

Class16:RNAseq_analysis

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```
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
```

```
## Loading required package: GenomicRanges
```

```
## Warning: package 'GenomicRanges' was built under R version 4.1.2
```

```
## 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, colAvgPerRowSet, 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, rowAvgPerColSet,
##   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"

```

```
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
```

```
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
```

#Q:

```
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
```

#Q:

```
countData = countData[-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

```
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
```

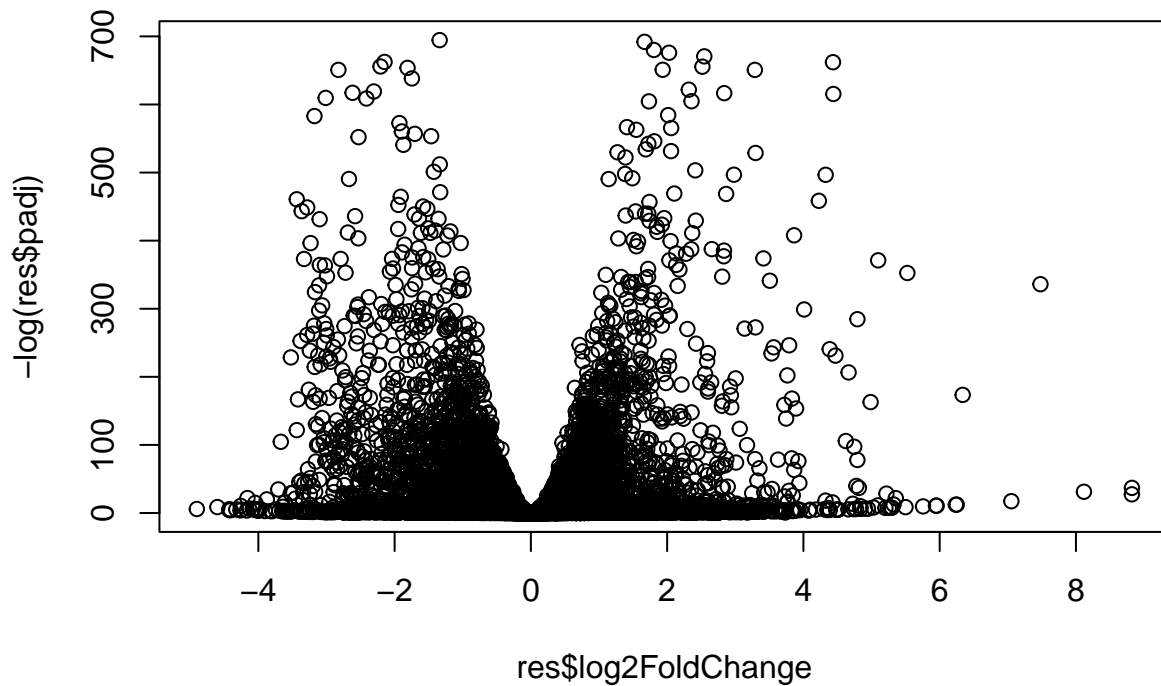
```
dds
```

```
## 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, contrast=c("condition", "hoxa1_kd", "control_sirna"))
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
```

