

Class 14: RNA Seq Mini-Project

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Table of contents

Setup for DESeq	4
Running DESeq	5
Add gene annotation data (gene names etc.)	7
Results Visualization	9
Save our Results	12
Pathway Analysis (KEGG, GO, Reactome)	12

Here, we'll run through a complete RNASeq analysis.

#Data Import

First, we'll library our necessary packages and import relevant data 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 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

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 <- "data/GSE37704_metadata.csv"
countFile <- "data/GSE37704_featurecounts.csv"
```

To take a quick look at the data we imported:

```
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
```

Setup for DESeq

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.

```
#Finds where there are "0" values in columns 1-6 in the countData dataset
#zero.vals = which(countData[,1:6]==0, arr.ind=TRUE)
#Finds unique instances
#to.rm = unique(zero.vals[,1])
#removes the set of values with 0 from countData
#countData = countData[-to.rm, ]
#View the results
#head(countData)

to.keep.inds = rowSums(countData)>0
countData = countData[to.keep.inds,]
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 DESeq

```
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

```
head(dds)
```

```
class: DESeqDataSet
dim: 6 6
metadata(1): version
assays(4): counts mu H cooks
rownames(6): ENSG00000279457 ENSG00000187634 ... ENSG00000187583
           ENSG00000187642
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"))
head(res)
```

log2 fold change (MLE): condition hoxa1_kd vs control_sirna

Wald test p-value: condition hoxa1 kd vs control sirna

DataFrame with 6 rows and 6 columns

	baseMean	log2FoldChange	lfcSE	stat	pvalue
	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
ENSG00000279457	29.9136	0.1792571	0.3248216	0.551863	5.81042e-01
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
      <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01

```

```
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

```

4349 genes are upregulated and 4396 genes are downregulated with a p-value cutoff of 0.1.

Add gene annotation data (gene names etc.)

```

library("AnnotationDbi")
library("org.Hs.eg.db")

```

```
columns(org.Hs.eg.db)
```

[1]	"ACCNUM"	"ALIAS"	"ENSEMBL"	"ENSEMBLPROT"	"ENSEMBLTRANS"
[6]	"ENTREZID"	"ENZYME"	"EVIDENCE"	"EVIDENCEALL"	"GENENAME"
[11]	"GENETYPE"	"GO"	"GOALL"	"IPI"	"MAP"
[16]	"OMIM"	"ONTOLOGY"	"ONTOLOGYALL"	"PATH"	"PFAM"
[21]	"PMID"	"PROSITE"	"REFSEQ"	"SYMBOL"	"UCSCKG"
[26]	"UNIPROT"				

```
res$symbol = mapIds(org.Hs.eg.db,
  keys=row.names(res),
  keytype="ENSEMBL",
  column="SYMBOL",
  multiVals="first")
```

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

```
res$entrez = mapIds(org.Hs.eg.db,
  keys=row.names(res),
  keytype="ENSEMBL",
  column="ENTREZID",
  multiVals="first")
```

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

```
res$name = mapIds(org.Hs.eg.db,
  keys=row.names(res),
  keytype="ENSEMBL",
  column="GENENAME",
  multiVals="first")
```

