

HW/Lab Class 14 DESeq 2 Mini Project

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Section 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, 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 object is masked from 'package:utils':

findMatches

The following objects are masked from 'package:base':

expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Warning: package 'GenomeInfoDb' was built under R version 4.3.2

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 <- "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

Q1. 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

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).

```
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
```

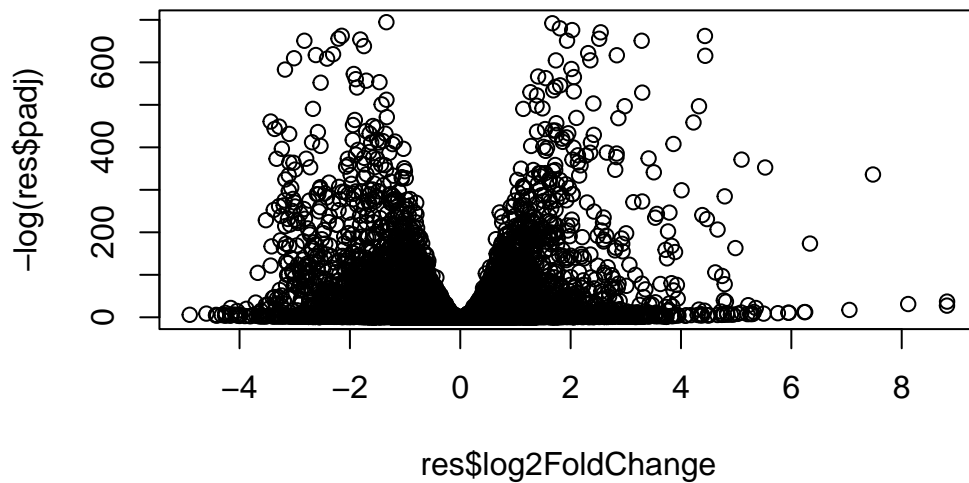
```
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

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.

```
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) )
```



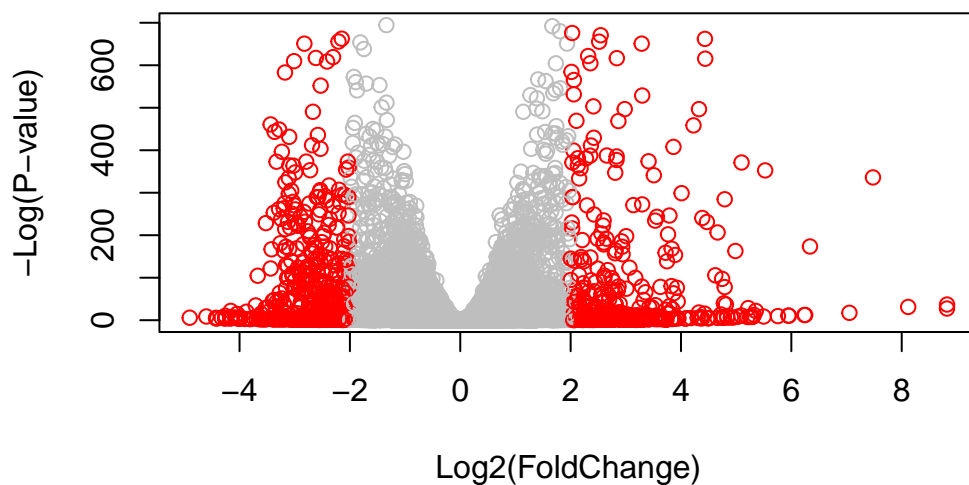
Q4. Improve this plot by completing the below code, which adds color and axis labels