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



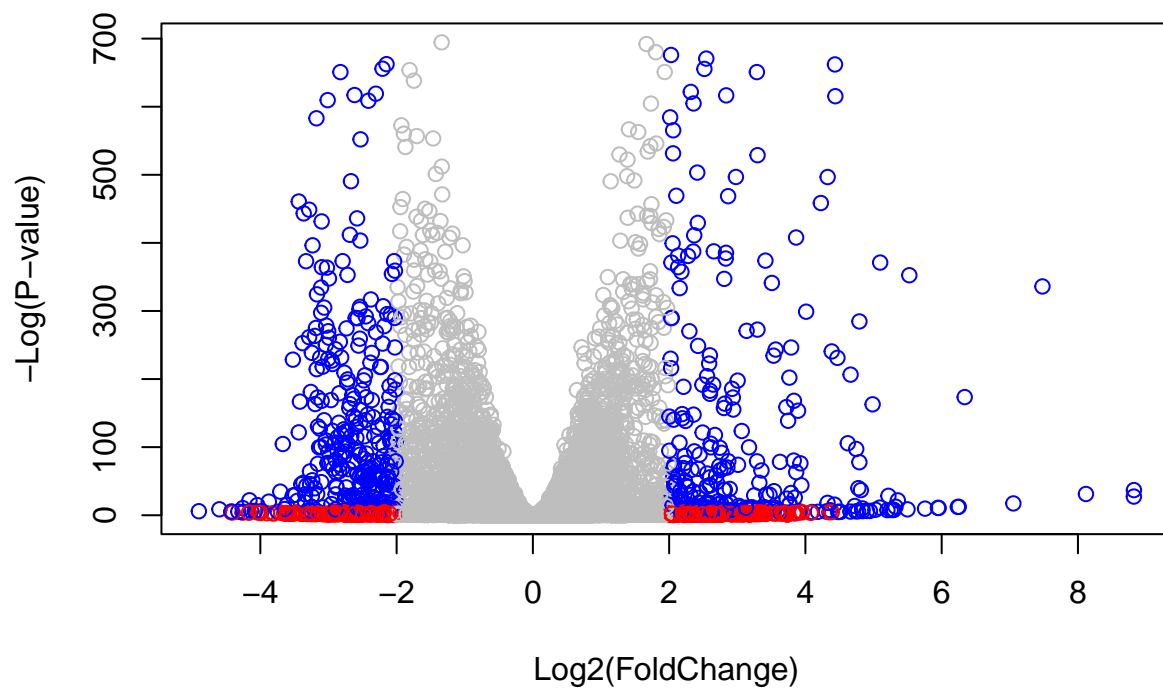
#Q:

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

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

# Color blue those with adjusted p-value less than 0.01
# and absolute fold change more than 2
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)" )
```



```
library(AnnotationDbi)
```

```
## Warning: package 'AnnotationDbi' was built under R version 4.1.2
```

```
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"
```

```
#Q:
```

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

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

```
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
```

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

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

```
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 ..	

```
#Q:
```

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

```
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)
```

```
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"
```

```
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
```