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

```
head(res, 10)
```

log2 fold change (MLE): condition hoxa1_kd vs control_sirna

Wald test p-value: condition hoxa1 kd vs control sirna

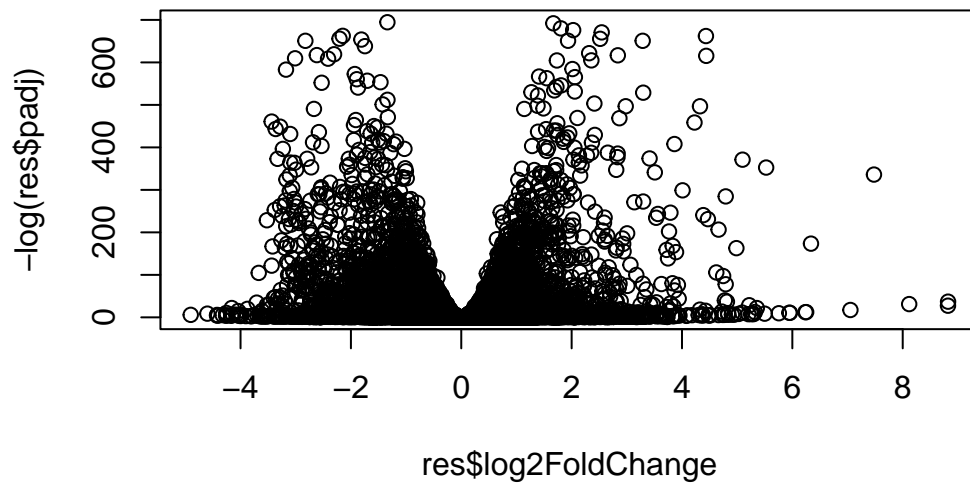
DataFrame with 10 rows and 9 columns

	baseMean	log2FoldChange	lfcSE	stat	pvalue
--	----------	----------------	-------	------	--------

	<numeric>	<numeric>	<numeric>	<numeric>	<numeric>
ENSG00000279457	29.913579	0.1792571	0.3248216	0.551863	5.81042e-01
ENSG00000187634	183.229650	0.4264571	0.1402658	3.040350	2.36304e-03
ENSG00000188976	1651.188076	-0.6927205	0.0548465	-12.630158	1.43990e-36
ENSG00000187961	209.637938	0.7297556	0.1318599	5.534326	3.12428e-08
ENSG00000187583	47.255123	0.0405765	0.2718928	0.149237	8.81366e-01
ENSG00000187642	11.979750	0.5428105	0.5215598	1.040744	2.97994e-01
ENSG00000188290	108.922128	2.0570638	0.1969053	10.446970	1.51282e-25
ENSG00000187608	350.716868	0.2573837	0.1027266	2.505522	1.22271e-02
ENSG00000188157	9128.439422	0.3899088	0.0467163	8.346304	7.04321e-17
ENSG00000237330	0.158192	0.7859552	4.0804729	0.192614	8.47261e-01
	padj	symbol	entrez	name	
	<numeric>	<character>	<character>	<character>	
ENSG00000279457	6.86555e-01	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 ..	

Results Visualization

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

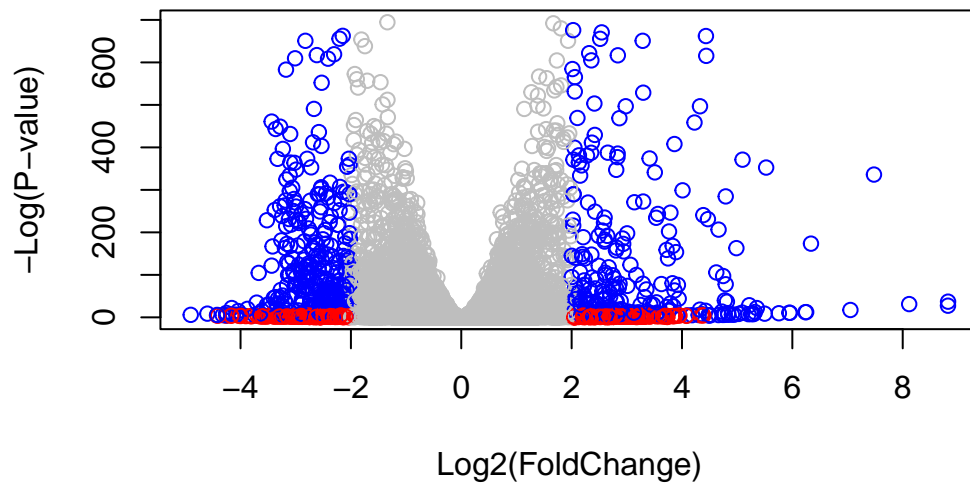


```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

# Color red the genes with absolute fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] = "blue"

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

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