```
mycols <- rep("gray", nrow(res) )
```

```
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

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

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



Q5. 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 4.3.2

```
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>
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	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 ..	

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.

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

Section 2. Pathway Analysis

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

Bioconductor version 3.18 (BiocManager 1.30.22), R 4.3.1 (2023-06-16)

Warning: package(s) not installed when version(s) same as or greater than current; use
`force = TRUE` to re-install: '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)
```

```
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/alexsimonyan/Desktop/BIMM 143/class14

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/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

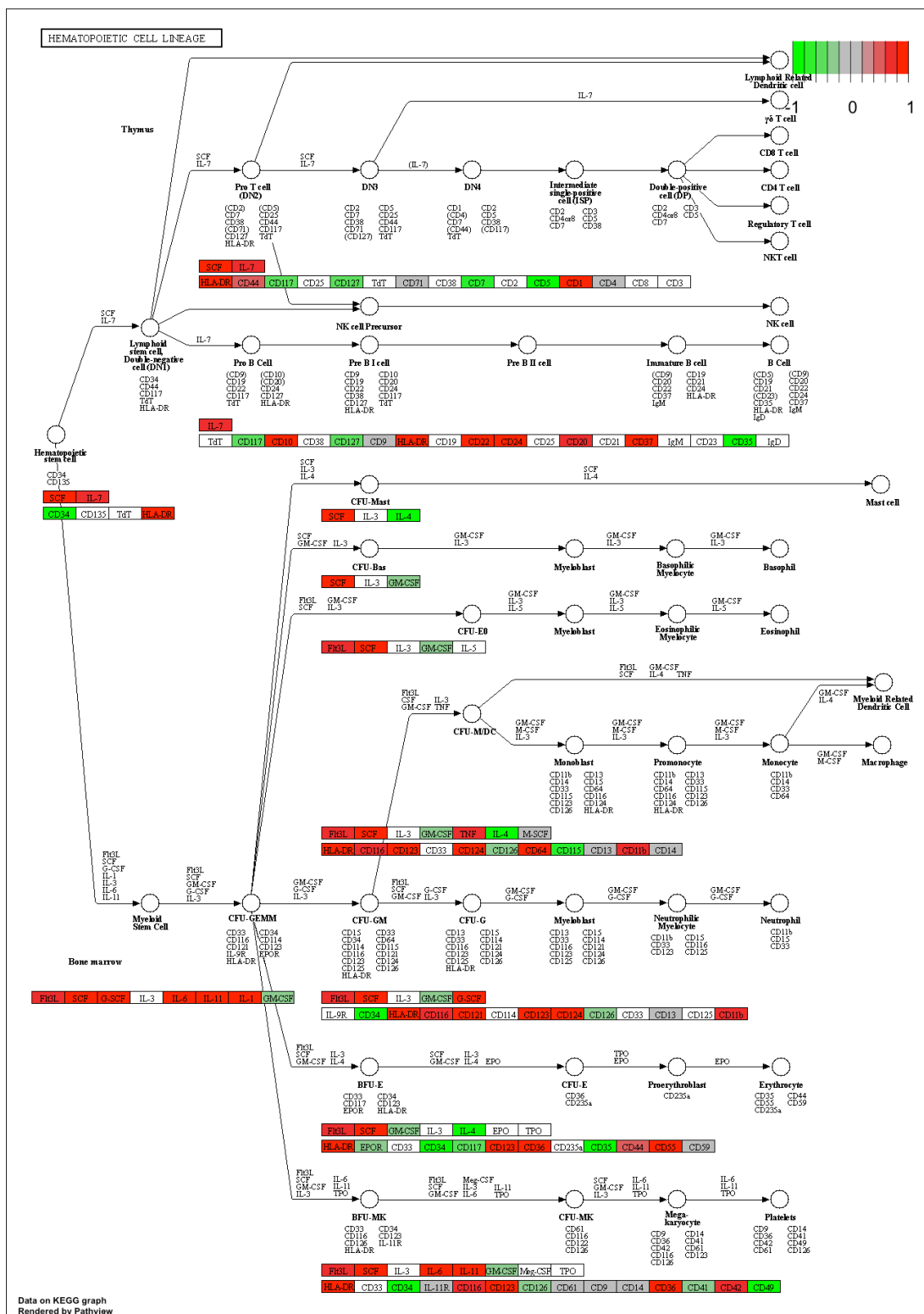
Info: Writing image file hsa04142.pathview.png

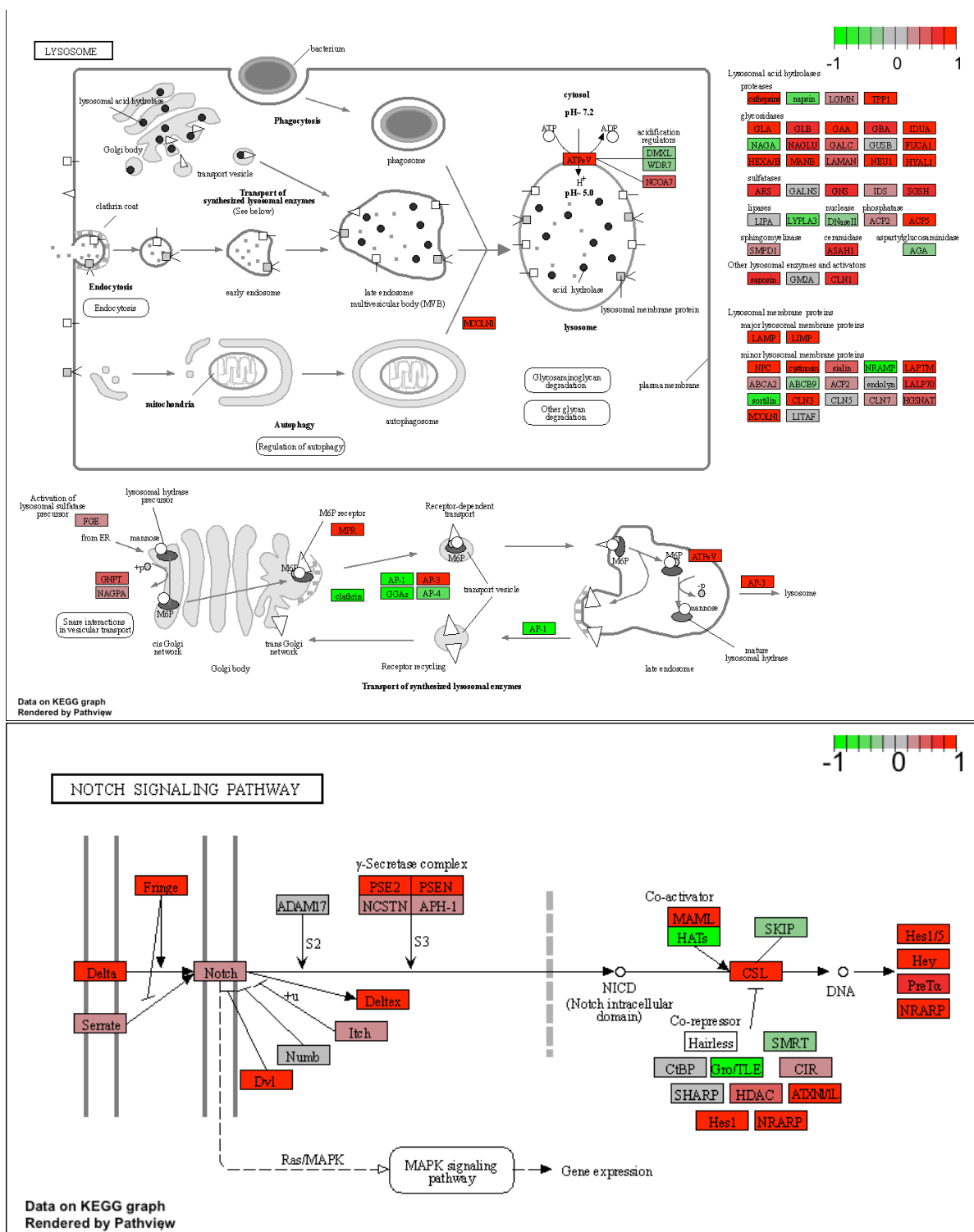
Info: some node width is different from others, and hence adjusted!

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa04330.pathview.png





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

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

keggresids_1 = substr(keggrespathways_downregulated, start=1, stop=8)
keggresids_1
```