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

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

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

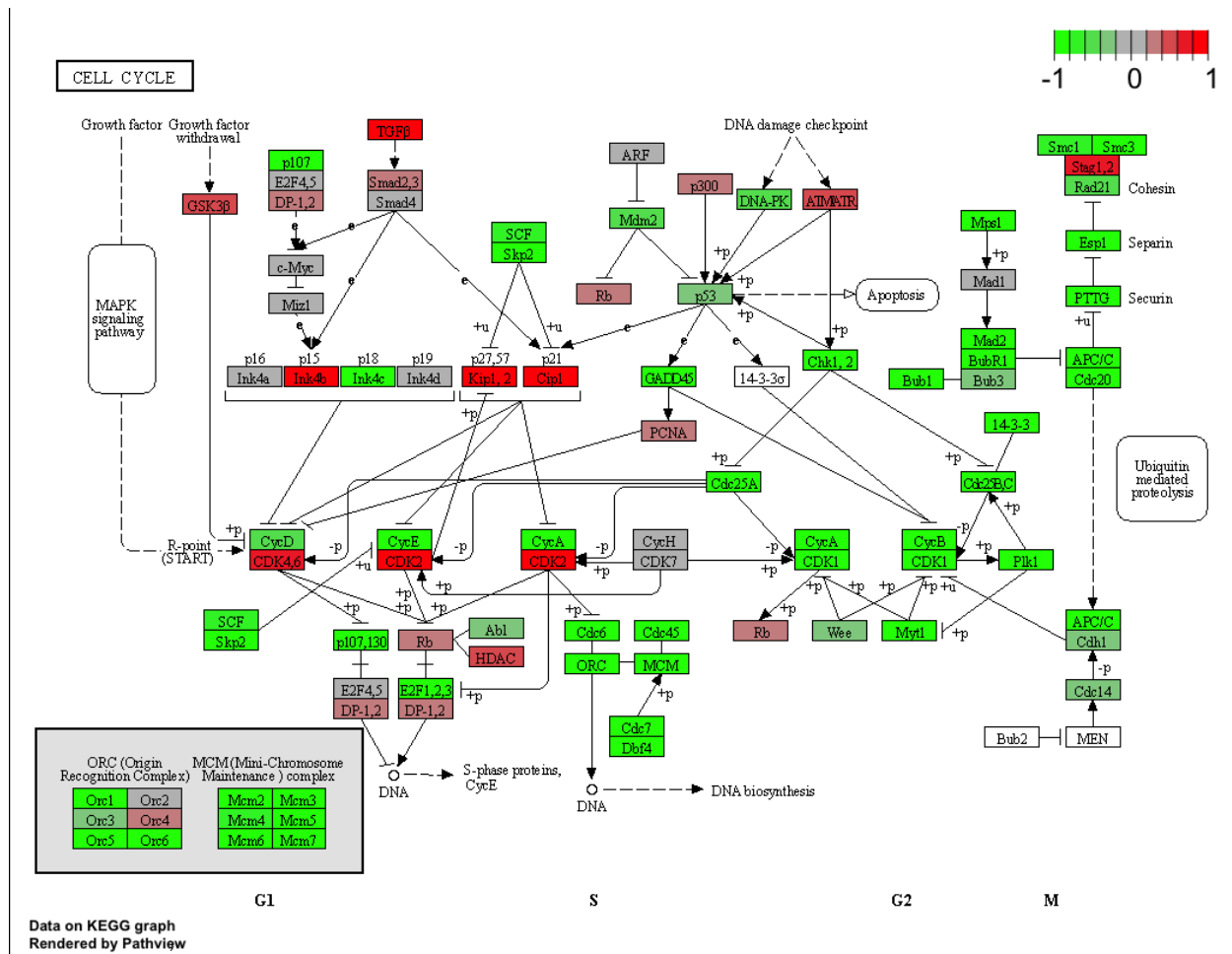
```
##                                p.geomean stat.mean      p.val
## hsa04110 Cell cycle             8.995727e-06 -4.378644 8.995727e-06
## hsa03030 DNA replication        9.424076e-05 -3.951803 9.424076e-05
## hsa03013 RNA transport          1.375901e-03 -3.028500 1.375901e-03
## hsa03440 Homologous 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
```

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

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

```
## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15
```

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



```
keggrespathways <- rownames(keggres$greater)[1:5]
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

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

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

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

```
## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15
```

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

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

```
## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15
```

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

```

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

## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15

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

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

## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15

## 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 /Users/apple/Desktop/bimm143_github/class15

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

keggrespathways1<- rownames(keggres$less)[1:5]
keggresids1 = substr(keggrespathways, start=1, stop=8)
keggresids1

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

pathview(gene.data=foldchanges, pathway.id=keggresids1, species="hsa")

