

Class16

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1. Differential Expression Analysis

```
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
```

```

## 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, colAveragesPerRowSet, 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, rowAveragesPerColSet,
##   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"

# 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
```

Q. 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
```

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?

```
count.nozero <- countData[rowSums(countData) != 0, ]
pca <- prcomp(count.nozero)
head(count.nozero)
```

```
##                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: 19808 6
## metadata(1): version
## assays(4): counts mu H cooks
## rownames(19808): ENSG00000186092 ENSG00000279928 ... ENSG00000277475
##      ENSG00000268674
## rowData names(22): baseMean baseVar ... deviance maxCooks
## colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
## colData names(2): condition sizeFactor
```

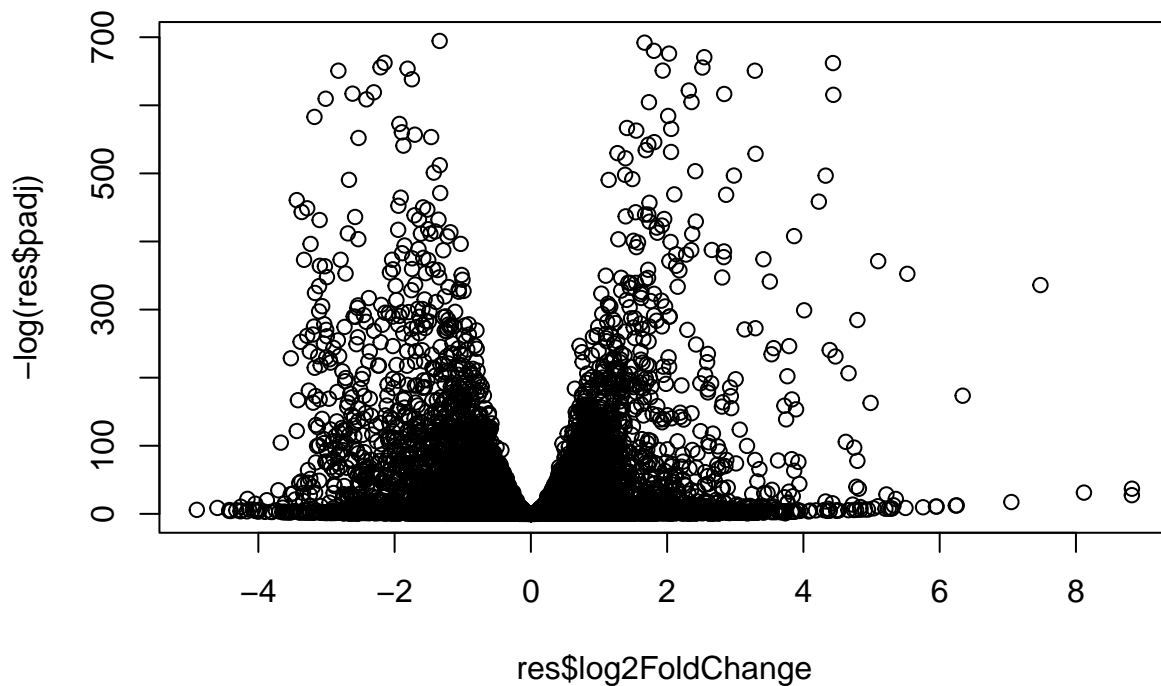
```
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

