Class 16 RNA Seq Mini Project

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Differential Expression Analysis

Download the data

```
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, 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
```

```
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"
```

Take a look at metadata

```
colData = read.csv(metaFile, row.names = 1)
head(colData)
```

```
##                 condition
## SRR493366 control_sirna
## SRR493367 control_sirna
## SRR493368 control_sirna
## SRR493369      hoxa1_kd
## SRR493370      hoxa1_kd
## SRR493371      hoxa1_kd
```

Take a look at countData

```
countData = read.csv(countFile, row.names = 1)
head(countData)
```

```
##                 length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
## ENSG00000186092    918         0         0         0         0         0
## ENSG00000279928    718         0         0         0         0         0
## ENSG00000279457   1982        23        28        29        29        28
## ENSG00000278566    939         0         0         0         0         0
## ENSG00000273547    939         0         0         0         0         0
## ENSG00000187634   3214       124       123       205       207       212
##                 SRR493371
## ENSG00000186092         0
## ENSG00000279928         0
## ENSG00000279457        46
## ENSG00000278566         0
## ENSG00000273547         0
## ENSG00000187634       258
```

We do not want the first column of the count data called “length”. All columns must be the same as the rows of our meta data.

```
countData <- as.matrix(countData[,-1])
head(countData)
```

```
##                 SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
## ENSG00000186092         0         0         0         0         0         0
## ENSG00000279928         0         0         0         0         0         0
## ENSG00000279457        23        28        29        29        28        46
## ENSG00000278566         0         0         0         0         0         0
## ENSG00000273547         0         0         0         0         0         0
## ENSG00000187634       124       123       205       207       212       258
```

Let’s get rid of the zero data so it doesn’t mess up future calculations

```
countData = countData[-which(rowSums(countData)==0),]
head(countData)
```

```
##                 SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
## ENSG00000279457        23        28        29        29        28        46
## ENSG00000187634       124       123       205       207       212       258
## ENSG00000188976      1637      1831      2383      1226      1326      1504
## ENSG00000187961       120       153       180       236       255       357
## ENSG00000187583        24        48        65        44        48        64
## ENSG00000187642         4         9        16        14        16        16
```

Running DESeq2

Set up the DESeqDataSet object required for the function

```
dds = DESeqDataSetFromMatrix(countData = countData, colData = colData, design =~condition)
```

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

```
dds = DESeq(dds)
```

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing
```

```
dds
```

```
## class: DESeqDataSet
## dim: 15975 6
## metadata(1): version
## assays(4): counts mu H cooks
## rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
##   ENSG00000271254
## rowData names(22): baseMean baseVar ... deviance maxCooks
## colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
## colData names(2): condition sizeFactor
```

