lab15

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

```
## Bioconductor version 3.11 (BiocManager 1.30.10), ?BiocManager::install for help

## Bioconductor version '3.11' is out-of-date; the current release version '3.14'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, which.max, which.min
```

```
##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:base':
##
##     expand.grid

## Loading required package: IRanges

## Loading required package: GenomicRanges

## Loading required package: GenomeInfoDb

## Loading required package: SummarizedExperiment

## 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")'.

## Loading required package: DelayedArray

## Loading required package: matrixStats

##
## Attaching package: 'matrixStats'

## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians

##
## Attaching package: 'DelayedArray'

## The following objects are masked from 'package:matrixStats':
##
##     colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges

## The following objects are masked from 'package:base':
##
##     aperm, apply, rowsum
```

#input the data for RNAseq

```
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
```

```
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
```

#Check the correspondence of the metadata and count data

```
colnames(counts) == metadata$id
```

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

```
all(colnames(counts)==metadata$id) #check whether they are matched
```

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

#Q1. How many genes are in this dataset? 38694

```
nrow(counts)
```

```
## [1] 38694
```

#Q2. How many 'control' cell lines do we have? 4

```
length(which(metadata$dex=="control"))
```

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

#Compare control to treated first we need to access all the control columns in our counts data.

```
control.inds <- metadata$dex=="control"
control.ids<-metadata[control.inds,]$id
metadata$dex=="treated"
```

```
## [1] FALSE  TRUE FALSE  TRUE FALSE  TRUE FALSE  TRUE
```

#Use the ids to access just the control fcolumns of our 'counts' data

```
head(counts[,control.ids])
```

```
##                 SRR1039508 SRR1039512 SRR1039516 SRR1039520
## ENSG00000000003        723        904       1170        806
## ENSG00000000005          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(counts[,control.ids])
head(control.mean)
```

```
## ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
##          900.75            0.00          520.50          339.75           97.25
## ENSG00000000938
##            0.75
```

#Do the same for the drug treated

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

```
##                 SRR1039509 SRR1039513 SRR1039517 SRR1039521
## ENSG00000000003        486        445       1097        604
## ENSG00000000005          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(counts[,treated.ids])
head(treated.mean)
```

```
## ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
##          658.00            0.00          546.00          316.50           78.75
## ENSG00000000938
##            0.00
```

#combine our meancount data for bookkeeping

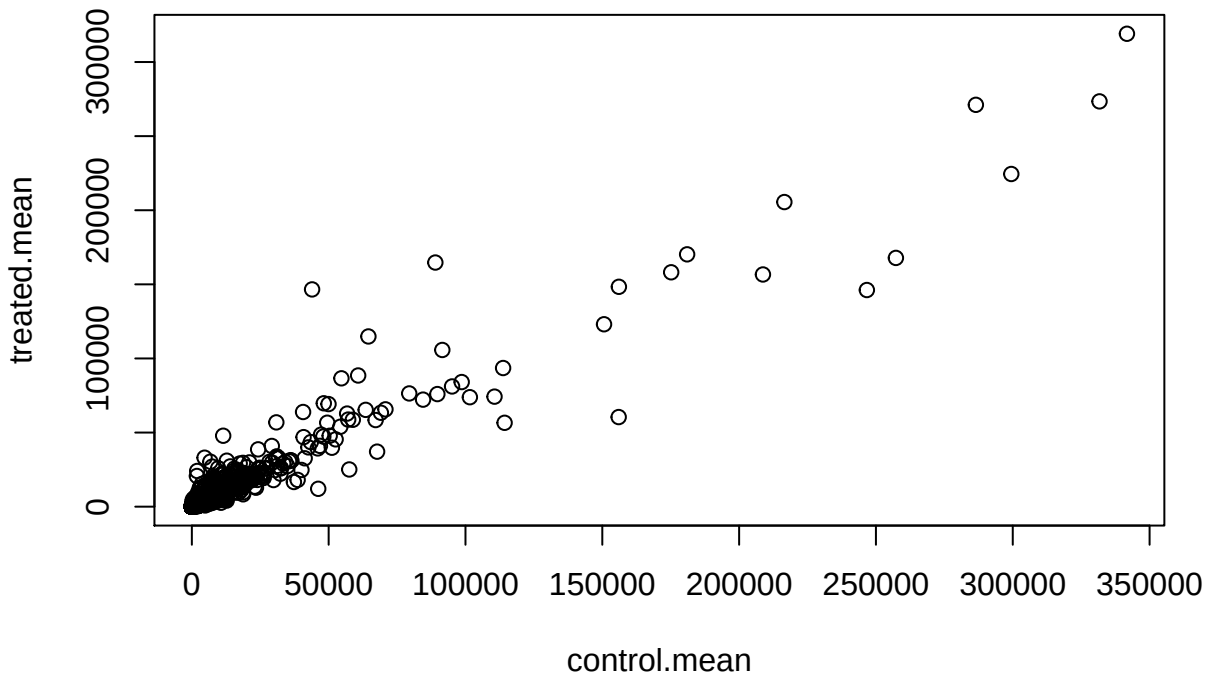
```
meancounts <- data.frame(control.mean,treated.mean)
nrow(counts)
```

```
## [1] 38694
```

