 7907 | 7913 | 7923 | 7928 | 7964 | 7966 | 7974 | 7984 | 7992 | 8007 |
| ## | [661] | 8012 | 8019 | 8033 | 8056 | 8062 | 8064 | 8095 | 8097 | 8141 | 8148 | 8156 | 8176 |
| ## | [673] | 8177 | 8190 | 8195 | 8209 | 8221 | 8224 | 8227 | 8230 | 8234 | 8235 | 8244 | 8247 |
| ## | [685] | 8248 | 8273 | 8291 | 8294 | 8301 | 8305 | 8306 | 8318 | 8324 | 8342 | 8360 | 8402 |
| ## | [697] | 8408 | 8412 | 8423 | 8430 | 8479 | 8486 | 8493 | 8511 | 8513 | 8517 | 8526 | 8528 |
| ## | [709] | 8612 | 8633 | 8645 | 8649 | 8652 | 8669 | 8670 | 8708 | 8710 | 8711 | 8744 | 8745 |
| ## | [721] | 8746 | 8749 | 8750 | 8752 | 8762 | 8776 | 8779 | 8780 | 8781 | 8790 | 8814 | 8821 |
| ## | [733] | 8825 | 8826 | 8827 | 8829 | 8841 | 8844 | 8853 | 8859 | 8867 | 8877 | 8880 | 8886 |
| ## | [745] | 8888 | 8891 | 8903 | 8917 | 8922 | 8929 | 8936 | 8939 | 8941 | 8946 | 8947 | 8956 |
| ## | [757] | 8957 | 8977 | 8987 | 9012 | 9014 | 9017 | 9020 | 9032 | 9033 | 9037 | 9043 | 9044 |
| ## | [769] | 9066 | 9071 | 9073 | 9086 | 9091 | 9096 | 9097 | 9111 | 9123 | 9140 | 9143 | 9155 |
| ## | [781] | 9190 | 9192 | 9203 | 9227 | 9231 | 9232 | 9237 | 9259 | 9267 | 9268 | 9269 | 9270 |
| ## | [793] | 9272 | 9277 | 9310 | 9311 | 9334 | 9340 | 9344 | 9359 | 9376 | 9381 | 9385 | 9395 |
| ## | [805] | 9408 | 9411 | 9447 | 9461 | 9462 | 9463 | 9472 | 9474 | 9484 | 9487 | 9489 | 9498 |
| ## | [817] | 9500 | 9505 | 9542 | 9543 | 9544 | 9551 | 9552 | 9574 | 9584 | 9585 | 9587 | 9618 |
| ## | [829] | 9625 | 9629 | 9634 | 9652 | 9658 | 9662 | 9665 | 9678 | 9690 | 9709 | 9714 | 9718 |
| ## | [841] | 9725 | 9736 | 9740 | 9745 | 9765 | 9771 | 9807 | 9831 | 9835 | 9855 | 9865 | 9870 |
| ## | [853] | 9894 | 9910 | 9911 | 9938 | 9940 | 9948 | 9949 | 9958 | 9983 | 9985 | 9994 | 10000 |
| ## | [865] | 10001 | 10014 | 10016 | 10033 | 10036 | 10040 | 10041 | 10046 | 10047 | 10055 | 10059 | 10111 |
| ## | [877] | 10131 | 10139 | 10201 | 10203 | 10206 | 10208 | 10209 | 10210 | 10217 | 10221 | 10226 | 10236 |
| ## | [889] | 10238 | 10239 | 10242 | 10283 | 10287 | 10289 | 10290 | 10305 | 10306 | 10328 | 10331 | 10340 |
| ## | [901] | 10342 | 10343 | 10354 | 10387 | 10411 | 10413 | 10415 | 10424 | 10459 | 10461 | 10478 | 10485 |
| ## | [913] | 10493 | 10528 | 10547 | 10554 | 10558 | 10595 | 10617 | 10621 | 10622 | 10628 | 10636 | 10645 |
| ## | [925] | 10649 | 10650 | 10653 | 10686 | 10692 | 10718 | 10720 | 10725 | 10745 | 10759 | 10761 | 10775 |
| ## | [937] | 10777 | 10783 | 10791 | 10802 | 10803 | 10806 | 10807 | 10809 | 10814 | 10823 | 10829 | 10836 |
| ## | [949] | 10840 | 10853 | 10889 | 10894 | 10898 | 10900 | 10919 | 10920 | 10921 | 10922 | 10924 | 10925 |
| ## | [961] | 10926 | 10928 | 10929 | 10930 | 10931 | 10934 | 10936 | 10937 | 10940 | 10941 | 10942 | 10945 |
| ## | [973] | 10965 | 10976 | 10977 | 10982 | 10988 | 10999 | 11000 | 11001 | 11007 | 11010 | 11011 | 11016 |
| ## | [985] | 11026 | 11032 | 11034 | 11037 | 11038 | 11091 | 11092 | 11098 | 11106 | 11123 | 11149 | 11164 |
| ## | [997] | 11170 | 11171 | 11189 | 11204 | 11213 | 11226 | 11231 | 11233 | 11236 | 11237 | 11287 | 11290 |
