

Class13

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```
#load our files  
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, aperm, 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  
## 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 <- "https://marcos-diazg.github.io/BIMM143_SP23/class-material/class13/GSE37704_metadata.csv"
countFile <- "https://marcos-diazg.github.io/BIMM143_SP23/class-material/class13/GSE37704_featurecounts"

#View 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

# Import and View countdata
countData = read.csv(countFile, row.names=1)
head(countData)

##              length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
## ENSG00000186092    918         0         0         0         0
## ENSG00000279928    718         0         0         0         0
## ENSG00000279457   1982        23        28        29        29        28
## ENSG00000278566    939         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
```

Q1. Complete the code below to remove the troublesome first column from countData

```
# 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
```

Q2. 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).

```
# Filter count data where you have 0 read count across all samples.
countData = countData[rowSums(countData>0)>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
```

```
#setup the DESeqDataSet
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
```

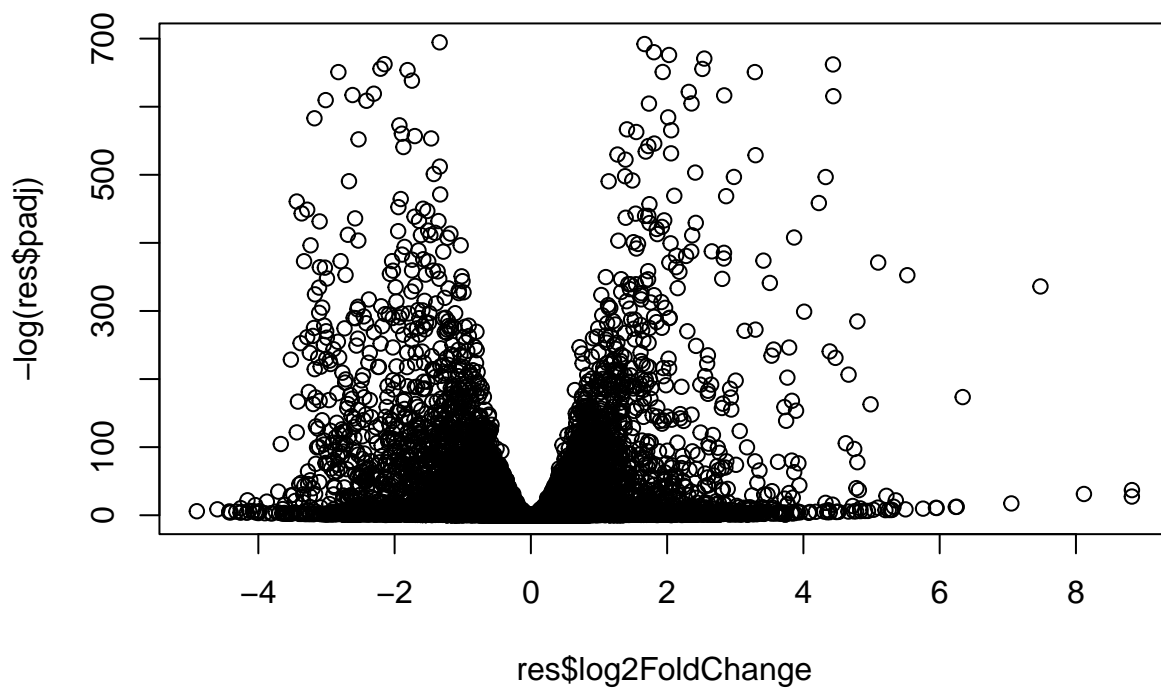
Q3. Call the `summary()` function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
#Making results and running summary()
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
```

Volcano plot

```
#Make volcano plot
plot( res$log2FoldChange, -log(res$padj) )
```



