

Class 14: Mini RNA-Seq Analysis

AUTHOR

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```
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, colAvgPerRowSet, 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, rowAvgPerColSet,
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

```

```

# Note we need to remove the odd first $length col
countData <- as.matrix(countData[,2:7])
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.

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

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, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

```
summary(res)
```

out of 15975 with nonzero total read count

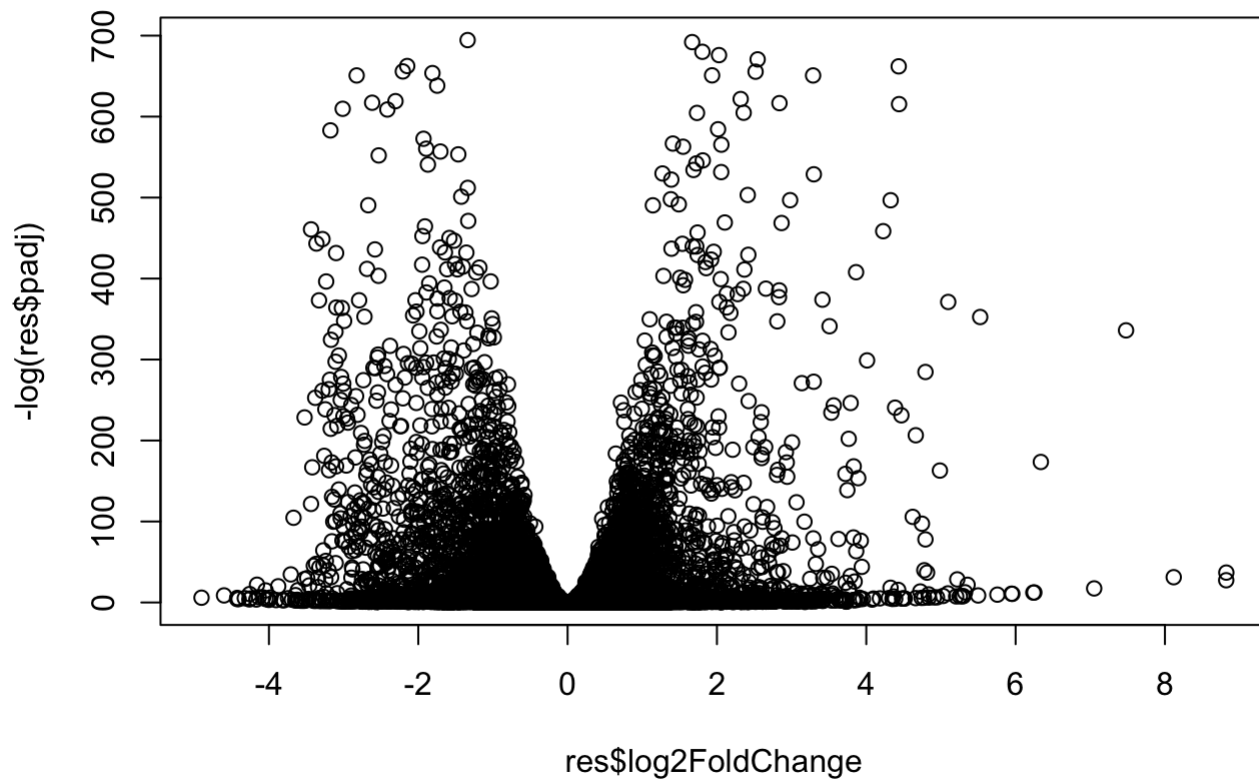
adjusted p-value < 0.1

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
```

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

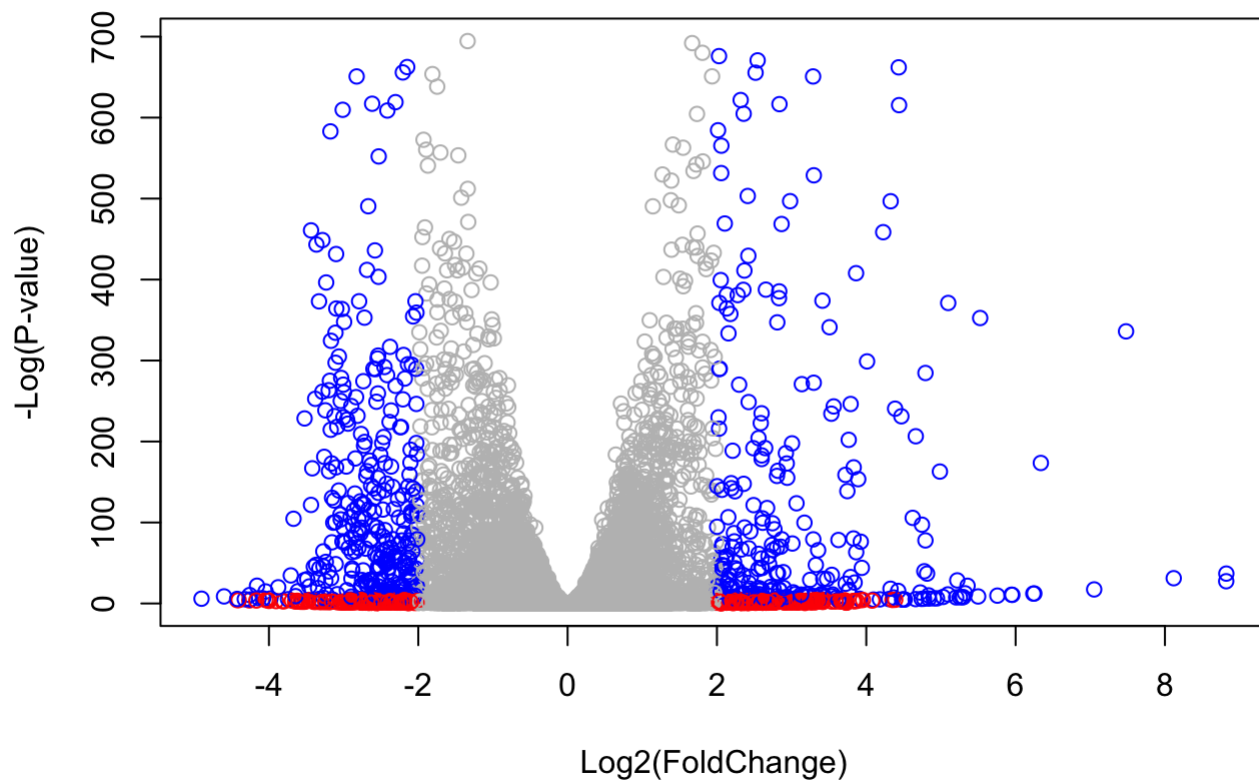


```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

# 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 <- (res$padj < .01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