| ## | [1009] | 11298 | 11299 | 11305 | 11311 | 11312 | 11317 | 11329 | 11332 | 11333 | 11339 | 11340 | 11342 |
| ## | [1021] | 11344 | 11349 | 11354 | 11361 | 11371 | 11375 | 11378 | 11388 | 11397 | 11399 | 11400 | 11419 |
| ## | [1033] | 11421 | 11422 | 11424 | 11431 | 11434 | 11440 | 11444 | 11456 | 11482 | 11485 | 11488 | 11500 |
| ## | [1045] | 11504 | 11509 | 11510 | 11514 | 11525 | 11527 | 11530 | 11550 | 11555 | 11570 | 11572 | 11573 |
| ## | [1057] | 11583 | 11596 | 11597 | 11599 | 11604 | 11605 | 11607 | 11611 | 11618 | 11630 | 11639 | 11650 |
| ## | [1069] | 11671 | 11683 | 11696 | 11699 | 11701 | 11709 | 11710 | 11712 | 11720 | 11722 | 11725 | 11727 |
| ## | [1081] | 11731 | 11740 | 11744 | 11746 | 11747 | 11765 | 11766 | 11781 | 11794 | 11804 | 11807 | 11808 |
| ## | [1093] | 11811 | 11819 | 11822 | 11854 | 11856 | 11862 | 11870 | 11875 | 11877 | 11886 | 11892 | 11894 |
| ## | [1105] | 11896 | 11902 | 11932 | 11935 | 11952 | 11954 | 11959 | 11961 | 11963 | 11969 | 11988 | 11991 |
| ## | [1117] | 12004 | 12006 | 12014 | 12015 | 12020 | 12033 | 12043 | 12044 | 12045 | 12052 | 12059 | 12069 |
| ## | [1129] | 12079 | 12094 | 12106 | 12110 | 12111 | 12116 | 12121 | 12151 | 12152 | 12154 | 12157 | 12168 |
| ## | [1141] | 12171 | 12176 | 12177 | 12180 | 12181 | 12195 | 12199 | 12241 | 12246 | 12253 | 12255 | 12256 |
| ## | [1153] | 12257 | 12261 | 12282 | 12284 | 12303 | 12305 | 12309 | 12329 | 12338 | 12348 | 12349 | 12353 |
| ## | [1165] | 12355 | 12357 | 12363 | 12370 | 12372 | 12373 | 12379 | 12392 | 12395 | 12400 | 12405 | 12418 |
| ## | [1177] | 12419 | 12424 | 12433 | 12442 | 12444 | 12459 | 12460 | 12470 | 12474 | 12476 | 12477 | 12495 |
| ## | [1189] | 12497 | 12508 | 12510 | 12526 | 12530 | 12544 | 12545 | 12548 | 12555 | 12556 | 12557 | 12562 |
| ## | [1201] | 12564 | 12572 | 12573 | 12574 | 12575 | 12581 | 12582 | 12585 | 12589 | 12593 | 12600 | 12604 |
| ## | [1213] | 12611 | 12613 | 12618 | 12622 | 12626 | 12639 | 12643 | 12644 | 12645 | 12646 | 12651 | 12652 |
| ## | [1225] | 12658 | 12661 | 12665 | 12667 | 12668 | 12670 | 12672 | 12705 | 12713 | 12717 | 12721 | 12724 |
| ## | [1237] | 12728 | 12736 | 12741 | 12751 | 12753 | 12757 | 12771 | 12772 | 12775 | 12782 | 12787 | 12795 |
| ## | [1249] | 12799 | 12807 | 12809 | 12815 | 12817 | 12823 | 12832 | 12835 | 12851 | 12853 | 12859 | 12863 |
| ## | [1261] | 12865 | 12866 | 12868 | 12872 | 12873 | 12874 | 12875 | 12885 | 12895 | 12902 | 12904 | 12905 |
| ## | [1273] | 12907 | 12908 | 12910 | 12912 | 12913 | 12916 | 12921 | 12923 | 12924 | 12932 | 12933 | 12943 |
| ## | [1285] | 12955 | 12957 | 12967 | 12969 | 12970 | 12971 | 12976 | 13003 | 13008 | 13010 | 13011 | |

```

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Now remove these from our ‘meancounts’ dataframe

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mycounts <- meancounts[-to.rm,]  
head(mycounts)
```

```
## control.mean treated.mean      log2fc  
## ENSG000000000003    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
```

```
nrow(mycounts)
```