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.

```
summary(res)
```

```
##
## out of 15975 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)      : 4349, 27%
## LFC < 0 (down)    : 4393, 27%
## outliers [1]      : 0, 0%
## low counts [2]    : 1221, 7.6%
## (mean count < 0)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

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



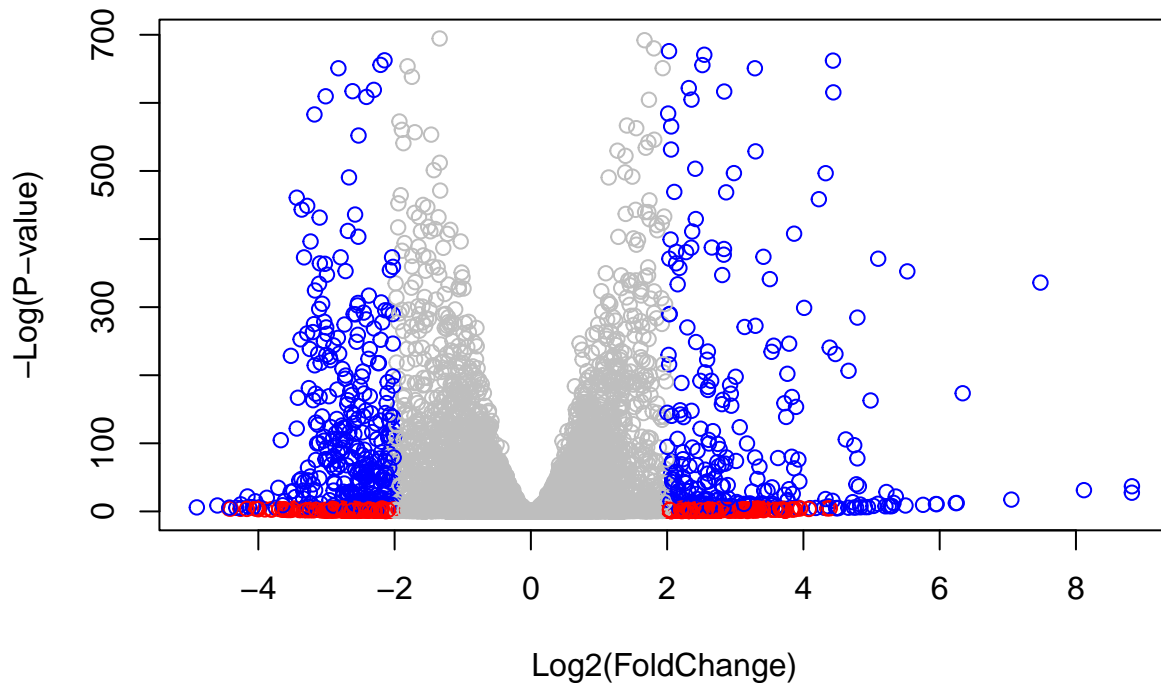
Q. Improve this plot by completing the below code, which adds color and axis labels.

```
# 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)" )
```



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

```
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"
```

```
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>
## ENSG00000186092	0.0000	NA	NA	NA	NA
## ENSG00000279928	0.0000	NA	NA	NA	NA
## ENSG00000279457	29.9136	0.1792571	0.3248216	0.551863	5.81042e-01
## ENSG00000278566	0.0000	NA	NA	NA	NA
## ENSG00000273547	0.0000	NA	NA	NA	NA
## ENSG00000187634	183.2296	0.4264571	0.1402658	3.040350	2.36304e-03
## ENSG00000188976	1651.1881	-0.6927205	0.0548465	-12.630158	1.43990e-36
## ENSG00000187961	209.6379	0.7297556	0.1318599	5.534326	3.12428e-08
## ENSG00000187583	47.2551	0.0405765	0.2718928	0.149237	8.81366e-01
## ENSG00000187642	11.9798	0.5428105	0.5215598	1.040744	2.97994e-01
	padj	symbol	entrez	name	
	<numeric>	<character>	<character>	<character>	
## ENSG00000186092	NA	OR4F5	79501	olfactory receptor f..	
## ENSG00000279928	NA	NA	NA		NA
## ENSG00000279457	6.87080e-01	WASH9P	102723897	WAS protein family h..	
## ENSG00000278566	NA	NA	NA		NA
## ENSG00000273547	NA	NA	NA		NA
## ENSG00000187634	5.16278e-03	SAMD11	148398	sterile alpha motif ..	
## ENSG00000188976	1.76741e-35	NOC2L	26155	NOC2 like nucleolar ..	
## ENSG00000187961	1.13536e-07	KLHL17	339451	kelch like family me..	
## ENSG00000187583	9.18988e-01	PLEKHN1	84069	pleckstrin homology ..	
## ENSG00000187642	4.03817e-01	PERM1	84808	PPARGC1 and ESRR ind..	