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



Adding gene notation

```
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 ..

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

Section 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	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

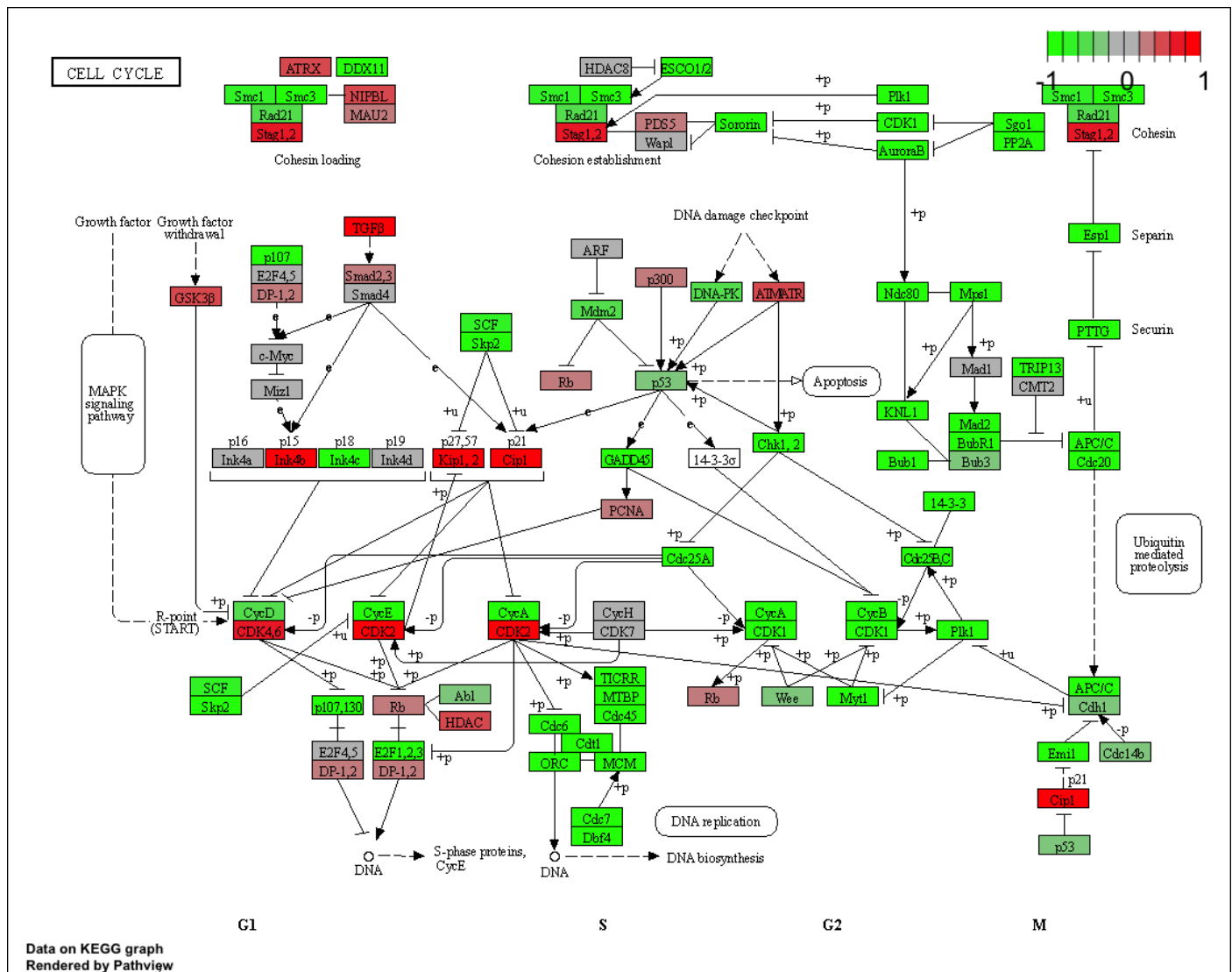
hsa03030 DNA replication	0.007586381	36	9.424076e-05
hsa03013 RNA transport	0.073840037	144	1.375901e-03
hsa03440 Homologous recombination	0.121861535	28	3.066756e-03