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## [1] 21817
```

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 purpose of **which()** function with the ‘arr.ind=TRUE’ argument is to get the columns and rows where the TRUE values are (i.e the zero counts in our case). We use the unique() function to remove any duplicate rows

```
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?

```
sum(up.ind == TRUE)
```

```
## [1] 250
```

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?

```
sum(down.ind == TRUE)
```

```
## [1] 367
```

There are 367 genes that are down regulated

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

No, I do not trust these results. We have been doing our analysis based on fold change which can be large without statistically significant. We have not done anything to determine if the differences we are seeing are significant. Therefore, these data can be very misleading.

```
##DESeq2

library(DESeq2)
citation("DESeq2")

##
## 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},
## }

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): ENSG00000000003 ENSG00000000005 ... ENSG00000283120
##   ENSG00000283123
## rowData names(0):
## colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
## colData names(4): id dex celltype geo_id
```

Now we can run DESeq analysis

```
## estimating size factors  
## estimating dispersions  
## gene-wise dispersion estimates  
## mean-dispersion relationship  
## final dispersion estimates  
## fitting model and testing
```

To get at the results here we use the deseq ‘results()’ function:

```
res <- results(dds)
res

## 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>
## ENSG000000000003  747.1942    -0.3507030  0.168246 -2.084470 0.0371175
## ENSG000000000005   0.0000       NA        NA        NA        NA
## ENSG000000000419  520.1342    0.2061078  0.101059  2.039475 0.0414026
## ENSG000000000457  322.6648    0.0245269  0.145145  0.168982 0.8658106
## ENSG000000000460   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>
## ENSG000000000003  0.163035
## ENSG000000000005   NA
## ENSG000000000419  0.176032
## ENSG000000000457  0.961694
## ENSG000000000460  0.815849
## ...
##           ...
## ENSG00000283115   NA
## ENSG00000283116   NA
## ENSG00000283119   NA
## ENSG00000283120   NA
## ENSG00000283123   NA
```

