

lab16

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#Differential expression gene analysis

```
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

metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"
# Import metadata and take a peak
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

```

Import 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
```

Note we need to remove the odd first \$length col

```
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
```

Filter count data where you have 0 read count across all samples.

Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns). Tip: What will rowSums() of countData return and how could you use it in this context?

```
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
```

```
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
```

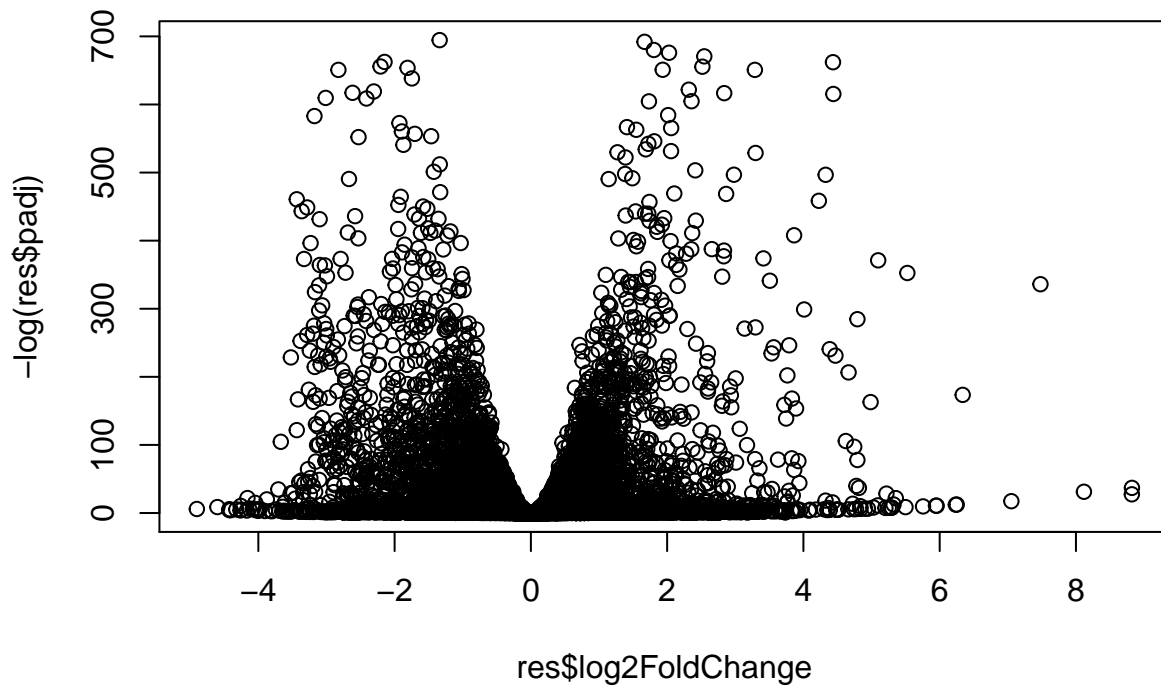
```
## class: DESeqDataSet  
## dim: 15975 6  
## metadata(1): version  
## assays(4): counts mu H cooks  
## rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345  
##      ENSG00000271254  
## rowData names(22): baseMean baseVar ... deviance maxCooks  
## colnames(6): SRR493366 SRR493367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

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

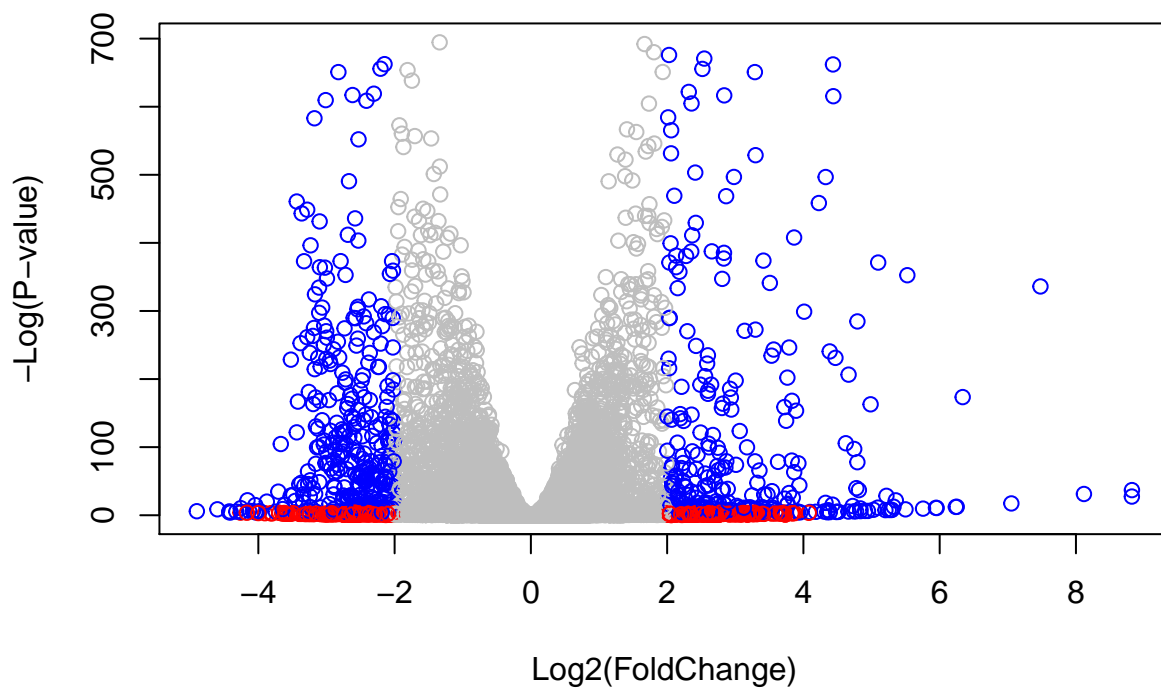


```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

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

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