```
res = results(dds)
```

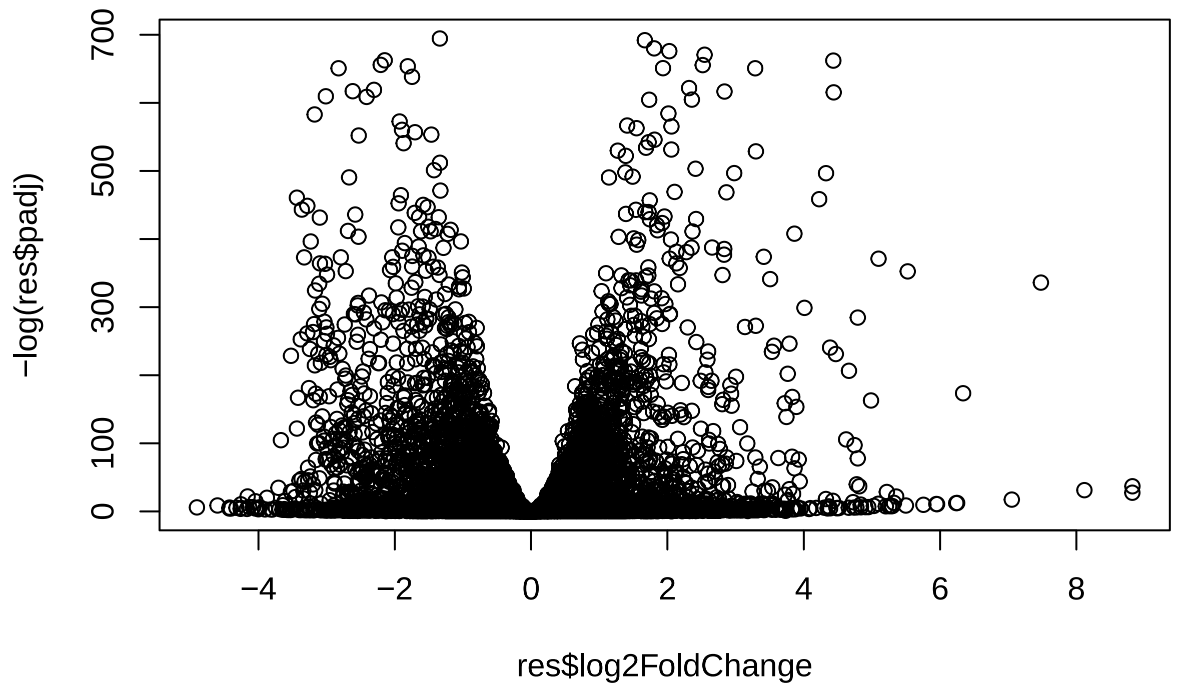
Use summary function to get a feel of how many genes are up or down regulated

```
summary(res)
```

```
##
## out of 15975 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)       : 4349, 27%
## LFC < 0 (down)     : 4396, 28%
## outliers [1]       : 0, 0%
## low counts [2]     : 1237, 7.7%
## (mean count < 0)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

Volcano Plot

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



Add code to polish the graph

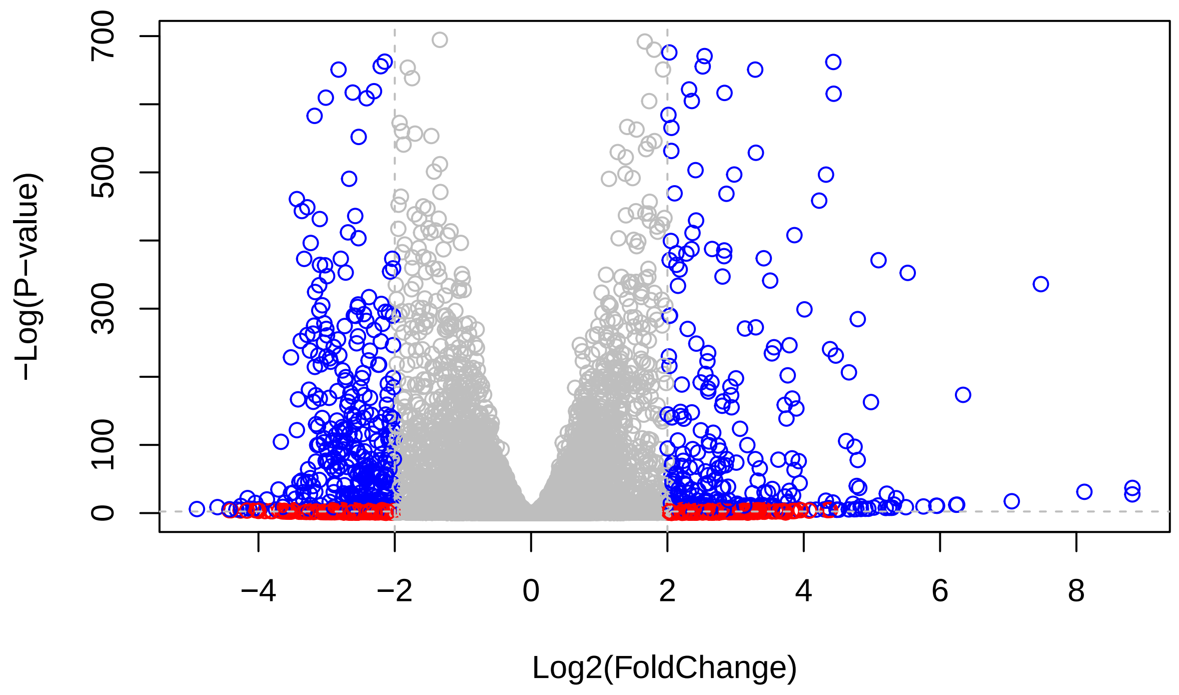
```
mycols <- rep("gray", nrow(res) )

# genes with absolute fold change above 2 will be red
mycols[abs(res$log2FoldChange) > 2] <- "red"
```

```
# genes with adjusted p-value less than 0.01 and absolute fold change more than 2 will be blue
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2)
mycols[inds] <- "blue"

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

# add some cut off lines
abline(v = c(-2,2), col = "gray", lty = 2)
abline(h = -log(0.1), col = "gray", lty = 2)
```