Q11. Run the mapIds() function two more times to add the Entrez ID and UniProt accession and GENENAME as new columns called res\$entrez, res\$uniprot and res\$genename

```

library("AnnotationDbi")
library("org.Hs.eg.db")

##

res$entrez <- mapIds(org.Hs.eg.db,
                      keys=row.names(res),
                      column="ENTREZID",
                      keytype="ENSEMBL",
                      multiVals="first")

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

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

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

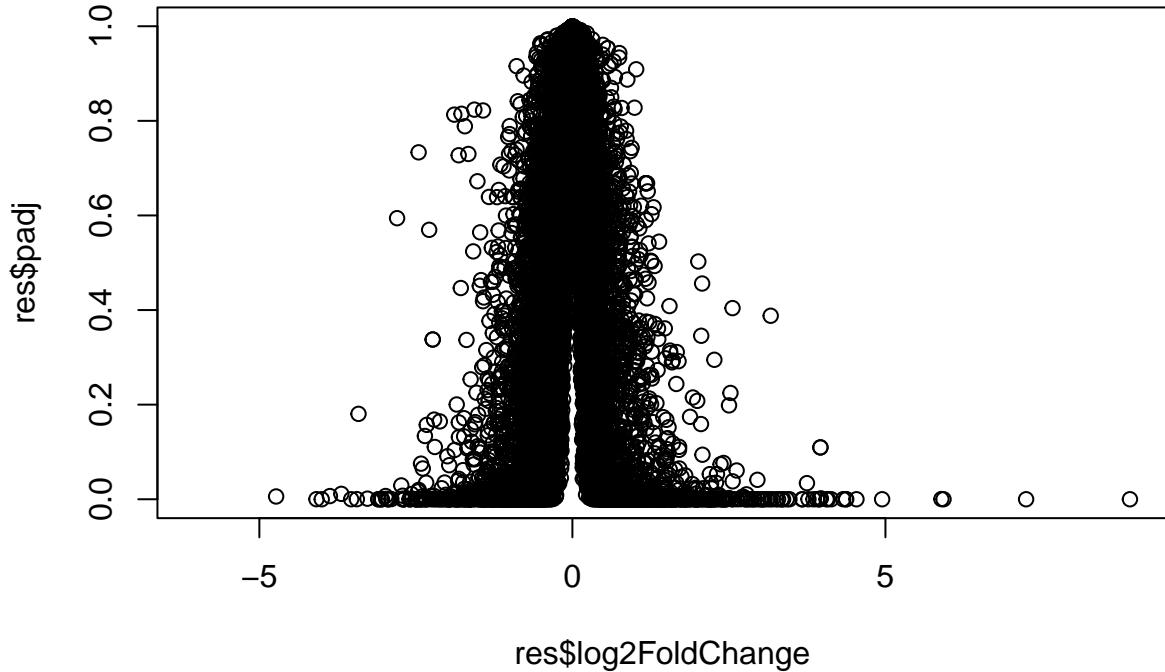
head(res)

## 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      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      entrez      uniprot      genename
##            <numeric> <character> <character> <character>
## ENSG00000000003   0.163035      7105 AOA024RCI0      tetraspanin 6
## ENSG00000000005      NA      64102 Q9H2S6      tenomodulin
## ENSG00000000419   0.176032      8813 O60762 dolichyl-phosphate m..
## ENSG00000000457   0.961694      57147 Q8IZE3 SCY1 like pseudokina..
## ENSG00000000460   0.815849      55732 AOA024R922 chromosome 1 open re..
## ENSG00000000938      NA      2268 P09769 FGR proto-oncogene, ..