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

```
pathview(gene.data = foldchanges, pathway.id = keggresids_1, species = "hsa")
```

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/alexsimonyan/Desktop/BIMM 143/class14

Info: Writing image file hsa03440.pathview.png

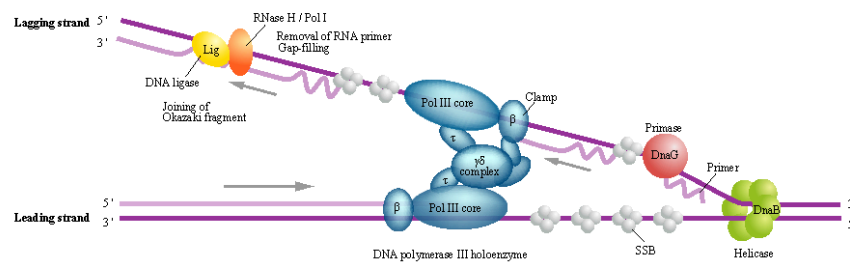
'select()' returned 1:1 mapping between keys and columns

Info: Writing image file hsa04114.pathview.png



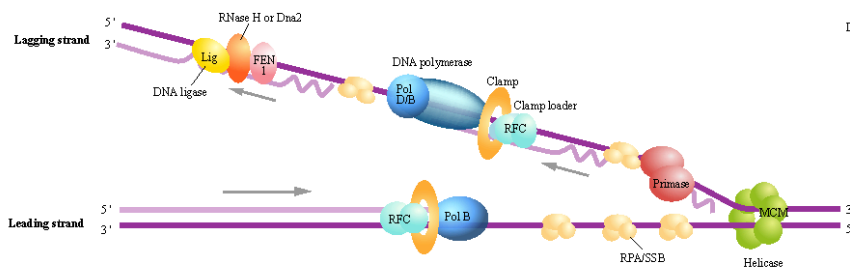
DNA REPLICATION

Replication complex (Bacteria)



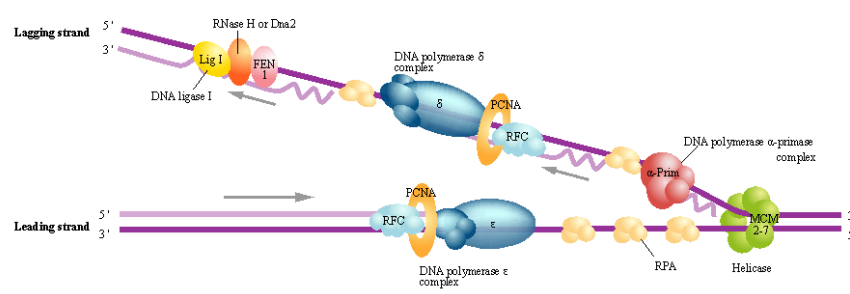
DNA polymerase III holoenzyme			
	θ	Pol III core	
	ε		
	α		
Clamp	γ δ τ	γδ complex	
	β		
Helicase	DnaB	Primase	DnaG
		SSB	
RNaseH	DNA polymerase I	DNA ligase	
RNaseHII	Dpol	Lig	
RNaseHIII			
RNaseHIII			

Replication complex (Archaea)



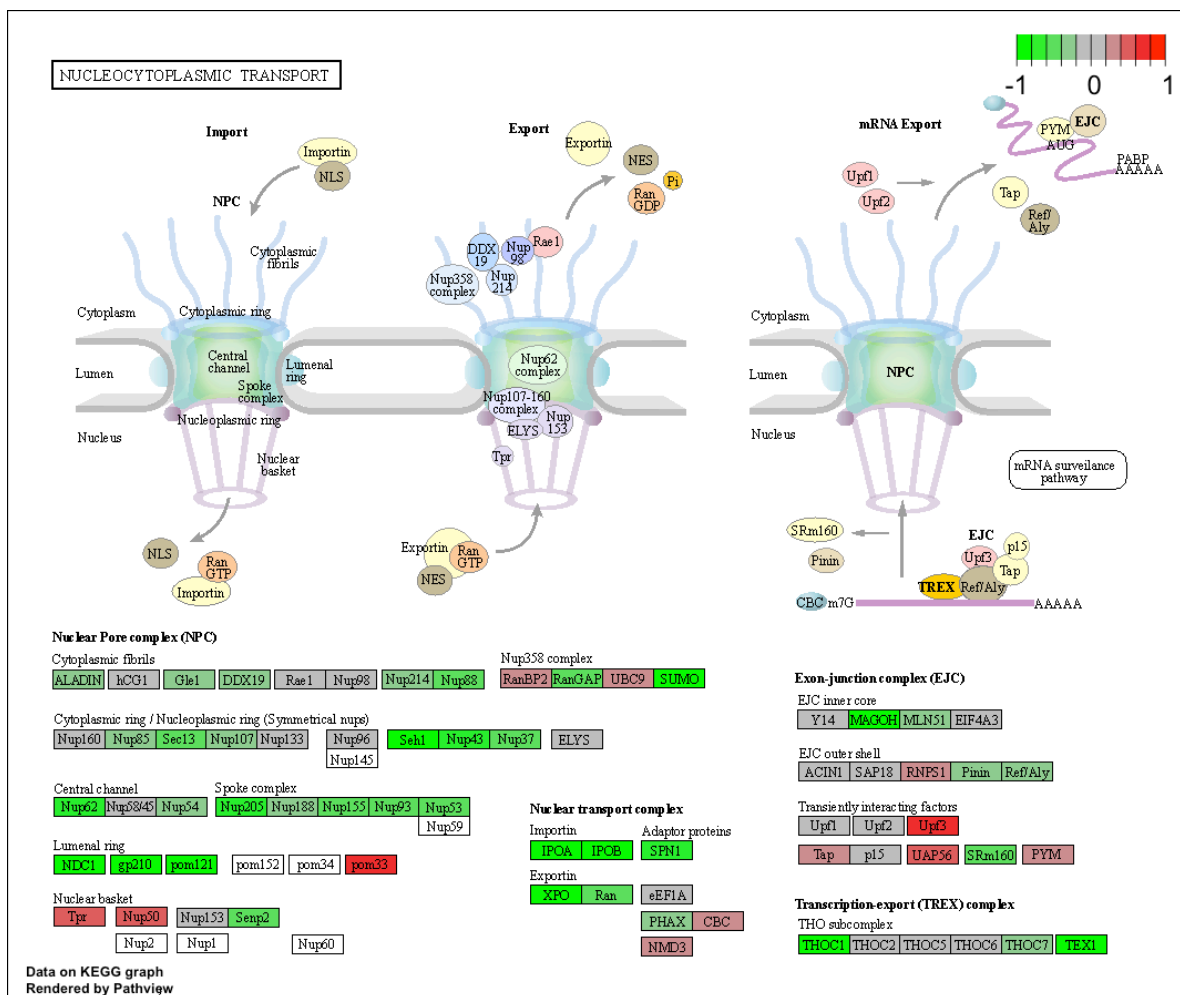
DNA polymerase B		DNA polymerase D	
	PolB		PolD1
			PolD2
Helicase	MCM	Primase	Pri1
			Pri2
Clamp	PCNA	RPA/SSB	RPA
		Clamp loader	RNaseH
			RNaseHII
			RNaseHIII
Helicase	Dna2	DNA ligase	Lig