Adding some gene annotation

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

```
##
```

```
columns(org.Hs.eg.db)
```

```
##  [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"
```

```r
res$symbol = mapIds(org.Hs.eg.db, keys = row.names(res), keytype = "ENSEMBL", column = "SYMBOL", multiVa
```

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

```r
res$entrez = mapIds(org.Hs.eg.db, keys = row.names(res), keytype = "ENSEMBL", column = "ENTREZID", multi
```

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

```r
res$name = mapIds(org.Hs.eg.db, keys = row.names(res), keytype = "ENSEMBL", column = "GENENAME", multiVa
```

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

```r
head(res, 10)
```

```
## log2 fold change (MLE): condition hoxa1 kd vs control sirna
## Wald test p-value: condition hoxa1 kd vs control sirna
## DataFrame with 10 rows and 9 columns
##                    baseMean log2FoldChange     lfcSE       stat      pvalue
##                   <numeric>      <numeric> <numeric>  <numeric>   <numeric>
## ENSG00000279457   29.913579      0.1792571 0.3248216   0.551863 5.81042e-01
## ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
## ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630158 1.43990e-36
## ENSG00000187961  209.637938      0.7297556 0.1318599   5.534326 3.12428e-08
## ENSG00000187583   47.255123      0.0405765 0.2718928   0.149237 8.81366e-01
## ENSG00000187642   11.979750      0.5428105 0.5215598   1.040744 2.97994e-01
## ENSG00000188290  108.922128      2.0570638 0.1969053  10.446970 1.51282e-25
## ENSG00000187608  350.716868      0.2573837 0.1027266   2.505522 1.22271e-02
## ENSG00000188157 9128.439422      0.3899088 0.0467163   8.346304 7.04321e-17
## ENSG00000237330    0.158192      0.7859552 4.0804729   0.192614 8.47261e-01
##                        padj      symbol      entrez                   name
##                   <numeric> <character> <character>            <character>
## ENSG00000279457 6.86555e-01     WASH9P   102723897 WAS protein family h..
## ENSG00000187634 5.15718e-03     SAMD11      148398 sterile alpha motif ..
## ENSG00000188976 1.76549e-35      NOC2L       26155 NOC2 like nucleolar ..
## ENSG00000187961 1.13413e-07     KLHL17      339451 kelch like family me..
## ENSG00000187583 9.19031e-01    PLEKHN1       84069 pleckstrin homology ..
## ENSG00000187642 4.03379e-01      PERM1       84808 PPARGC1 and ESRR ind..
## ENSG00000188290 1.30538e-24       HES4       57801 hes family bHLH tran..
## ENSG00000187608 2.37452e-02      ISG15        9636 ISG15 ubiquitin like..
## ENSG00000188157 4.21963e-16       AGRN      375790                  agrin
## ENSG00000237330          NA     RNF223      401934 ring finger protein ..
```

Reorder by p-value and save them to our current directory

```r
res = res[order(res$pvalue),]
write.csv(res, file = "deseq_results.csv")
```

Pathway Analysis

Time to use the **gage** pathway for pathway analysis. First we find a list of enriched pathways, then we use **pathview** to draw pathway diagrams

