class12

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Differential Expression Analysis

Work on a complete differential expression analysis project using DESeq2.

```
library(DESeq2)
library(ggplot2)
library(AnnotationDbi)
library(org.Hs.eg.db)
```

Input the count and metadata files

```
countData <- read.csv("GSE37704_featurecounts.csv", row.names = 1)
colData <- read.csv("GSE37704_metadata.csv", row.names = 1)

head(countData[,-1])
```

```
##                 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
```

```
head(colData)
```

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

> Q. Complete the code below to remove the troublesome first column from countData

```
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
```

Check correspondence of colData and countData

```
all(colData$id == colnames(count))
```

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

> 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).

```
counts <- countData[rowSums(countData) !=0, ]
head(counts)
```

```
##                 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
```

Run DESeq analysis

```
dds <-  DESeqDataSetFromMatrix(countData=counts,
                               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
```

```
res <- results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

> Q. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff. 4349 up-regulated, 4396 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
```

Add annotation

```
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. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
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"
```

```
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 ..
```

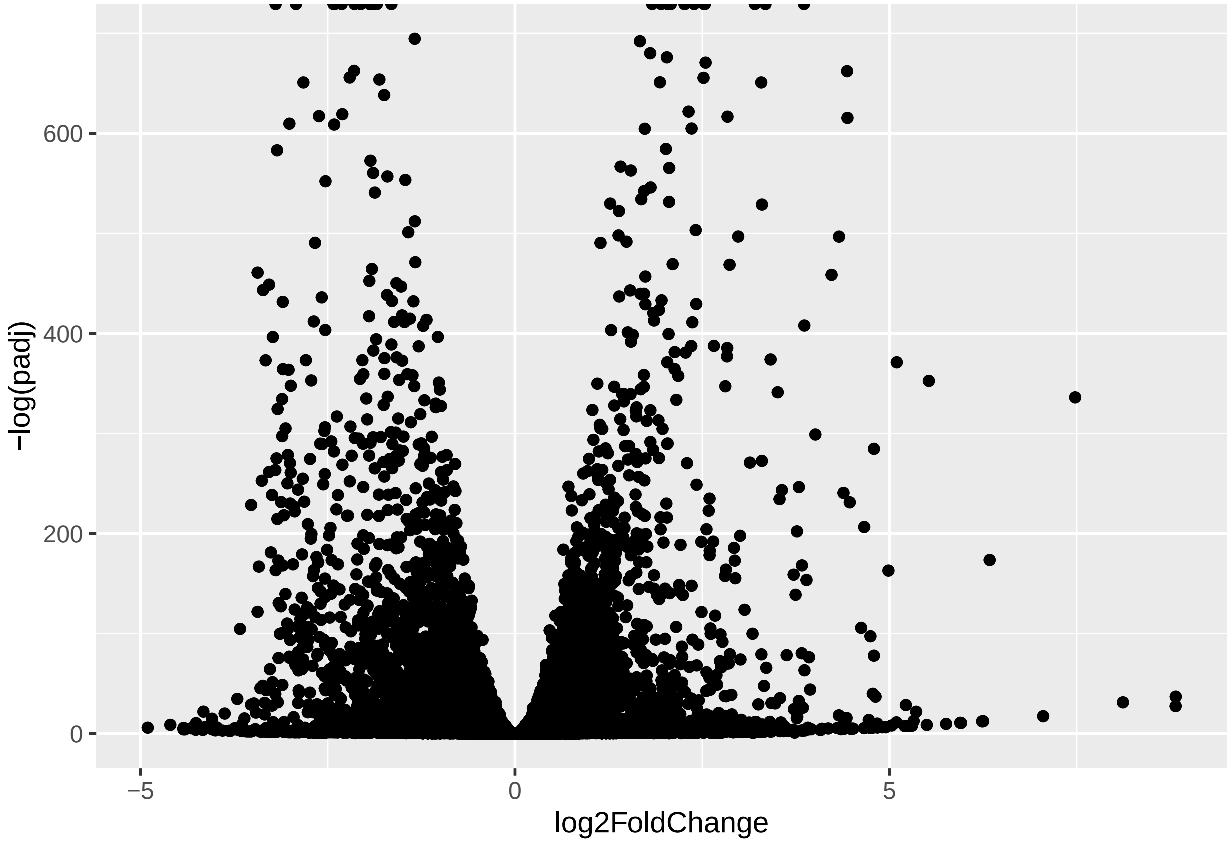
Volcano Plot

Try ggplot

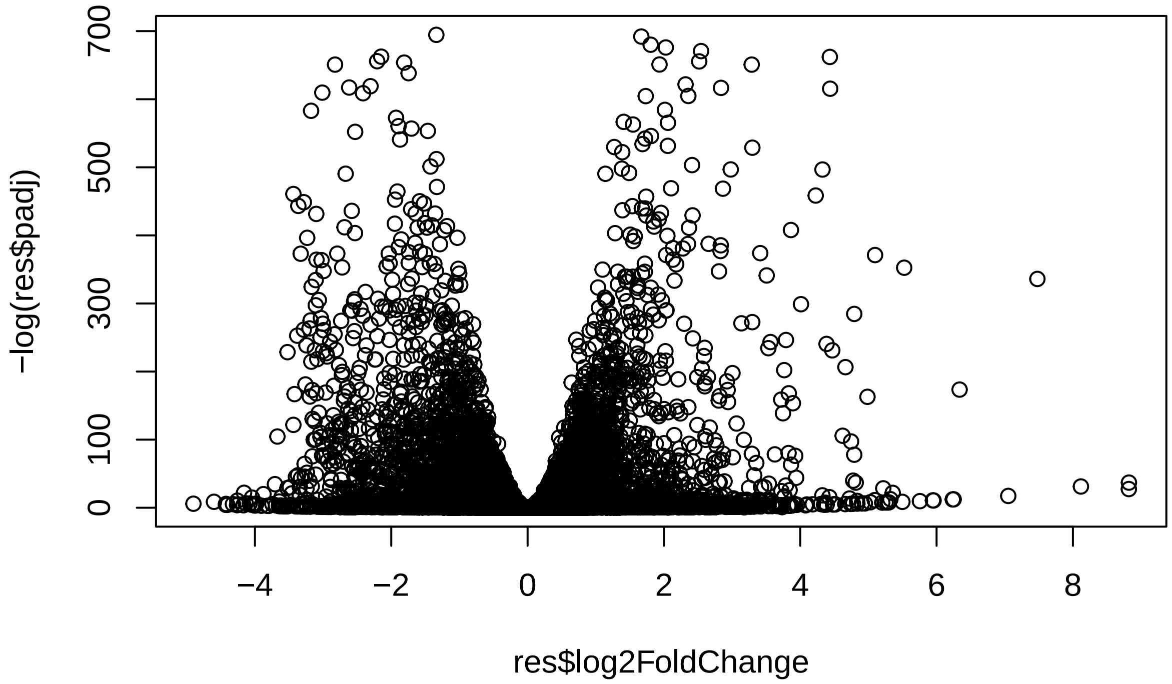
```
tmp <- as.data.frame(res)

ggplot(tmp) +
  aes(log2FoldChange, -log(padj)) +
  geom_point()
```

```
## Warning: Removed 1237 rows containing missing values (geom_point).
```