hsa04114 Oocyte meiosis	0.121861535	102	3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis	0.212222694	53	8.961413e-03

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

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

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

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/emilyrosehickey/Desktop/BIMM 143/class14

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/emilyrosehickey/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/emilyrosehickey/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/emilyrosehickey/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/emilyrosehickey/Desktop/BIMM 143/class14

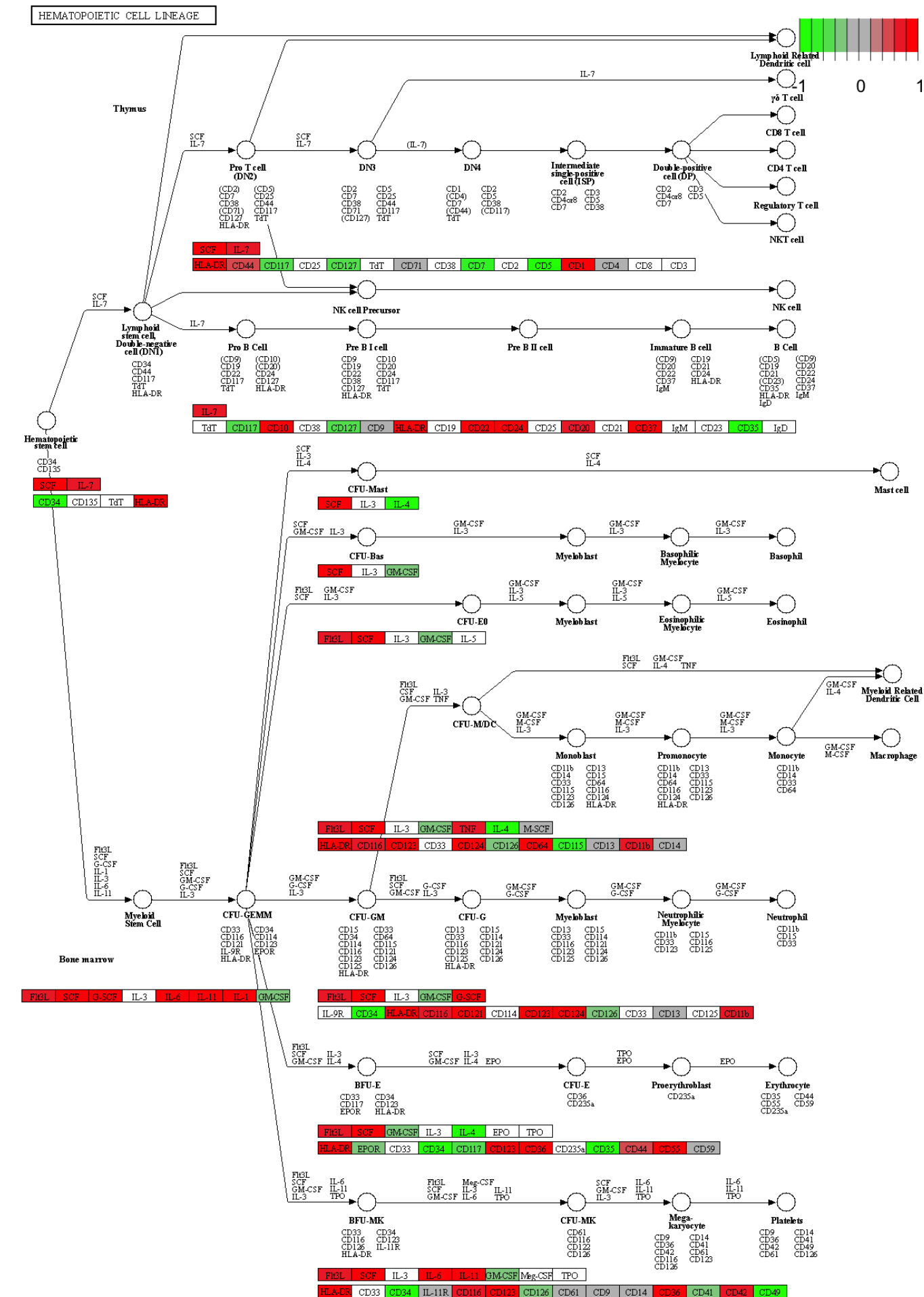
Info: Writing image file hsa04142.pathview.png

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

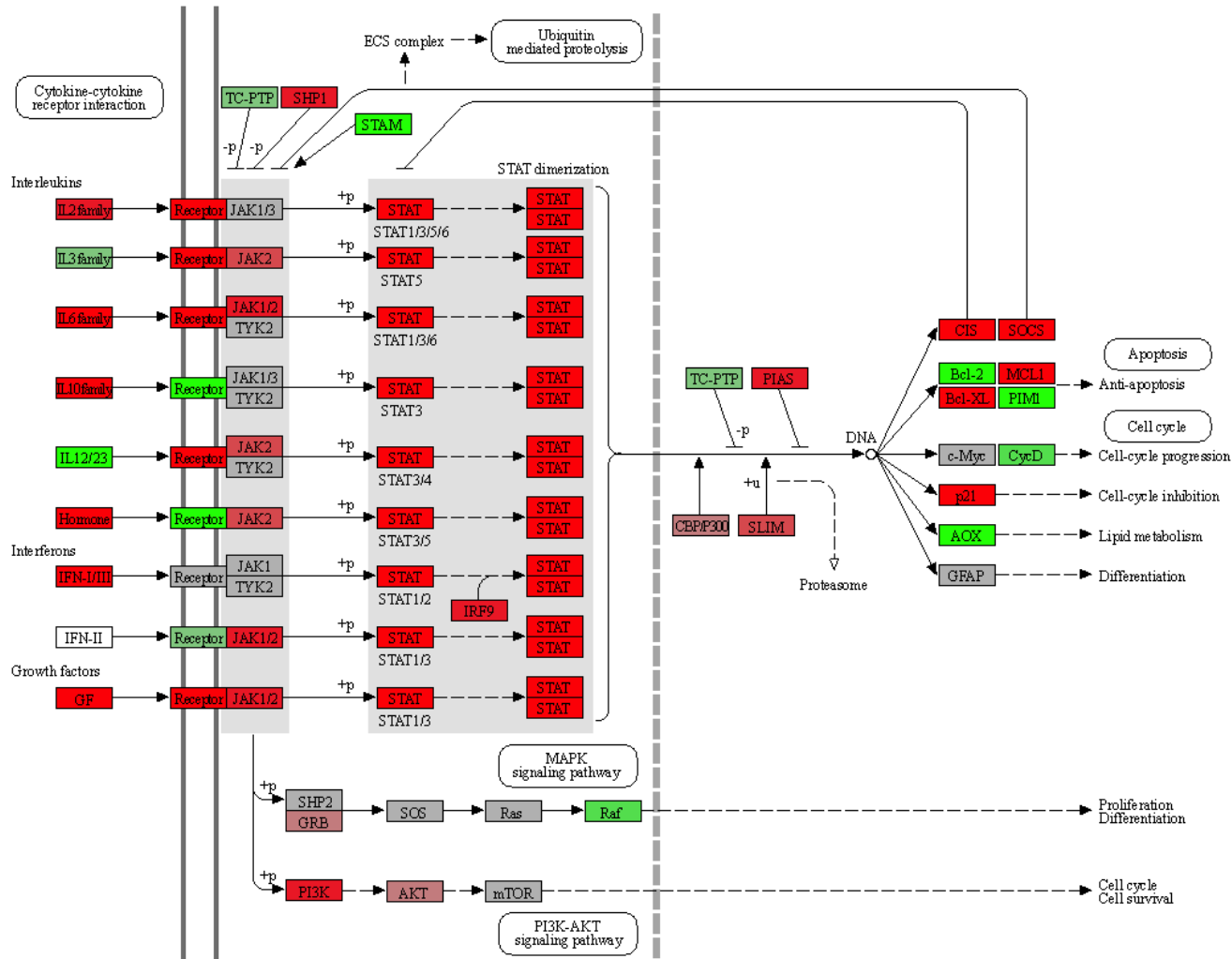
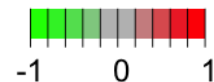
'select()' returned 1:1 mapping between keys and columns

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

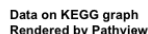
Info: Writing image file hsa04330.pathview.png



JAK-STAT SIGNALING PATHWAY



Data on KEGG graph
Rendered by Pathview