```
library(EnhancedVolcano)
```

Loading required package: ggplot2

Loading required package: ggrepel

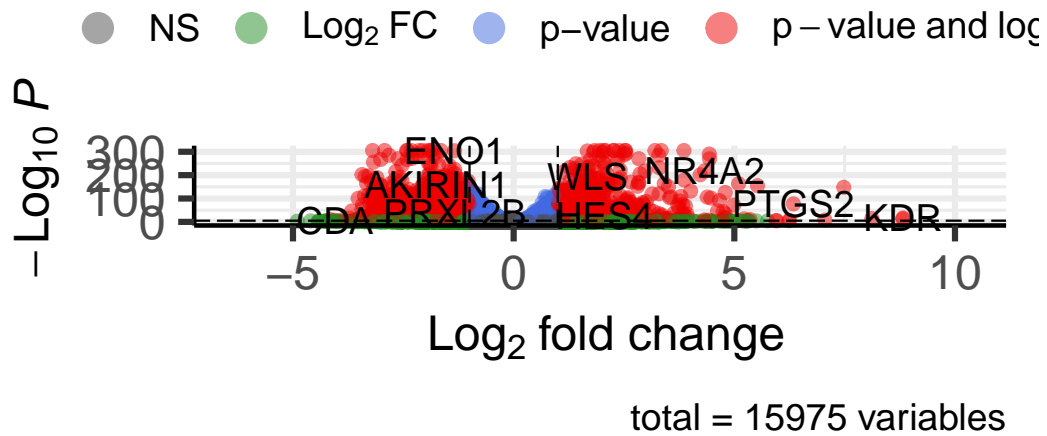
```
x <- as.data.frame(res)

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

Warning: One or more p-values is 0. Converting to $10^{-1} \times$ current lowest non-zero p-value...

Volcano plot

Enhanced Volcano



Save our Results

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

Pathway Analysis (KEGG, GO, Reactome)

```
#Library the necessary packages  
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)
```

```
#Helps us focus in on metabolic pathways
```

```
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
```

```
#Look at 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

```

Now we run the gage analysis

```

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

```

```

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

```

```

head(keggres$less)

```

	p.geomean	stat.mean	p.val
hsa04110 Cell cycle	8.995727e-06	-4.378644	8.995727e-06
hsa03030 DNA replication	9.424076e-05	-3.951803	9.424076e-05
hsa03013 RNA transport	1.375901e-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/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04110.pathview.png