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")
```

2. Pathway Analysis

```
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 7.077982e-06 -4.432593 7.077982e-06
## hsa03030 DNA replication 9.424076e-05 -3.951803 9.424076e-05
## hsa03013 RNA transport 1.121279e-03 -3.090949 1.121279e-03
## hsa04114 Oocyte meiosis 2.563806e-03 -2.827297 2.563806e-03
## hsa03440 Homologous recombination 3.066756e-03 -2.852899 3.066756e-03
## hsa00010 Glycolysis / Gluconeogenesis 4.360092e-03 -2.663825 4.360092e-03
## q.val set.size exp1
## hsa04110 Cell cycle 0.001160789 124 7.077982e-06
## hsa03030 DNA replication 0.007727742 36 9.424076e-05
## hsa03013 RNA transport 0.061296597 150 1.121279e-03
```

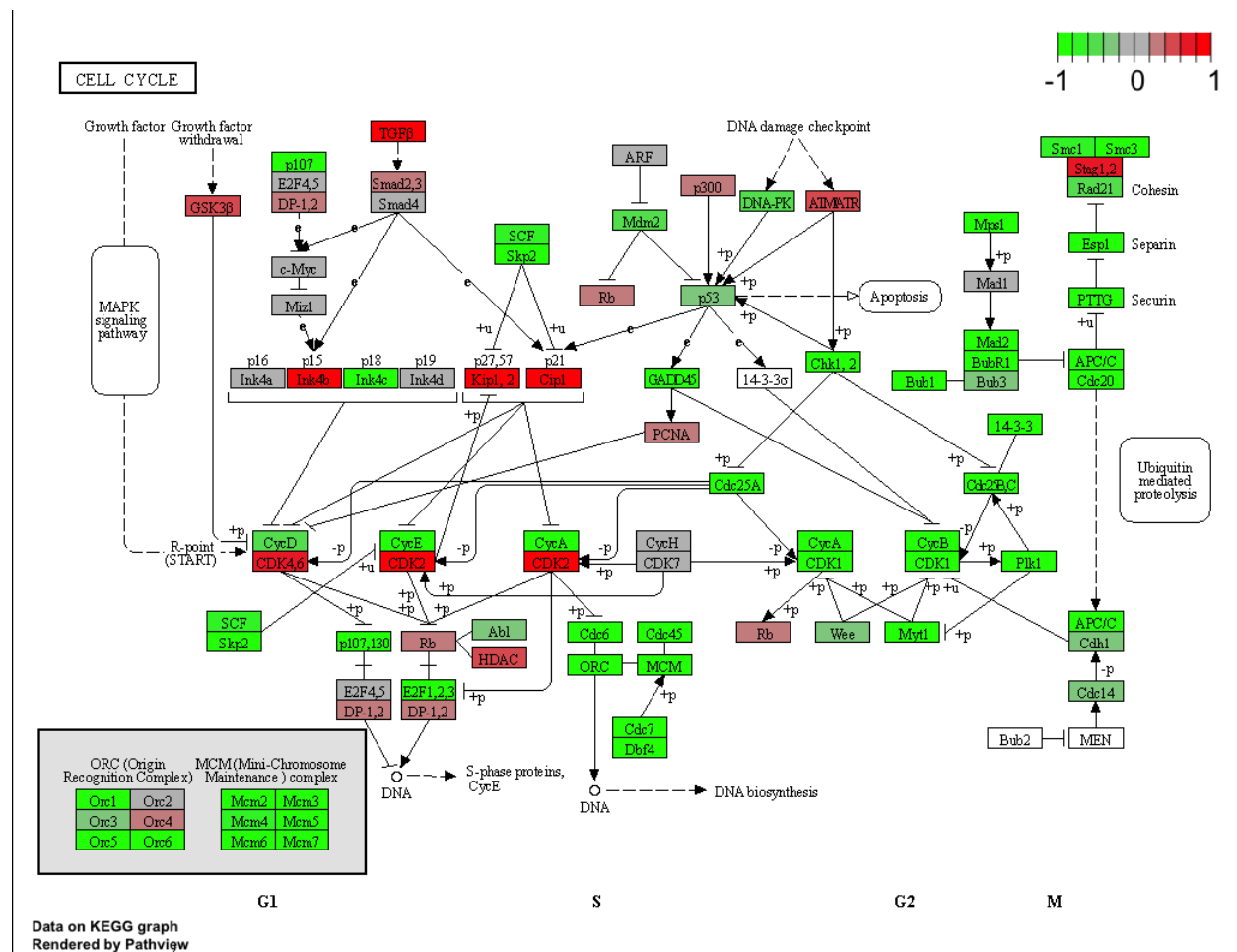
```
## hsa04114 Oocyte meiosis 0.100589607 112 2.563806e-03
## hsa03440 Homologous recombination 0.100589607 28 3.066756e-03
## hsa00010 Glycolysis / Gluconeogenesis 0.119175854 65 4.360092e-03
```

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

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