```
keggresidsless = substr(keggrespathwaysless, start=1, stop=8)
keggresidsless
```

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

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

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

```
Info: Working in directory /Users/emilyrosehickey/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/emilyrosehickey/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/emilyrosehickey/Desktop/BIMM 143/class14
```

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

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

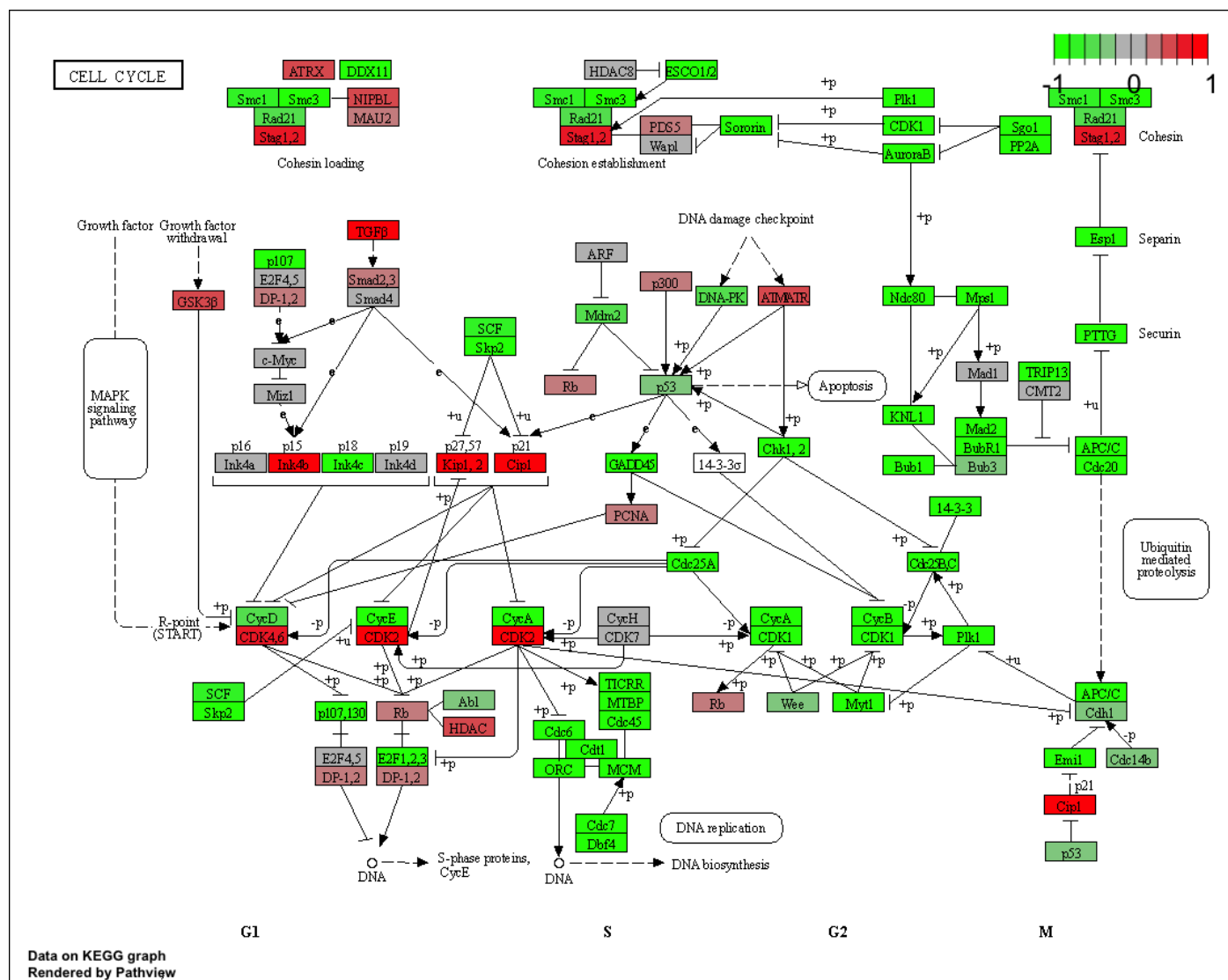