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

a quick plot of our progress so far

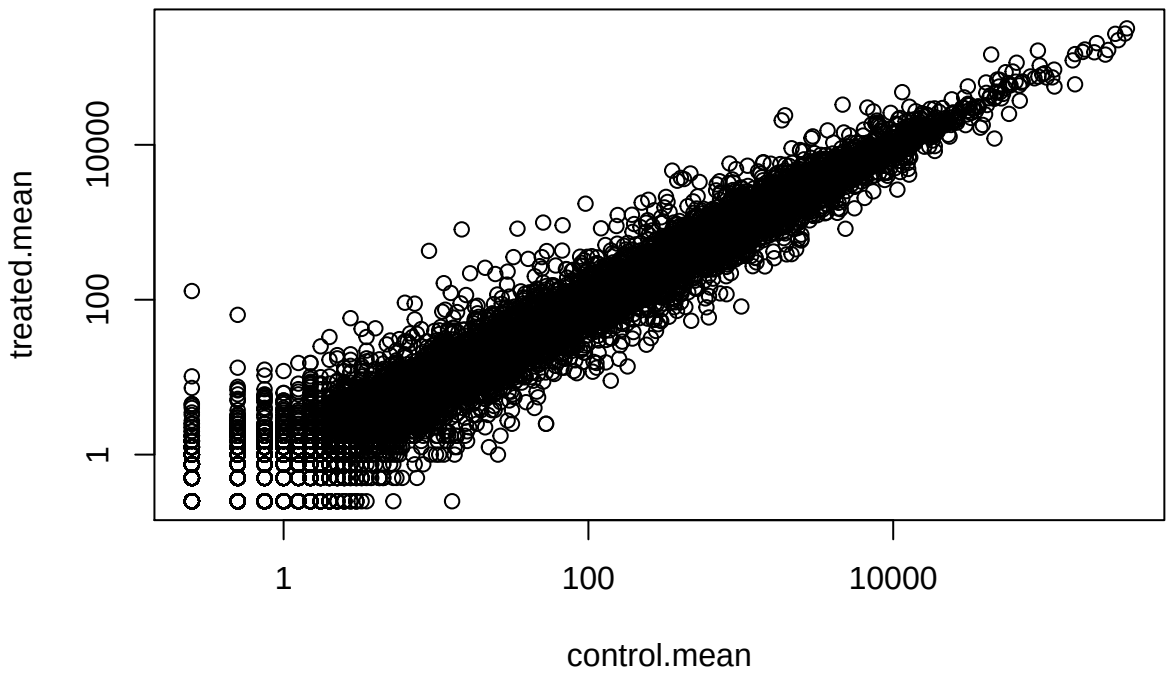
```
plot(meancounts)
```




```
#this would benefit from a single log transform from a plot
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
```




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

```
##                 control.mean treated.mean      log2fc
## ENSG00000000003       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
```

#we need to drop the zero count genes/rows #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? arr.ind is a logical statement that judge whether should array indices be returned when x is an array. Calling unique() will ensure we dont count any row twice.

```
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
```

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

```
to.rm<-unique(sort(inds[,"row"]))
mycounts<-meancounts[-to.rm,]
head(mycounts)
```

```
##                 control.mean treated.mean      log2fc
## ENSG00000000003       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
```

#we now have genes remaining as 'r nrow(mycounts)'

```
nrow(mycounts)
```

```
## [1] 21817
```

```
sum(mycounts < -2)
```

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

#how many of the genes are up regualted at the log2 fold-change threshold of +2 or greater and how to calculate the percentage

```
round((sum(mycounts$log2fc > +2) / nrow(mycounts))*100,2)
```

```
## [1] 1.15
```

#we first need to setup the DEseq object and run the pipeline

```
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 <- 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)
```