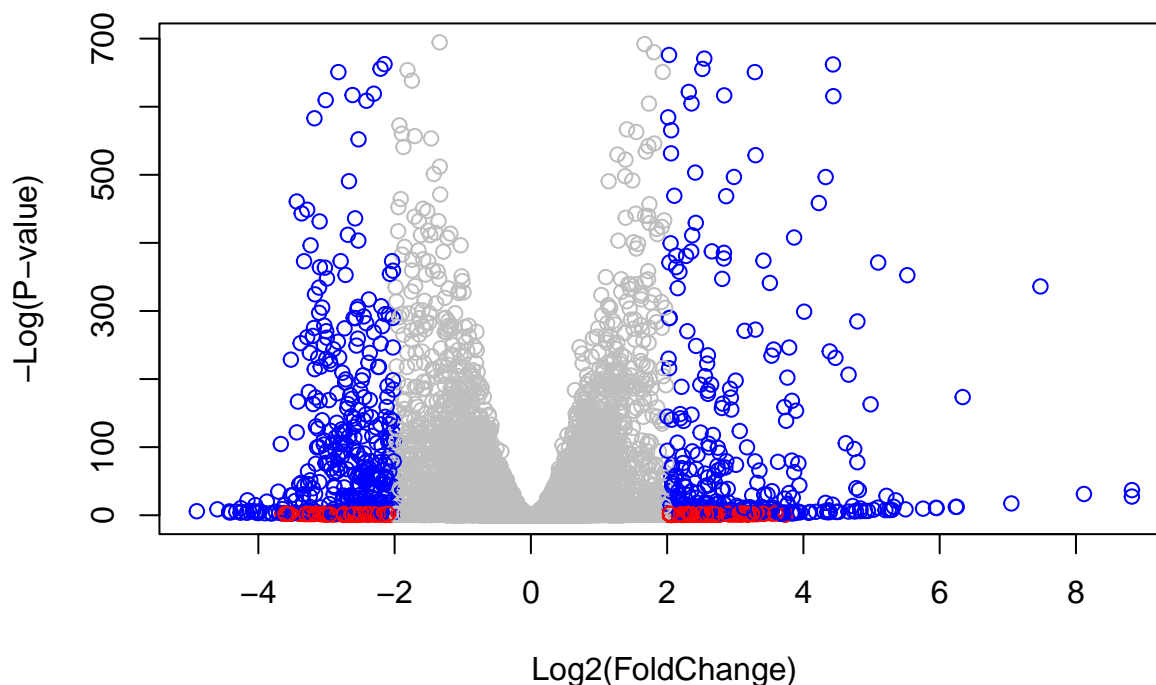
>Improve this plot by completing the below code, which adds color and axis labels

```
# 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 ] <- "blue"

# Color blue those with adjusted p-value less than 0.01
# and absolute fold change more than 2
inds <- (res$pvalue>0.05) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "red"

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



Q5. 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="ENTREZID",
                    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="ENTREZID",
                  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      NA      NA      NA
## ENSG00000187634 5.15718e-03    148398    148398    148398
## ENSG00000188976 1.76549e-35     26155     26155     26155
## ENSG00000187961 1.13413e-07    339451    339451    339451
## ENSG00000187583 9.19031e-01     84069     84069     84069
## ENSG00000187642 4.03379e-01     84808     84808     84808
## ENSG00000188290 1.30538e-24     57801     57801     57801
## ENSG00000187608 2.37452e-02      9636      9636      9636
## ENSG00000188157 4.21963e-16    375790    375790    375790
## ENSG00000237330      NA    401934    401934    401934

```

Q6. 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.

```

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

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
library(gage)
```

```
##
```

```
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
```

```
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).
```

```
## #####
```

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

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

```
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
```

```
## 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
```

```
## Warning: reconcile groups sharing member nodes!
```

```
##      [,1] [,2]
## [1,] "9"  "300"
## [2,] "9"  "306"
```

```
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
```

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

Q7. Can you do the same procedure as above to plot the pathview figures for the top 5 down-regulated pathways?

```
## Focus on top 5 downregulated pathways
```

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

```

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

## [1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")

## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
## Info: Writing image file hsa04110.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
## Info: Writing image file hsa03030.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
## Info: Writing image file hsa03013.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
## Info: Writing image file hsa03440.pathview.png
## 'select()' returned 1:1 mapping between keys and columns
## Info: Working in directory /Users/zainabashir/Desktop/BIMM 143/Week07
## Info: Writing image file hsa04114.pathview.png