```
Info: Working in directory /Users/emilyrosehickey/Desktop/BIMM 143/class14
```

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

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

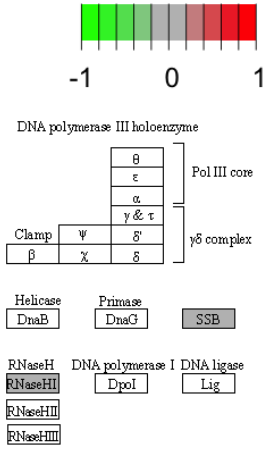
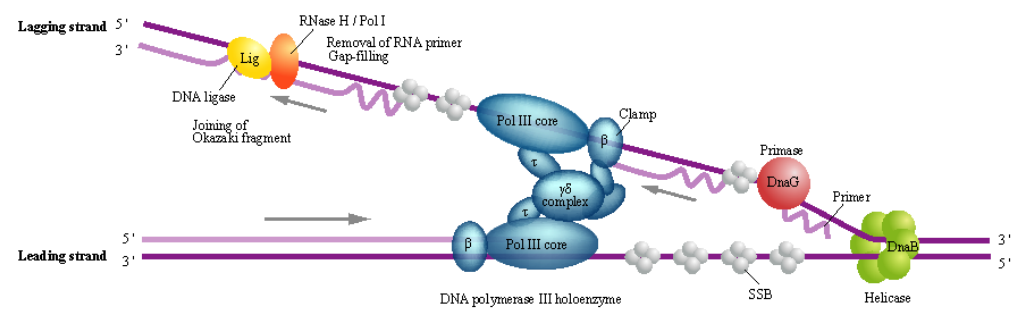
```
Info: Working in directory /Users/emilyrosehickey/Desktop/BIMM 143/class14
```

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

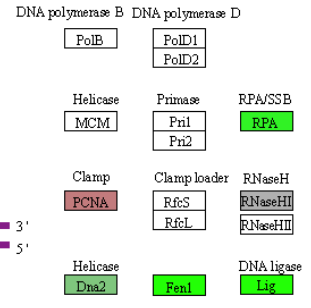
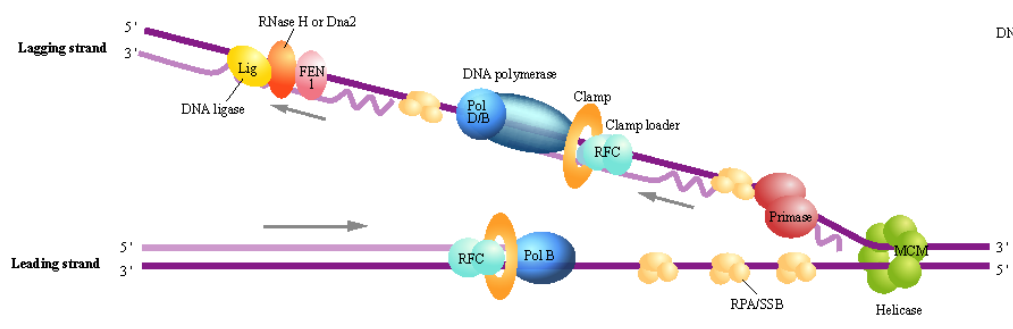



DNA REPLICATION

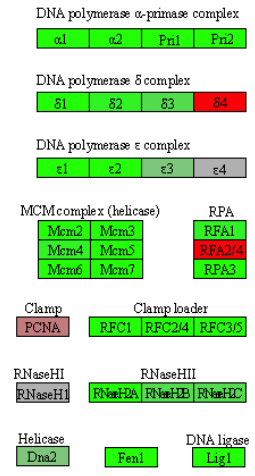
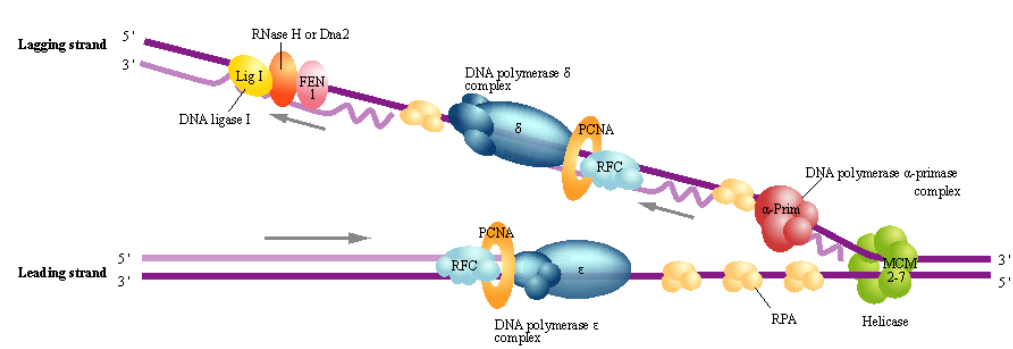
Replication complex (Bacteria)