```

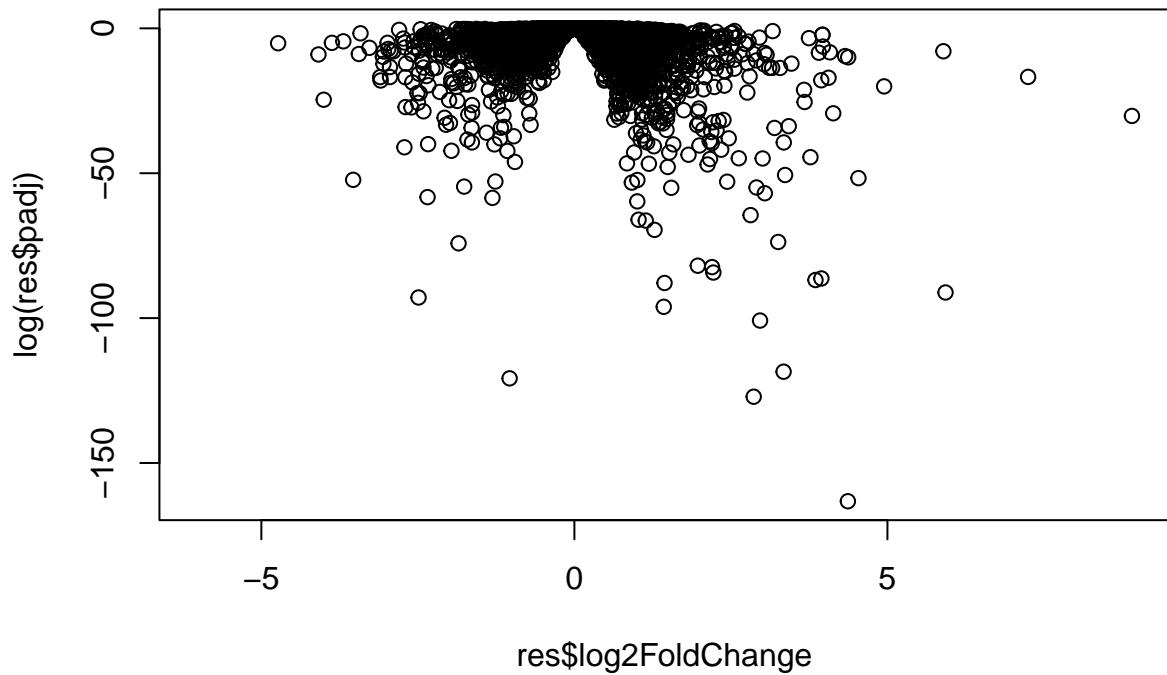
Volcano plots

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



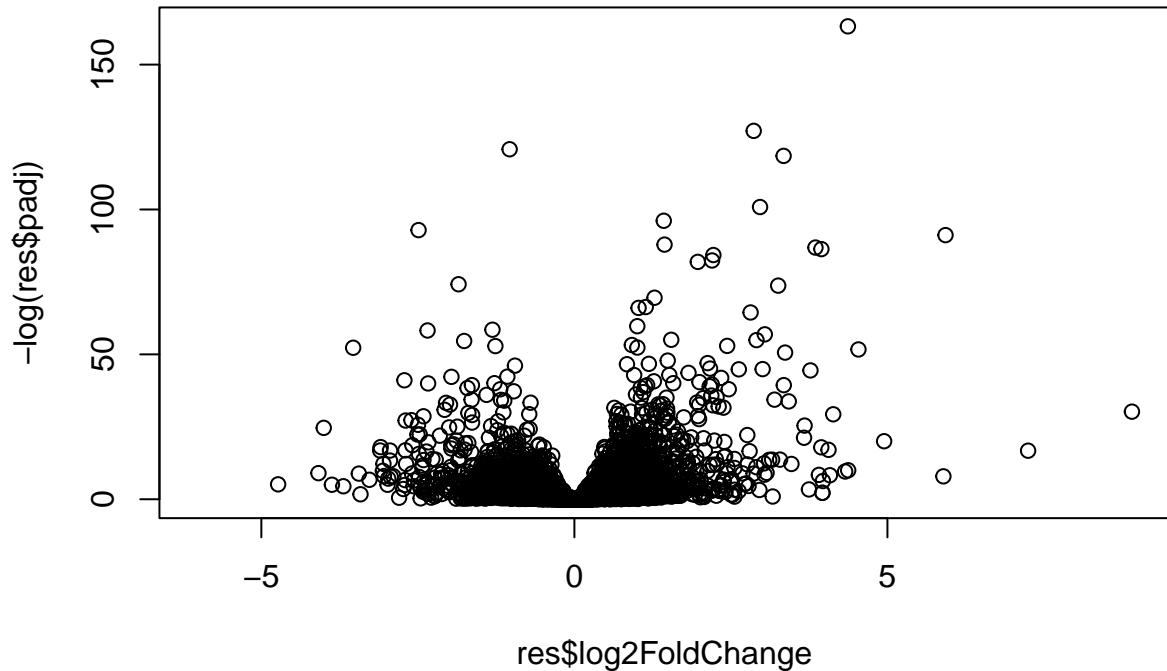
That is not a useful plot because all the small p-values are hidden at the bottom of the plot and we can't really see them. Log will help.

```
plot(res$log2FoldChange, log(res$padj))
```



We can flip this p-value axis by just putting a minus sign on it. Then we will have the classic volconia plot that the rest of the world uses