```
#Adding 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] "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),
                     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		
	<numeric>	<character>	<character>		
## ENSG00000279457	6.86555e-01	NA	NA		
## ENSG00000187634	5.15718e-03	SAMD11	148398		
## ENSG00000188976	1.76549e-35	NOC2L	26155		
## ENSG00000187961	1.13413e-07	KLHL17	339451		
## ENSG00000187583	9.19031e-01	PLEKHN1	84069		
## ENSG00000187642	4.03379e-01	PERM1	84808		
## ENSG00000188290	1.30538e-24	HES4	57801		
## ENSG00000187608	2.37452e-02	ISG15	9636		
## ENSG00000188157	4.21963e-16	AGRN	375790		
## ENSG00000237330	NA	RNF223	401934		
				name	
				<character>	
## ENSG00000279457				NA	
## ENSG00000187634				sterile alpha motif domain containing 11	
## ENSG00000188976	NOC2			like nucleolar associated transcriptional repressor	
## ENSG00000187961				kelch like family member 17	
## ENSG00000187583				pleckstrin homology domain containing N1	

```
## ENSG00000187642          PPARGC1 and ESRR induced regulator, muscle 1
## ENSG00000188290          hes family bHLH transcription factor 4
## ENSG00000187608          ISG15 ubiquitin like modifier
## ENSG00000188157          agrin
## ENSG00000237330          ring finger protein 223
```

#Q. Finally for this section let's reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

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

#Pathway analysis

```
#BiocManager::install( c("pathview", "gage", "gageData") )
```

For old versions of R only (R < 3.5.0)!

```
#source("http://bioconductor.org/biocLite.R") #biocLite( 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)
```

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

```
# Focus on signaling and metabolic pathways only
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
```

```
# Examine the first 3 pathways
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
```

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
attributes(keggres)
```

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

```
# Look at the first few down (less) pathways
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/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignment/2021Fall Courses/1
```

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

A different PDF based output of the same data

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

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

```
## Info: Working in directory /Users/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignment/2021Fall Courses/1
```

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

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] "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/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignment/2021Fall Courses/1
```

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

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

## Info: Working in directory /Users/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignent/2021Fall Courses/1

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

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

## Info: Working in directory /Users/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignent/2021Fall Courses/1

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

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

## Info: Working in directory /Users/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignent/2021Fall Courses/1

## 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/ziyuanhan/Desktop/UCSD PhD/Courses VS Assignent/2021Fall Courses/1

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

```
#Gene Ontology
```

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

# Focus on Biological Process subset of GO
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 0.1951475      113 8.519724e-05
```

```
## G0:0002009 morphogenesis of an epithelium 0.1951475      339 1.396681e-04
## G0:0048729 tissue morphogenesis          0.1951475      424 1.432451e-04
## G0:0007610 behavior                      0.2243246      427 2.195494e-04
## G0:0060562 epithelial tube morphogenesis 0.3710482      257 5.932837e-04
## G0:0035295 tube development              0.3710482      391 5.953254e-04
##
## $less
##
##           p.geomean stat.mean      p.val
## G0:0048285 organelle fission 1.536227e-15 -8.063910 1.536227e-15
## G0:0000280 nuclear division  4.286961e-15 -7.939217 4.286961e-15
## G0:0007067 mitosis           4.286961e-15 -7.939217 4.286961e-15
## G0:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
## G0:0007059 chromosome segregation 2.028624e-11 -6.878340 2.028624e-11
## G0:0000236 mitotic prometaphase 1.729553e-10 -6.695966 1.729553e-10
##
##           q.val set.size      exp1
## G0:0048285 organelle fission 5.840269e-12      376 1.536227e-15
## G0:0000280 nuclear division  5.840269e-12      352 4.286961e-15
## G0:0007067 mitosis           5.840269e-12      352 4.286961e-15
## G0:0000087 M phase of mitotic cell cycle 1.195380e-11      362 1.169934e-14
## G0:0007059 chromosome segregation 1.658197e-08      142 2.028624e-11
## G0:0000236 mitotic prometaphase 1.178114e-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
```

#Reactome Analysis

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
## [1] "Total number of significant genes: 8147"
```

```
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quote=FALSE)
```