Replication complex (Eukaryotes)



DNA polymerase α-primase complex			
α1	α2	Pri1	Pri2
DNA polymerase δ complex			
δ1	δ2	δ3	δ4
DNA polymerase ε complex			
ε1	ε2	ε3	ε4
MCM complex (helicase)			
Mcm2	Mcm3	Mcm4	Mcm5
Mcm6	Mcm7	Mcm8	Mcm9
Clamp	PCNA	Clamp loader	RFC1
			RFC2
			RFC3
RNaseHII	RNaseHII	RNaseHII	RNaseHII
RNaseHII	RNaseHII	RNaseHII	RNaseHII
Helicase	Dna2	DNA ligase	Lig1

Data on KEGG graph
Rendered by Pathview



G0:0002009	morphogenesis of an epithelium	1.396681e-04	3.653886	1.396681e-04
G0:0048729	tissue morphogenesis	1.432451e-04	3.643242	1.432451e-04
G0:0007610	behavior	1.925222e-04	3.565432	1.925222e-04
G0:0060562	epithelial tube morphogenesis	5.932837e-04	3.261376	5.932837e-04
G0:0035295	tube development	5.953254e-04	3.253665	5.953254e-04

		q.val	set.size	exp1
G0:0007156	homophilic cell adhesion	0.1952430	113	8.519724e-05
G0:0002009	morphogenesis of an epithelium	0.1952430	339	1.396681e-04
G0:0048729	tissue morphogenesis	0.1952430	424	1.432451e-04
G0:0007610	behavior	0.1968058	426	1.925222e-04
G0:0060562	epithelial tube morphogenesis	0.3566193	257	5.932837e-04
G0:0035295	tube development	0.3566193	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	5.843127e-12	376	1.536227e-15
G0:0000280	nuclear division	5.843127e-12	352	4.286961e-15
G0:0007067	mitosis	5.843127e-12	352	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.195965e-11	362	1.169934e-14
G0:0007059	chromosome segregation	1.659009e-08	142	2.028624e-11
G0:0000236	mitotic prometaphase	1.178690e-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	3.565432	3.565432
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, quo
```

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 that has the most significant “Entities p-value is cell cycle. The most significant pathways listed does match my previous KEGG results. A factor that could cause differences between the two method is having more conditions.