```
## 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/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

```
## 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] "hsa04740" "hsa04640" "hsa00140" "hsa04630" "hsa04976"

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

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

## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16

## Info: Writing image file hsa04740.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/LeynaN/BIMM143_Fall2021/bimm143_github/Class16

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

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

## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16

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

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

## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16

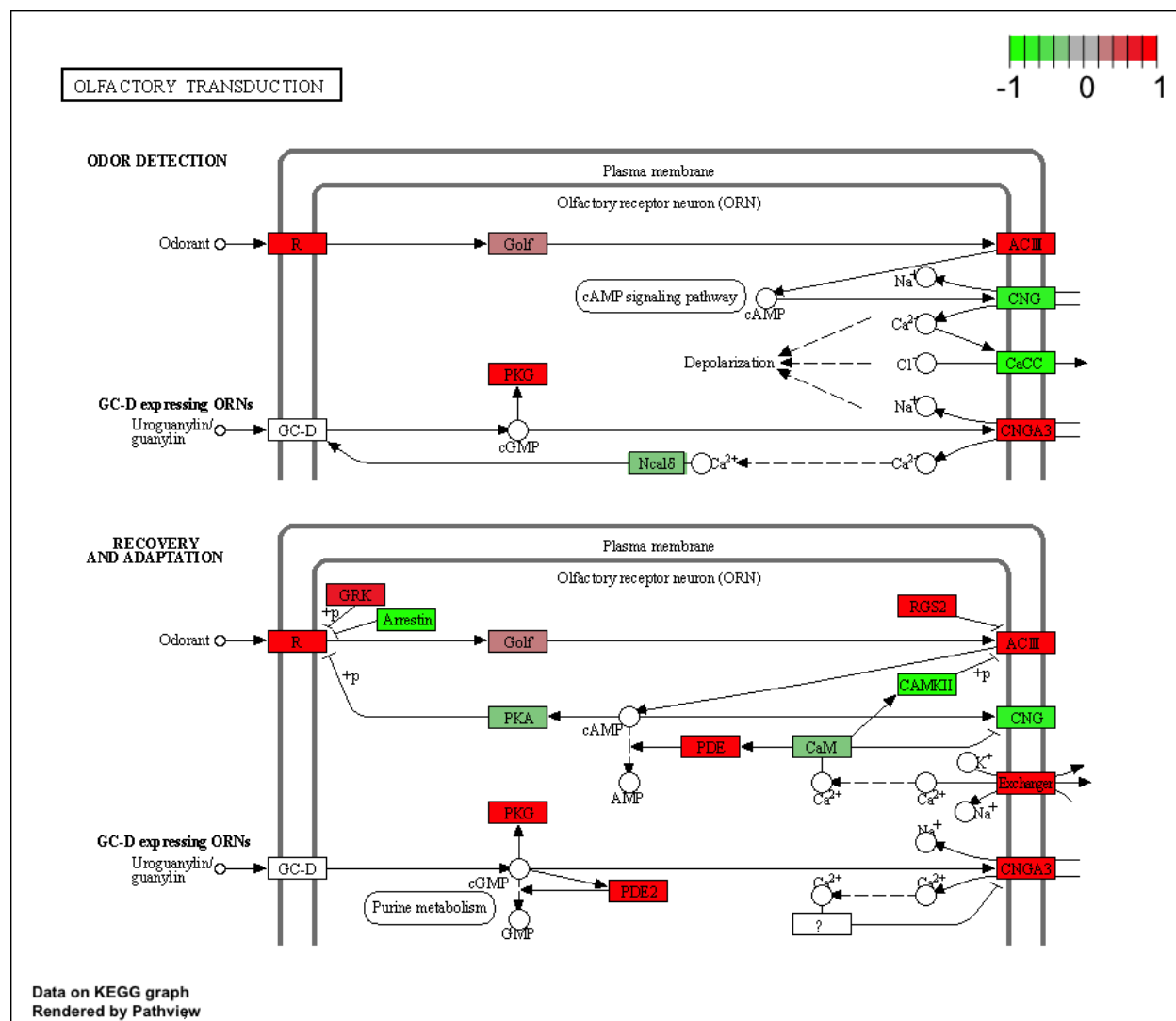
## Info: Writing image file hsa04630.pathview.png

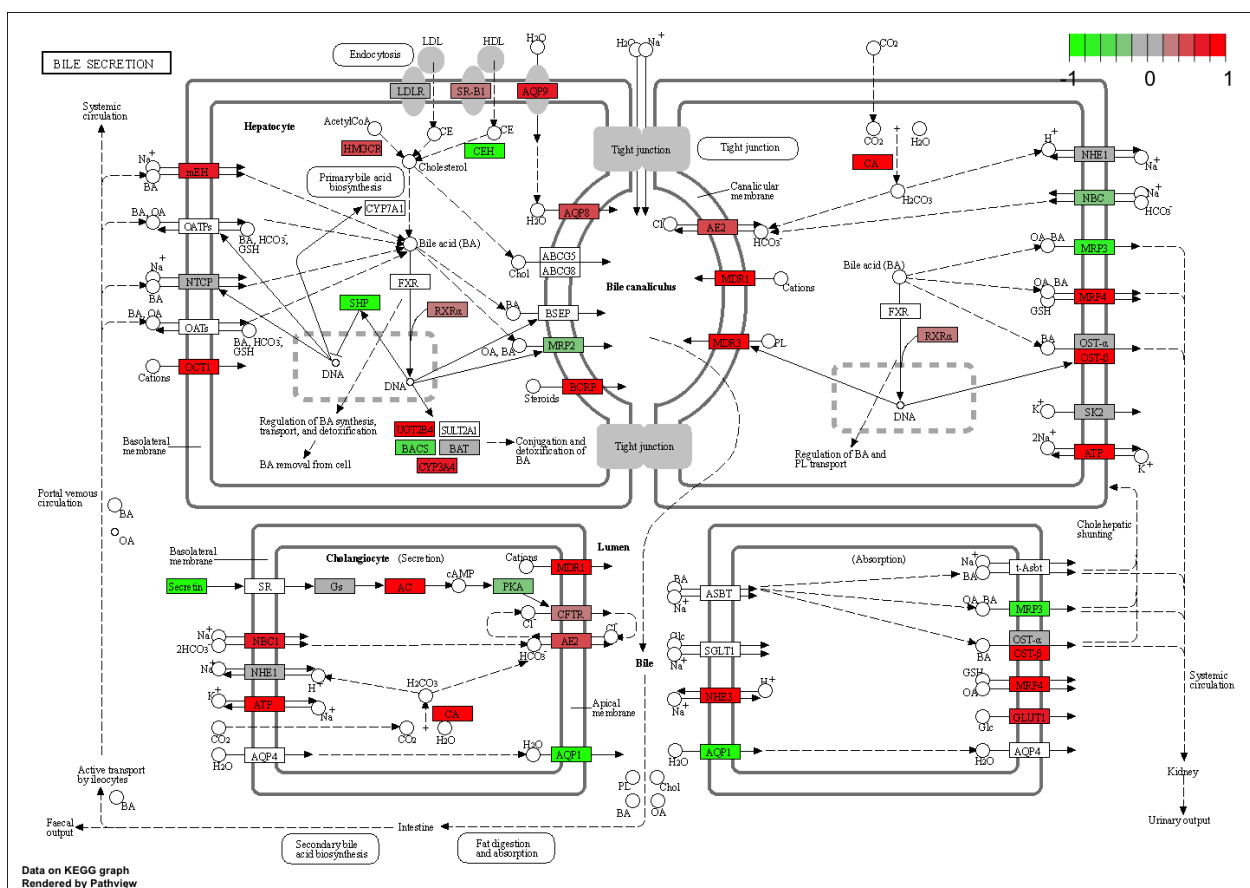
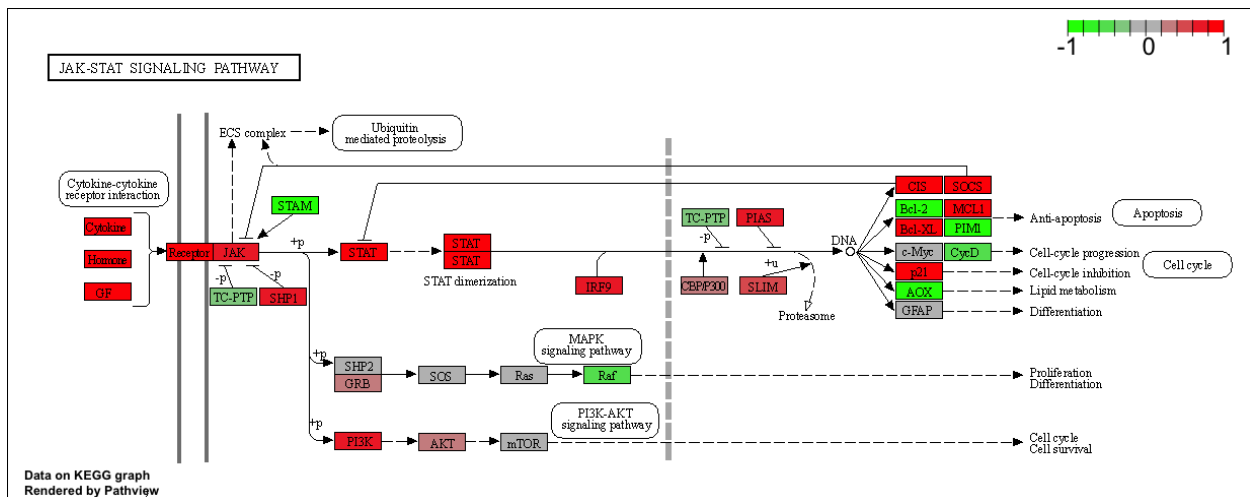
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16

## Info: Writing image file hsa04976.pathview.png