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



Finally let's add some color to this plot to draw attention to the genes (i.e. points) we care about - that is those with large fold-change and low pvalues (ie. high -log(pvalues))

```

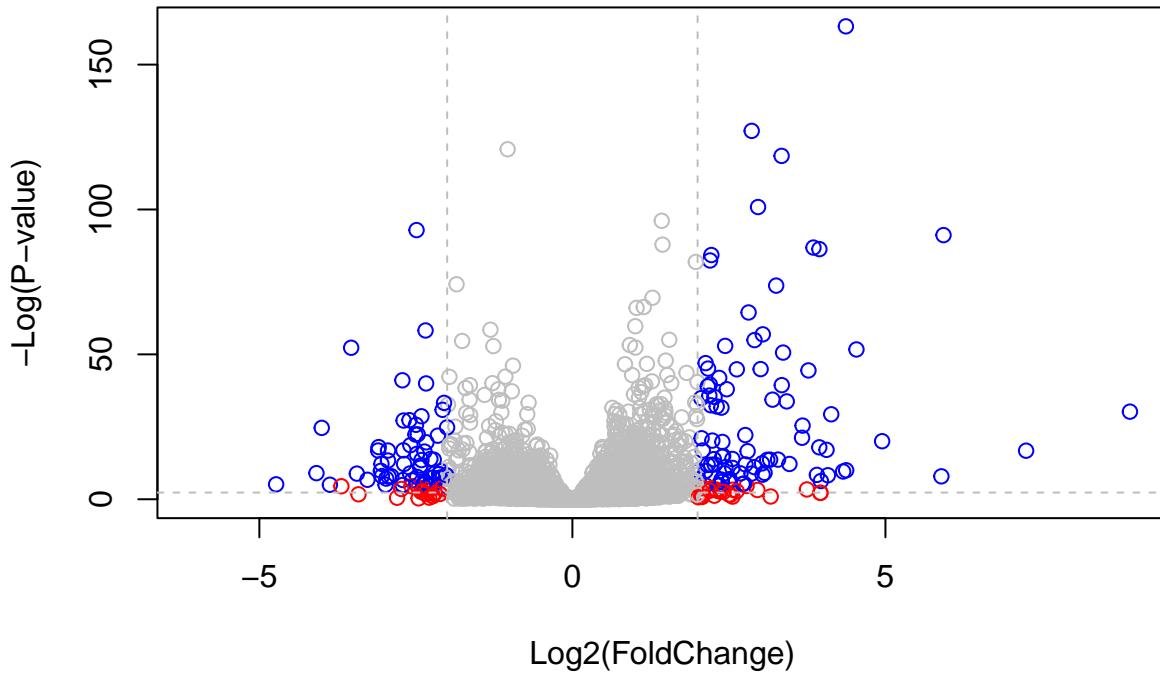
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

# Volcano plot with custom colors
plot( res$log2FoldChange, -log(res$padj),
      col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )

# Cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", lty=2)

```



```

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)

head(kegg.sets.hs, 2)

## $`hsa00232 Caffeine metabolism`

```

```

## [1] "10"    "1544"  "1548"  "1549"  "1553"  "7498"  "9"
##
## $`hsa00983 Drug metabolism - other enzymes`
## [1] "10"      "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"
## [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"
## [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"
## [41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799" "83549"
## [49] "8824"   "8833"   "9"      "978"

foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)

##          7105        64102        8813        57147        55732        2268
## -0.35070302           NA  0.20610777  0.02452695 -0.14714205 -1.73228897

keggres = gage(foldchanges, gsets=kegg.sets.hs)

attributes(keggres)

## $names
## [1] "greater" "less"     "stats"

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      exp1
## 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

pathview(gene.data=foldchanges, pathway.id="hsa05310")

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

## Info: Working in directory C:/bimm143_github/Class15_Transcriptomics and the analysis of RNA-Seq data

## Info: Writing image file hsa05310.pathview.png

```

Q12. Can you do the same procedure as above to plot the pathview figures for the top 2 down-regulated pathways?

```
pathview(gene.data=foldchanges, pathway.id="hsa05332")

## Info: Downloading xml files for hsa05332, 1/1 pathways..

## Info: Downloading png files for hsa05332, 1/1 pathways..

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

## Info: Working in directory C:/bimm143_github/Class15 Transcriptomics and the analysis of RNA-Seq data

## Info: Writing image file hsa05332.pathview.png

pathview(gene.data=foldchanges, pathway.id="hsa04940")

## Info: Downloading xml files for hsa04940, 1/1 pathways..

## Info: Downloading png files for hsa04940, 1/1 pathways..

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

## Info: Working in directory C:/bimm143_github/Class15 Transcriptomics and the analysis of RNA-Seq data

## Info: Writing image file hsa04940.pathview.png
```