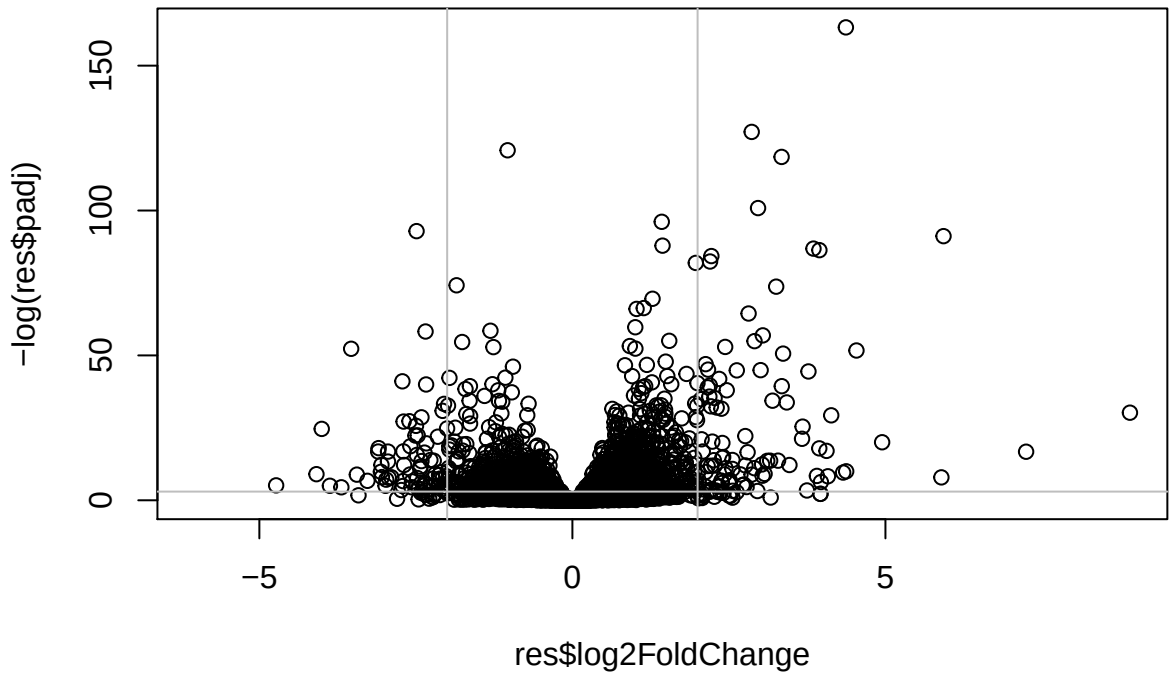
```
##
## out of 25258 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)       : 1563, 6.2%
## LFC < 0 (down)     : 1188, 4.7%
## outliers [1]       : 142, 0.56%
## low counts [2]     : 9971, 39%
## (mean count < 10)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

```
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
```

#A Volcano plot

```
plot(res$log2FoldChange,-log(res$padj))
abline(v=c(-2,2),col="gray")
abline(h=-log(0.05),col="gray")
```



#add annotations #BiocManager::install("org.Hs.eg.db") #BiocManager::install("AnnotationDbi")

```
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] "GO"           "GOALL"        "IPI"          "MAP"          "OMIM"
## [16] "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"         "PMID"
## [21] "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"       "UNIGENE"
## [26] "UNIPROT"
```

```
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")
```

```
## '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    C1orf112
## ENSG00000000938        NA         FGR
```

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

#Pathway analysis Let's try to bring some insights into this #BiocManager::install(c("pathview", "gage", "gageData"))

```
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"
```

Before we can use KEGG we need to get our gene identifiers in the correct format for KEGG which is ENTREZ format in this case

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

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

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

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