```





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

```
keggrespathways2 <- rownames(keggres$less)[1:5]
keggresids2 = substr(keggrespathways2, start=1, stop=8)
keggresids2
```

```
## [1] "hsa04110" "hsa03030" "hsa03013" "hsa04114" "hsa03440"
```

```
pathview(gene.data=foldchanges, pathway.id=keggresids2, species="hsa")
```

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

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

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

```
## Info: Writing image file hsa03030.pathview.png
```

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

```
## Info: Writing image file hsa03013.pathview.png
```

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

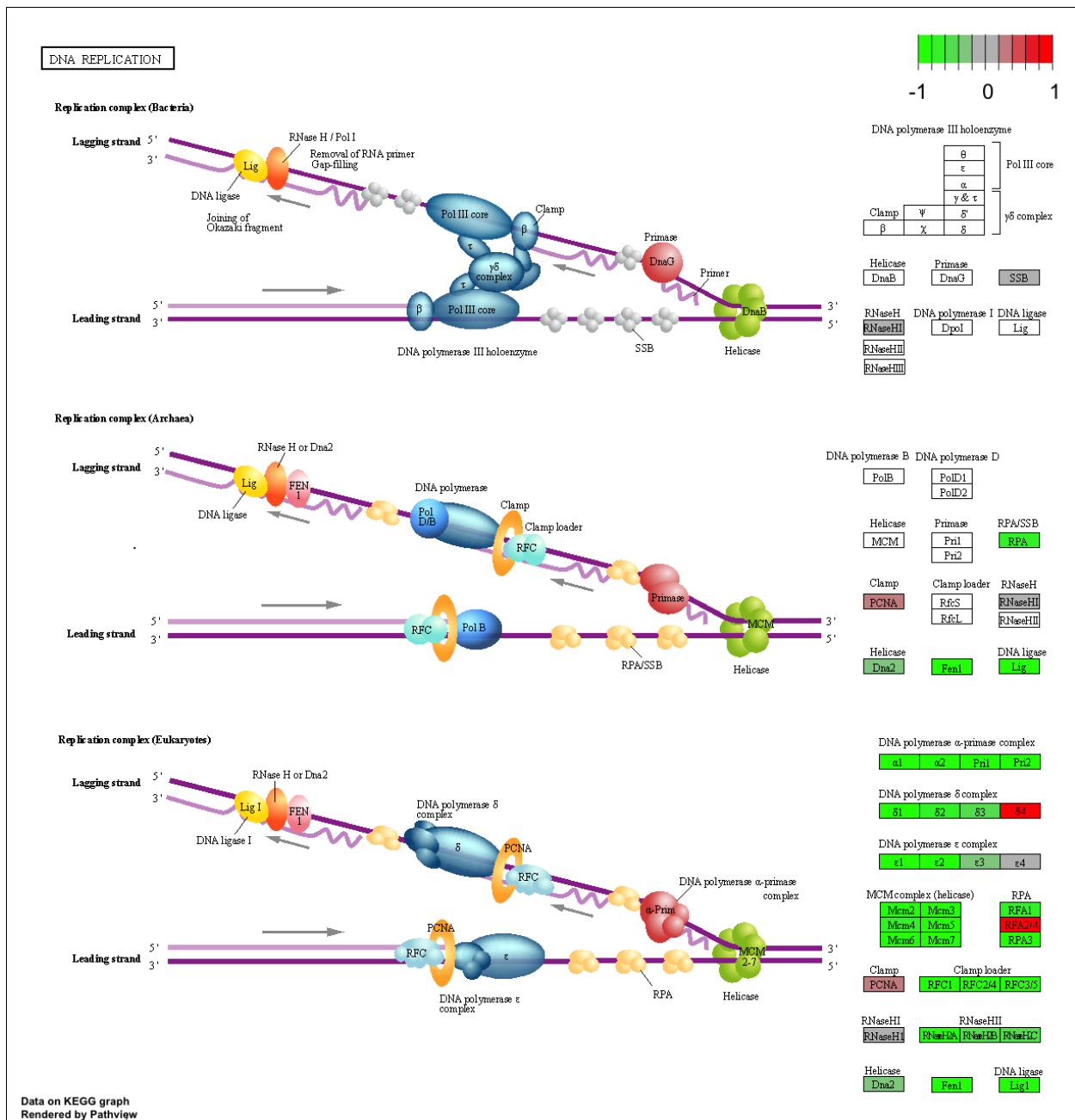
```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

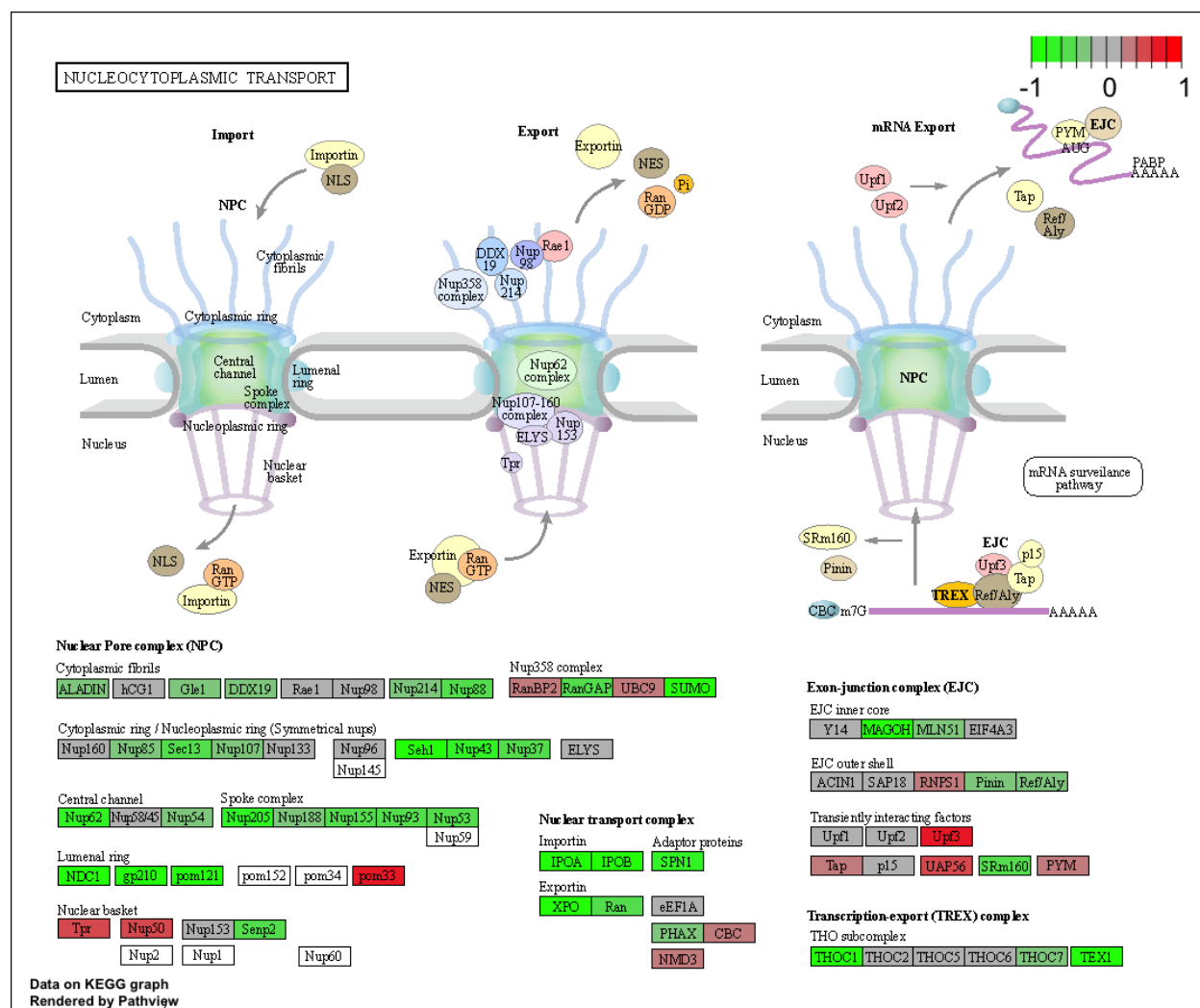
```
## Info: Writing image file hsa04114.pathview.png
```

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class16
```

```
## Info: Writing image file hsa03440.pathview.png
```




```