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



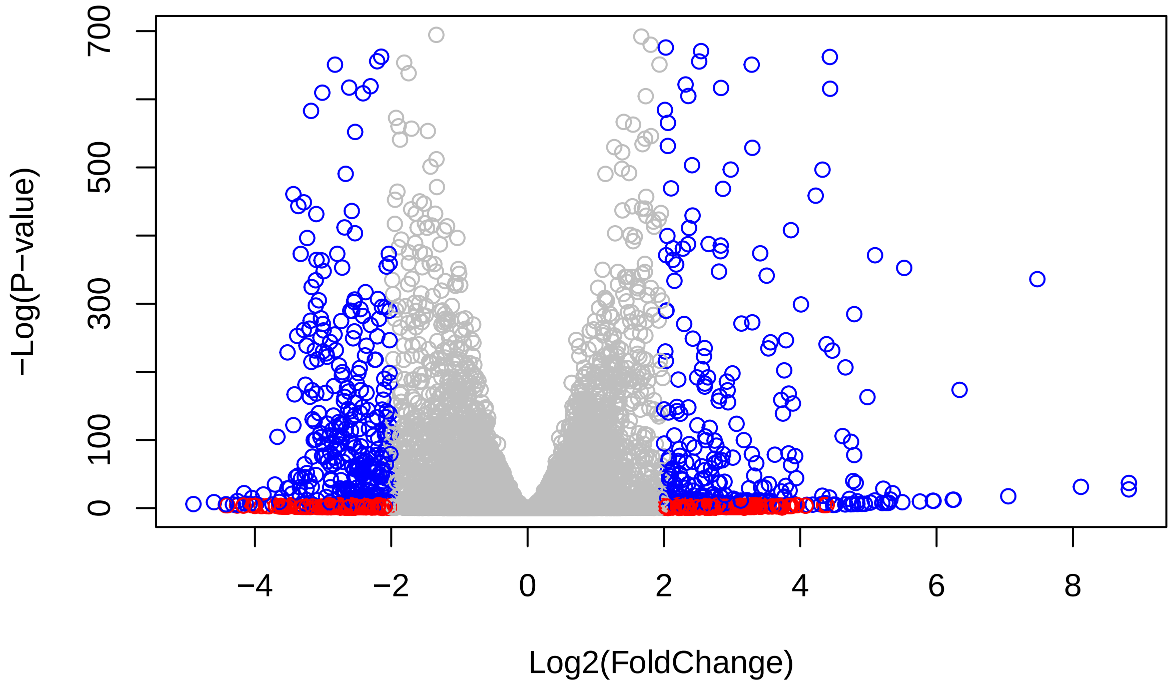
Q. Improve this plot by completing the below code, which adds color and axis labels

```
# 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)" )
```

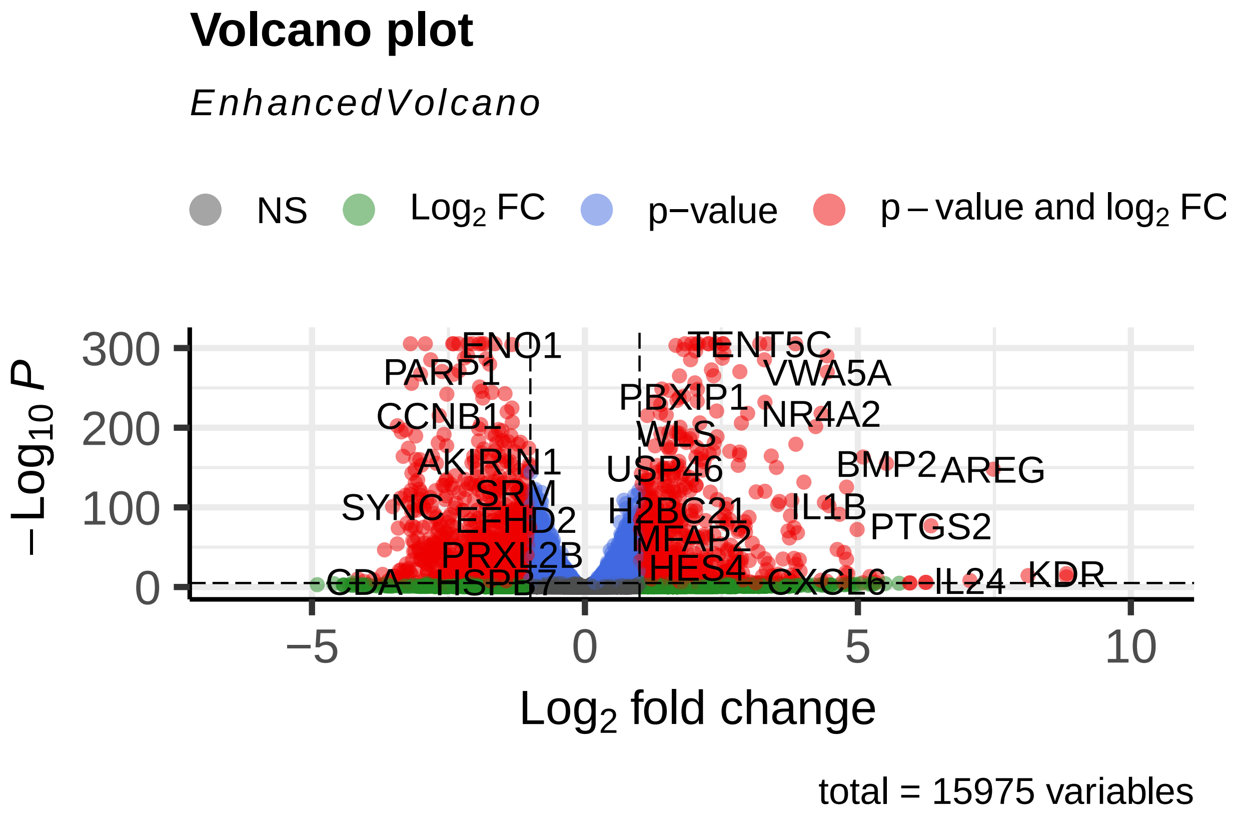


```
library(EnhancedVolcano)

x <- as.data.frame(res)

EnhancedVolcano(x,
    lab = x$symbol,
    x = 'log2FoldChange',
    y = 'pvalue')
```

```
## Warning: One or more p-values is 0. Converting to 10^-1 * current lowest non-
## zero p-value...
```