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

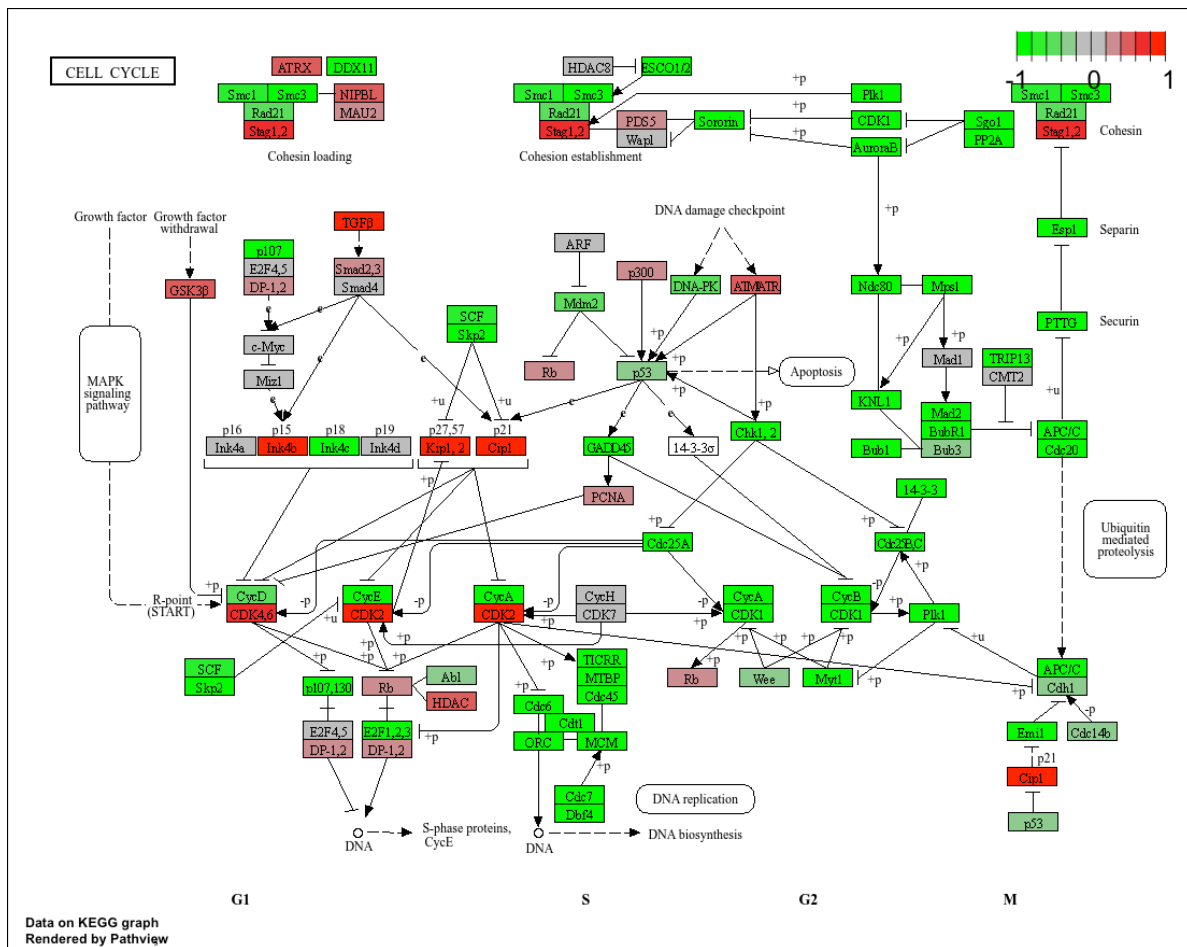
'select()' returned 1:1 mapping between keys and columns

Warning: reconcile groups sharing member nodes!