```

Section 3. Gene Ontology (GO)

```

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

```

```
## G0:0048729 tissue morphogenesis      0.1951953      424 1.432451e-04
## G0:0007610 behavior                   0.2243795      427 2.195494e-04
## G0:0060562 epithelial tube morphogenesis 0.3711390      257 5.932837e-04
## G0:0035295 tube development           0.3711390      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      5.841698e-12      376 1.536227e-15
## G0:0000280 nuclear division       5.841698e-12      352 4.286961e-15
## G0:0007067 mitosis                 5.841698e-12      352 4.286961e-15
## G0:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
## G0:0007059 chromosome segregation    1.658603e-08      142 2.028624e-11
## G0:0000236 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
```

Section 4. 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)
```

Q8: 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?

#It would be Disease pathway where it shows Defective factor VIII causes hemophilia A with lowest p_value. No it doesnt match it. A different factot can be the datatbase of the pathway where the previous results used GO and this one uses method of reactome website. Also, another difference is the data used.

Q9: 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?

#It is metabolic process. Yes it does. Differences could be data used and website used as before.

```
sessionInfo()
```

```
## R version 4.2.3 (2023-03-15)
```

```

## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] pathview_1.38.0      gage_2.48.0
## [3] gageData_2.36.0      org.Hs.eg.db_3.16.0
## [5] AnnotationDbi_1.60.2 DESeq2_1.38.3
## [7] SummarizedExperiment_1.28.0 Biobase_2.58.0
## [9] MatrixGenerics_1.10.0 matrixStats_0.63.0
## [11] GenomicRanges_1.50.2  GenomeInfoDb_1.34.9
## [13] IRanges_2.32.0        S4Vectors_0.36.2
## [15] BiocGenerics_0.44.0
##
## loaded via a namespace (and not attached):
## [1] httr_1.4.6           bit64_4.0.5           highr_0.10
## [4] blob_1.2.4           GenomeInfoDbData_1.2.9 yaml_2.3.7
## [7] pillar_1.9.0         RSQlite_2.3.1         lattice_0.21-8
## [10] glue_1.6.2           digest_0.6.31         RColorBrewer_1.1-3
## [13] XVector_0.38.0       colorspace_2.1-0      htmltools_0.5.5
## [16] Matrix_1.5-4.1       XML_3.99-0.14         pkgconfig_2.0.3
## [19] zlibbioc_1.44.0      xtable_1.8-4          GO.db_3.16.0
## [22] scales_1.2.1         BiocParallel_1.32.6   tibble_3.2.1
## [25] annotate_1.76.0      KEGGREST_1.38.0       generics_0.1.3
## [28] ggplot2_3.4.2        cachem_1.0.8          cli_3.6.1
## [31] magrittr_2.0.3       crayon_1.5.2          KEGGgraph_1.58.3
## [34] memoise_2.0.1        evaluate_0.21         fansi_1.0.4
## [37] graph_1.76.0         tools_4.2.3           lifecycle_1.0.3
## [40] munsell_0.5.0        locfit_1.5-9.7        DelayedArray_0.24.0
## [43] Biostrings_2.66.0    compiler_4.2.3        rlang_1.1.1
## [46] grid_4.2.3           RCurl_1.98-1.12       rstudioapi_0.14
## [49] bitops_1.0-7         rmarkdown_2.21        gtable_0.3.3
## [52] codetools_0.2-19     DBI_1.1.3             R6_2.5.1
## [55] knitr_1.42           dplyr_1.1.2           fastmap_1.1.1
## [58] bit_4.0.5            utf8_1.2.3            Rgraphviz_2.42.0
## [61] parallel_4.2.3       Rcpp_1.0.10           vctrs_0.6.2
## [64] geneplotter_1.76.0   png_0.1-8             tidyselect_1.2.0
## [67] xfun_0.39

```