Install one time only in console

```
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)
data(sigmet.idx.hs)
```

Narrow down to signaling and metabolic pathways only

```
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
head(kegg.sets.hs, 3)
```

```
## $`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"
##
## $`hsa00230 Purine metabolism`
##   [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"    "10714"
##   [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"    "113"
##  [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"    "159"
##  [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"    "221823"
##  [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"  "270"
##  [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"   "2984"  
##  [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"    "3251"  
##  [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"   "4831"  
##  [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"  "50940" 
##  [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"   "5140"  
##  [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"   "5148"  
##  [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"   "5169"  
##  [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"  "5422"  
## [105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"   "5433"  
## [113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"   "5441"  
## [121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"  "55821" 
## [129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"  "6240"  
## [137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"   "84172" 
## [145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"   "9060"  
## [153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"    "957"   
## [161] "9583"   "9615"
```

gage() requires a named vector of fold changes (from DESeq2 analysis) with names of values as Entrez gene IDs (obtained from mapIDs())

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

```
##      1266     54855      1465     51232      2034      2317 
## -2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

Use Gage

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

```
attributes(keggres)
```

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

```
head(keggres$less)
```

```
##                                          p.geomean stat.mean        p.val
## hsa04110 Cell cycle                   8.995727e-06 -4.378644 8.995727e-06
## hsa03030 DNA replication              9.424076e-05 -3.951803 9.424076e-05
## hsa03013 RNA transport                1.375901e-03 -3.028500 1.375901e-03
## hsa03440 Homologous recombination     3.066756e-03 -2.852899 3.066756e-03
## hsa04114 Oocyte meiosis               3.784520e-03 -2.698128 3.784520e-03
## hsa00010 Glycolysis / Gluconeogenesis 8.961413e-03 -2.405398 8.961413e-03
##                                             q.val set.size         exp1
## hsa04110 Cell cycle                   0.001448312      121 8.995727e-06
## hsa03030 DNA replication              0.007586381       36 9.424076e-05
## hsa03013 RNA transport                0.073840037      144 1.375901e-03
## hsa03440 Homologous recombination     0.121861535       28 3.066756e-03
## hsa04114 Oocyte meiosis               0.121861535      102 3.784520e-03
## hsa00010 Glycolysis / Gluconeogenesis 0.212222694       53 8.961413e-03
```

```r
pathview(gene.data = foldchanges, pathway.id = "hsa04110")
```

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04110.pathview.png
```

Another way to present the data…

```r
pathview(gene.data = foldchanges, pathway.id = "hsa04110", kegg.native = FALSE)
```

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04110.pathview.pdf
```

Let's try to pull out the top 5 upregulated pathways to use for future pathview plotting

Focus on top 5

```r
keggrespathways <- rownames(keggres$greater)[1:5]
```

Extract IDs of each string

```r
keggresids = substr(keggrespathways, start = 1, stop = 8)
keggresids
```

```
## [1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

Now use these IDs in pathview to show paths for the top 5

```r
pathview(gene.data = foldchanges, pathway.id = keggresids, species = "hsa")
```

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04640.pathview.png

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04630.pathview.png

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa00140.pathview.png

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04142.pathview.png

## Info: some node width is different from others, and hence adjusted!

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04330.pathview.png
```

Try to do this but with the 5 most downregulated genes Focus on top 5

```
keggrespathways.down <- rownames(keggres$less)[1:5]
```

Extract the IDs (8 characters) of each string

```
keggresids.down <- substr(keggrespathways.down, start = 1, stop = 8)
keggresids.down
```

```
## [1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

View the data

```
pathview(gene.data = foldchanges, pathway.id = keggresids.down, species = "hsa", low = "blue", mid = "g
```

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04110.pathview.png

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa03030.pathview.png

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16
## Info: Writing image file hsa03013.pathview.png

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa03440.pathview.png

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

## Info: Working in directory C:/Users/chapm/Documents/BGGN_213/BGGN_213/bggn213_github/class16

## Info: Writing image file hsa04114.pathview.png
```