```
foldchanges<-res$log2FoldChange
head(foldchanges)
```

```
## [1] -0.35070302          NA  0.20610777  0.02452695 -0.14714205 -1.73228897
```

```
names(foldchanges) <- res$entrez
head(foldchanges)
```

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

#get the restuls

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

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

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

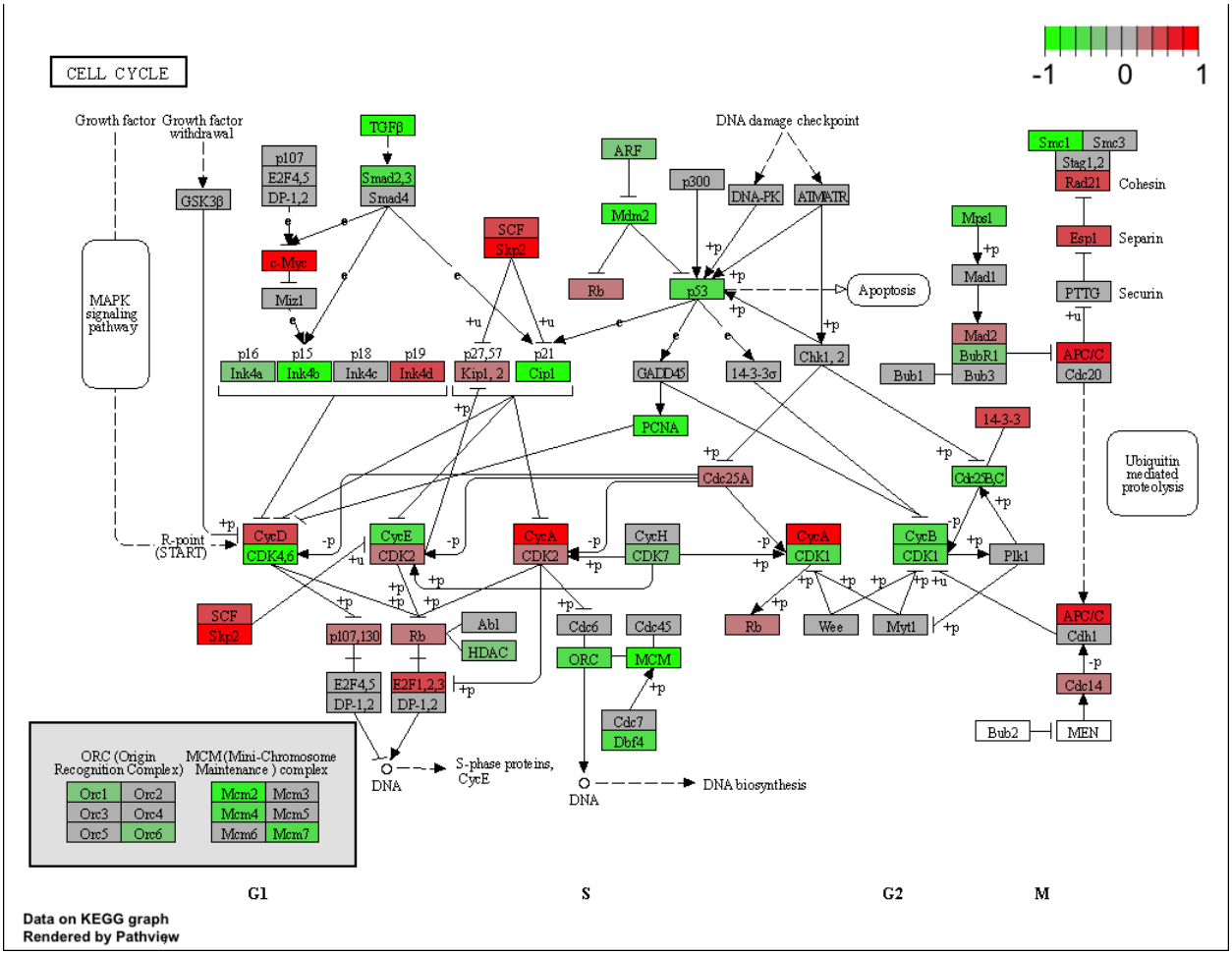
```
##                                                      p.geomean stat.mean
## hsa05332 Graft-versus-host disease                  0.0004250461 -3.473346
## hsa04940 Type I diabetes mellitus                   0.0017820293 -3.002352
## hsa05310 Asthma                                     0.0020045888 -3.009050
## hsa04672 Intestinal immune network for IgA production 0.0060434515 -2.560547
## hsa05330 Allograft rejection                        0.0073678825 -2.501419
## hsa04340 Hedgehog signaling pathway                 0.0133239547 -2.248547
##                                                            p.val      q.val
## hsa05332 Graft-versus-host disease                  0.0004250461 0.09053483
## hsa04940 Type I diabetes mellitus                   0.0017820293 0.14232581
## hsa05310 Asthma                                     0.0020045888 0.14232581
## hsa04672 Intestinal immune network for IgA production 0.0060434515 0.31387180
## hsa05330 Allograft rejection                        0.0073678825 0.31387180
## hsa04340 Hedgehog signaling pathway                 0.0133239547 0.47300039
##                                                     set.size         exp1
## hsa05332 Graft-versus-host disease                        40 0.0004250461
## hsa04940 Type I diabetes mellitus                         42 0.0017820293
## hsa05310 Asthma                                           29 0.0020045888
## hsa04672 Intestinal immune network for IgA production     47 0.0060434515
## hsa05330 Allograft rejection                              36 0.0073678825
## hsa04340 Hedgehog signaling pathway                       56 0.0133239547
```

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

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

```
## Info: Working in directory /Users/ziyuanhan/Desktop/UCSD  PhD/Courses VS Assignent/2021Fall Courses/
```

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




```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

```
## [1] "hsa00500" "hsa00330" "hsa04910" "hsa04510" "hsa04920"
```