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

## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15

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

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

## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15

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

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

## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15

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

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

```

```
## Info: Working in directory /Users/apple/Desktop/bimm143_github/class15

## 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 /Users/apple/Desktop/bimm143_github/class15

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

```
data(go.sets.hs)
data(go.subs.hs)

gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets, same.dir=TRUE)

lapply(gobpres, head)
```

```
## $greater
##
##           p.geomean stat.mean      p.val
## GO:0007156 homophilic cell adhesion      8.519724e-05  3.824205 8.519724e-05
## GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
## GO:0048729 tissue morphogenesis          1.432451e-04  3.643242 1.432451e-04
## GO:0007610 behavior                      2.195494e-04  3.530241 2.195494e-04
## GO:0060562 epithelial tube morphogenesis 5.932837e-04  3.261376 5.932837e-04
## GO:0035295 tube development              5.953254e-04  3.253665 5.953254e-04
##
##           q.val set.size      exp1
## GO:0007156 homophilic cell adhesion      0.1951953      113 8.519724e-05
## GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
## GO:0048729 tissue morphogenesis          0.1951953      424 1.432451e-04
## GO:0007610 behavior                      0.2243795      427 2.195494e-04
## GO:0060562 epithelial tube morphogenesis 0.3711390      257 5.932837e-04
## GO:0035295 tube development              0.3711390      391 5.953254e-04
##
## $less
##
##           p.geomean stat.mean      p.val
## GO:0048285 organelle fission             1.536227e-15 -8.063910 1.536227e-15
## GO:0000280 nuclear division              4.286961e-15 -7.939217 4.286961e-15
## GO:0007067 mitosis                      4.286961e-15 -7.939217 4.286961e-15
## GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
## GO:0007059 chromosome segregation         2.028624e-11 -6.878340 2.028624e-11
## GO:0000236 mitotic prometaphase          1.729553e-10 -6.695966 1.729553e-10
##
##           q.val set.size      exp1
## GO:0048285 organelle fission             5.841698e-12      376 1.536227e-15
## GO:0000280 nuclear division              5.841698e-12      352 4.286961e-15
## GO:0007067 mitosis                      5.841698e-12      352 4.286961e-15
## GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
## GO:0007059 chromosome segregation         1.658603e-08      142 2.028624e-11
## GO:0000236 mitotic prometaphase          1.178402e-07       84 1.729553e-10
```

```
##
## $stats
##
##          stat.mean    exp1
## G0:0007156 homophilic cell adhesion    3.824205 3.824205
## G0:0002009 morphogenesis of an epithelium 3.653886 3.653886
## G0:0048729 tissue morphogenesis    3.643242 3.643242
## G0:0007610 behavior    3.530241 3.530241
## G0:0060562 epithelial tube morphogenesis 3.261376 3.261376
## G0:0035295 tube development    3.253665 3.253665
```

```
res
```

```
## log2 fold change (MLE): condition hoxa1_kd vs control_sirna
## Wald test p-value: condition hoxa1 kd vs control sirna
## DataFrame with 15975 rows and 9 columns
##          baseMean log2FoldChange    lfcSE      stat    pvalue
##          <numeric>    <numeric> <numeric>    <numeric> <numeric>
## ENSG00000117519    4483.63    -2.42272 0.0600016    -40.3776      0
## ENSG00000183508    2053.88     3.20196 0.0724172     44.2154      0
## ENSG00000159176    5692.46    -2.31374 0.0575534    -40.2016      0
## ENSG00000150938    7442.99    -2.05963 0.0538449    -38.2512      0
## ENSG00000116016    4423.95    -1.88802 0.0431680    -43.7366      0
## ...          ...          ...          ...          ...
## ENSG00000166507    13.040331 -5.62340e-04 0.4927065 -1.14133e-03 0.999089
## ENSG00000175279    15.141148 -4.60008e-04 0.4506727 -1.02071e-03 0.999186
## ENSG00000133997    199.422283  1.99090e-05 0.1332356  1.49427e-04 0.999881
## ENSG00000160948    788.341963 -2.75437e-06 0.0694729 -3.96467e-05 0.999968
## ENSG00000140600     0.682106  8.31079e-05 2.2352961  3.71798e-05 0.999970
##          padj      symbol      entrez      name
##          <numeric> <character> <character>    <character>
## ENSG00000117519      0        CNN3      1266      calponin 3
## ENSG00000183508      0       TENT5C     54855 terminal nucleotidyl..
## ENSG00000159176      0       CSRP1      1465 cysteine and glycine..
## ENSG00000150938      0       CRIM1     51232 cysteine rich transm..
## ENSG00000116016      0       EPAS1      2034 endothelial PAS doma..
## ...          ...          ...          ...
## ENSG00000166507 0.999361      NDST2      8509 N-deacetylase and N-..
## ENSG00000175279 0.999389      CENPS     378708 centromere protein S
## ENSG00000133997 0.999970      MED6     10001 mediator complex sub..
## ENSG00000160948 0.999970      VPS28     51160 VPS28 subunit of ESC..
## ENSG00000140600 0.999970     SH3GL3      6457 SH3 domain containin..
```