Replication complex (Archaea)

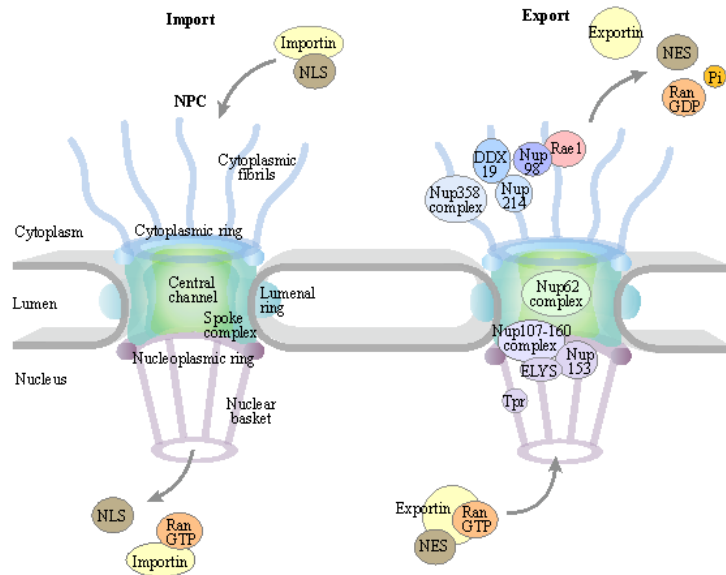


Replication complex (Eukaryotes)

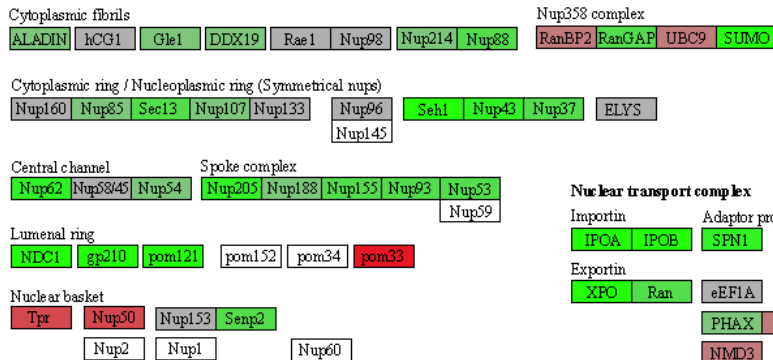


Data on KEGG graph
Rendered by Pathview

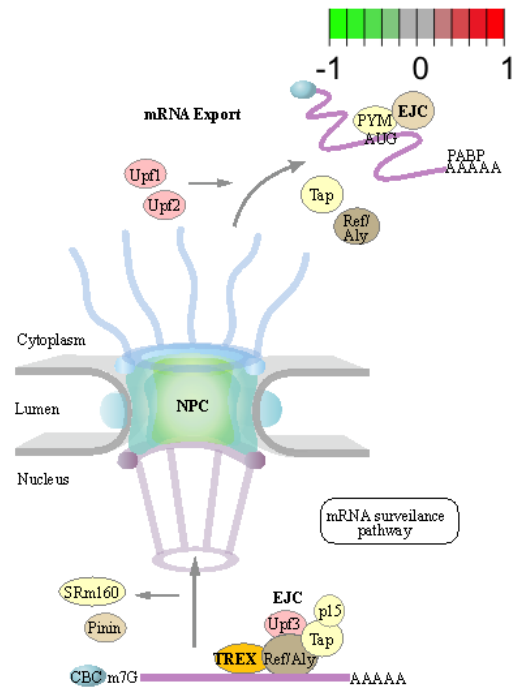
NUCLEOCYTOPLASMIC TRANSPORT



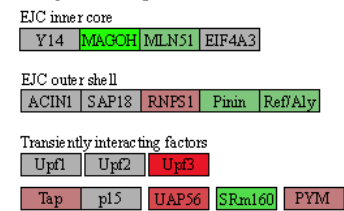
Nuclear Pore complex (NPC)



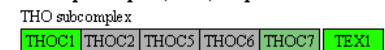
Data on KEGG graph
Rendered by Pathview



Exon-junction complex (EJC)

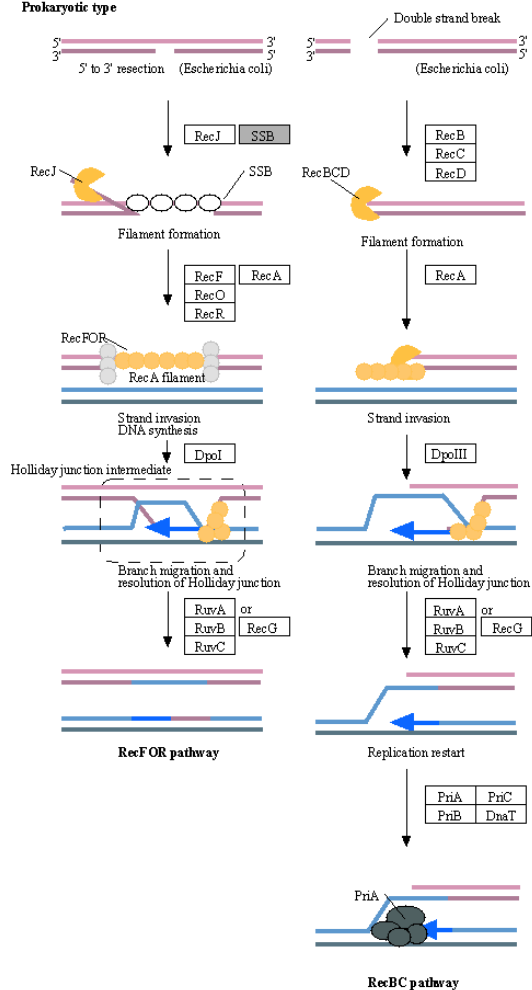


Transcription-export (TREX) complex

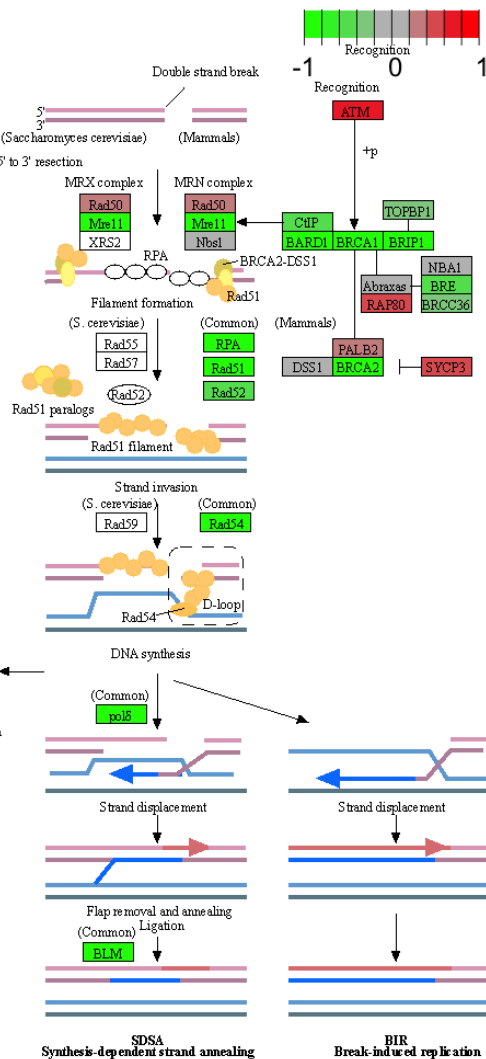
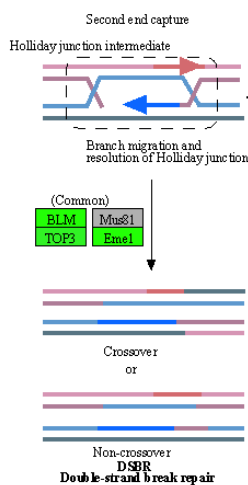
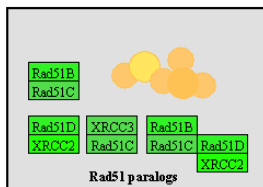


HOMOLOGOUS RECOMBINATION

Prokaryotic type



Eukaryotic type



Data on KEGG graph
Rendered by Pathview

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, qu
```

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?

Cell Cycle, Mitotic. Some of the most significant pathways match the previous KEGG results. Different methods will display results differently. The reactome website could be displaying the data in a different

order than ours. Another factor could potentially be that we adjusted the p-values for the sig_genes value, therefore slightly altering the data.