## G0:0060562 epithelial tube morphogenesis 2.927804e-04 3.458463 2.927804e-04
## G0:0048598 embryonic morphogenesis 2.959270e-04 3.446527 2.959270e-04
##
## q.val set.size exp1
## G0:0007156 homophilic cell adhesion 0.07103646 138 1.624062e-05
## G0:0048729 tissue morphogenesis 0.08350839 483 5.407952e-05
## G0:0002009 morphogenesis of an epithelium 0.08350839 382 5.727599e-05
## G0:0030855 epithelial cell differentiation 0.15370245 299 2.053700e-04
## G0:0060562 epithelial tube morphogenesis 0.15370245 289 2.927804e-04
## G0:0048598 embryonic morphogenesis 0.15370245 498 2.959270e-04
##
## $less
##
## p.geomean stat.mean p.val
## G0:0048285 organelle fission 6.626774e-16 -8.170439 6.626774e-16
## G0:0000280 nuclear division 1.797050e-15 -8.051200 1.797050e-15
## G0:0007067 mitosis 1.797050e-15 -8.051200 1.797050e-15
## G0:0000087 M phase of mitotic cell cycle 4.757263e-15 -7.915080 4.757263e-15
## G0:0007059 chromosome segregation 1.081862e-11 -6.974546 1.081862e-11
## G0:0051301 cell division 8.718528e-11 -6.455491 8.718528e-11
##
## q.val set.size exp1
## G0:0048285 organelle fission 2.620099e-12 386 6.626774e-16
## G0:0000280 nuclear division 2.620099e-12 362 1.797050e-15
## G0:0007067 mitosis 2.620099e-12 362 1.797050e-15
## G0:0000087 M phase of mitotic cell cycle 5.202068e-12 373 4.757263e-15
## G0:0007059 chromosome segregation 9.464127e-09 146 1.081862e-11
## G0:0051301 cell division 6.355807e-08 479 8.718528e-11
##
## $stats
##
## stat.mean exp1
## G0:0007156 homophilic cell adhesion 4.226117 4.226117
## G0:0048729 tissue morphogenesis 3.888470 3.888470
## G0:0002009 morphogenesis of an epithelium 3.878706 3.878706
## G0:0030855 epithelial cell differentiation 3.554776 3.554776
## G0:0060562 epithelial tube morphogenesis 3.458463 3.458463
## G0:0048598 embryonic morphogenesis 3.446527 3.446527
```

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: 8146"
```

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

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?

The pathway with the most significant Entities p-value is the endosomal/vacuolar pathway. They don’t match that much.