Pathway Analysis and Gene Set Enrichment

Which pathways and functions feature heavily in our differentially expressed genes.

```
library(pathview)
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"
```

Need a “vector of importance” as innput for GAGE that has ENTREZ IDs set as the names attribute.

```
foldchanges <-  res$log2FoldChange
names(foldchanges) <-  res$entrez
```

GAGE Pathway Analysis

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

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

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

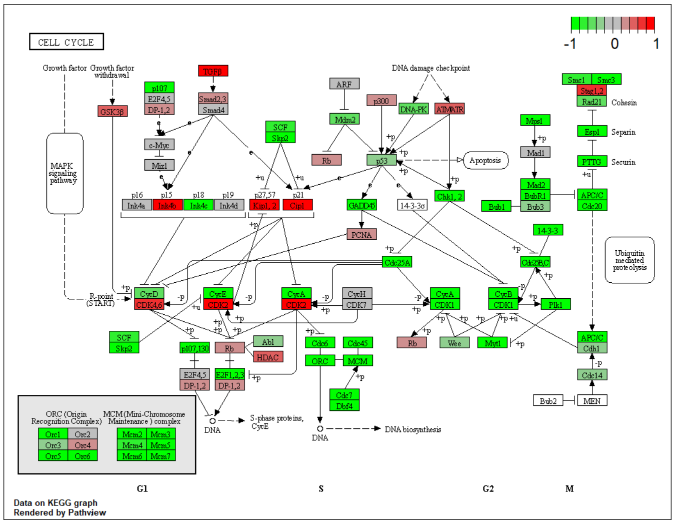
```
##                                          p.geomean stat.mean        p.val
## hsa04110 Cell cycle                   8.995727e-06 -4.378644 8.995727e-06
## hsa03030 DNA replication              9.424076e-05 -3.951803 9.424076e-05
## hsa03013 RNA transport                1.246882e-03 -3.059466 1.246882e-03
## 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                0.066915974      144 1.246882e-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 C:/Users/vince/Desktop/UCSD/Academic Years/Fourth Year/BIMM143/class12
```

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



Data on KEGG graph
Rendered by Pathview

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       0.1951953      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
## GO:0007156 homophilic cell adhesion        3.824205 3.824205
## GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
## GO:0048729 tissue morphogenesis            3.643242 3.643242
## GO:0007610 behavior                        3.530241 3.530241
## GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
## GO:0035295 tube development                3.253665 3.253665
```

Reactome Analysis

> Q: What pathway has the most significant "Entities p-value"? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods? Endosomal/Vacuolar pathway. The pathways differ from the previous KEGG results. Using different criteria to determine pathways could cause differences.

```r
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"
```

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

Save results

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