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04110.pathview.pdf



```
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/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa00140.pathview.png

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

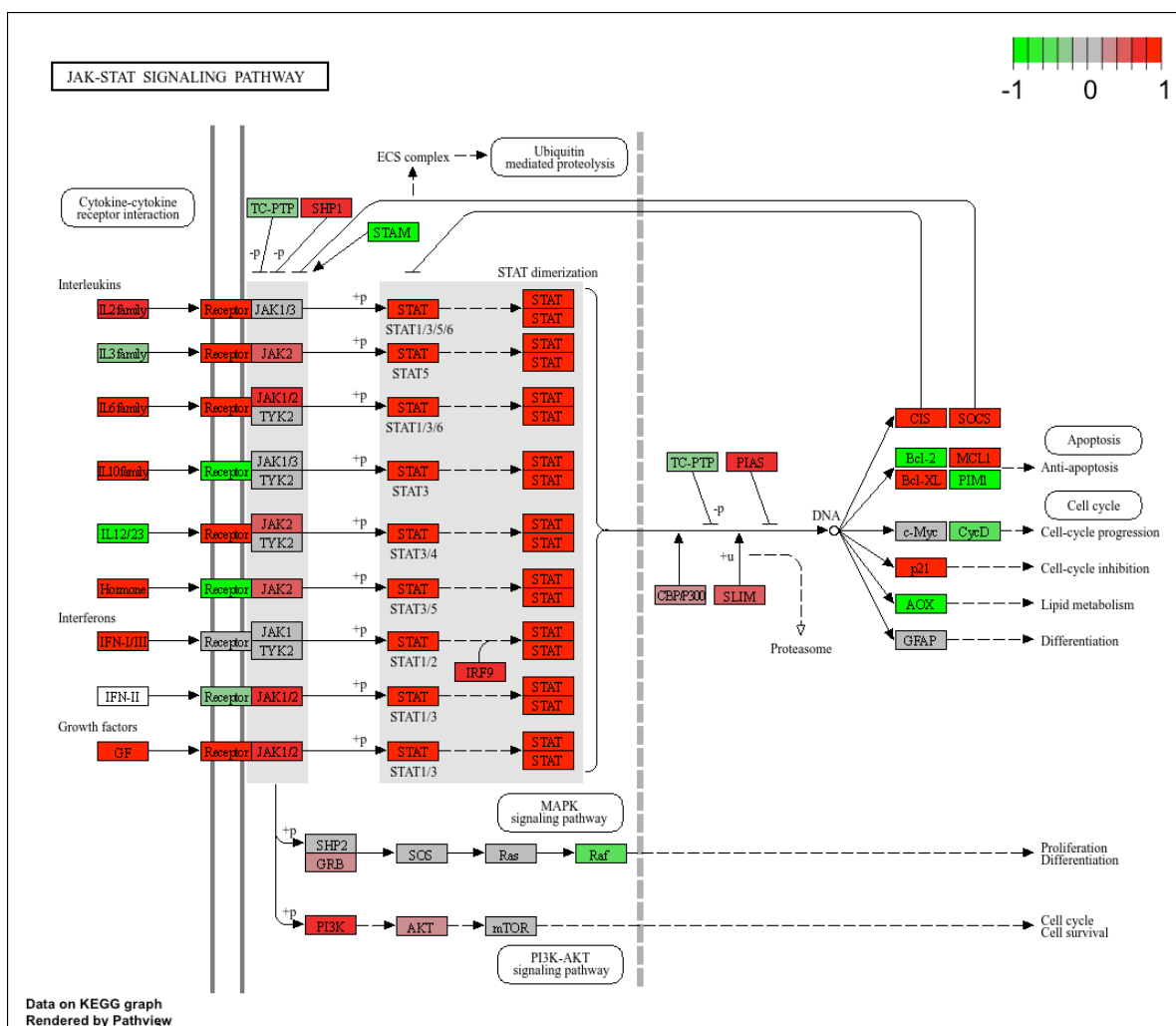
Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04330.pathview.png

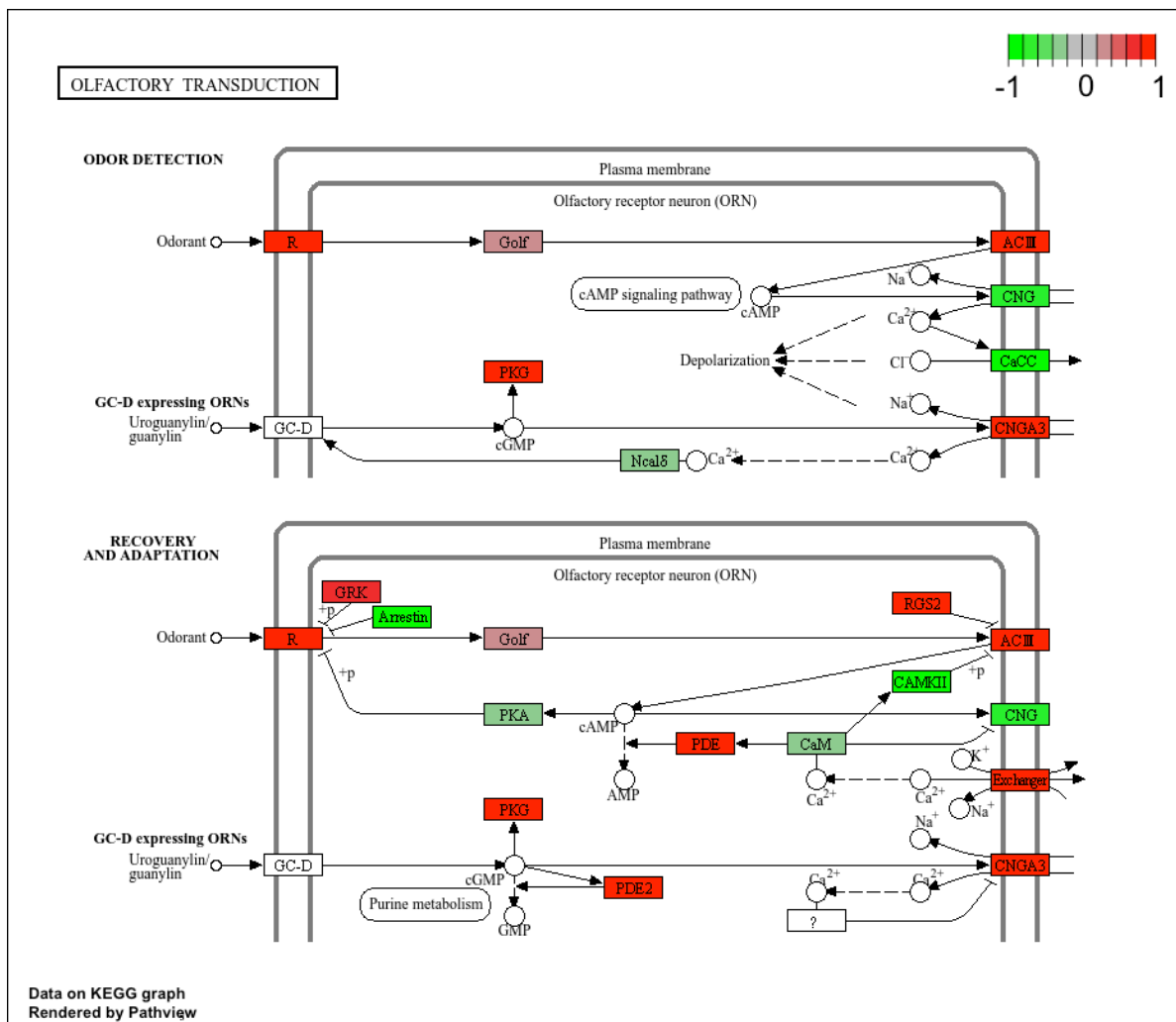



```
pathview(gene.data=foldchanges, pathway.id="hsa04740")
```

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04740.pathview.png



Here are the downregulated pathway diagrams:

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

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

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

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory /Users/lilith.sadil/Desktop/RStudio Projects/Class14

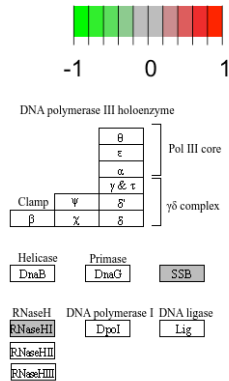
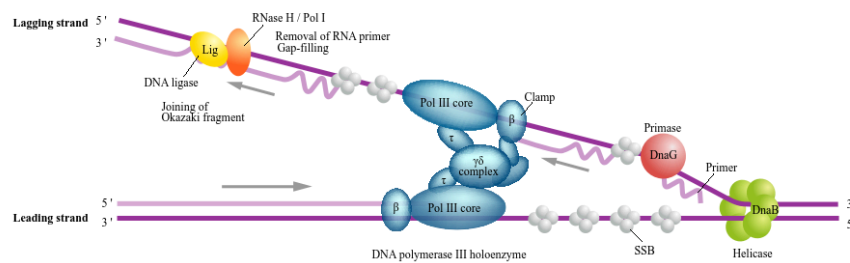
The diagram illustrates the cell cycle signaling pathways, showing the progression from G1 to S to G2 to M phases. Key components and interactions include:

- G1 Phase:** Growth factor withdrawal leads to GSK3 β activation, which inhibits c-Myc and Mxi1. TGF β leads to Smad2/3/4 activation. SCF/Skp2 complex is involved in ubiquitination of p16, p15, p18, p19, p27, p57, and p21. Cyclins D, E, and A are regulated by CDKs 4, 2, and 2, respectively.
- S Phase:** DNA replication is initiated by the R-point (START). S-phase proteins, including Cyclin E, are involved in DNA biosynthesis.
- G2 Phase:** DNA damage checkpoints involve ATM/ATR, DNA-PK, and p53. p53 leads to p21 induction, which inhibits Cyclin D/CDK4 and Cyclin E/CDK2. p53 also leads to Mdm2 activation, which inhibits p53. p53 also leads to GADD45, which is involved in DNA damage repair.
- M Phase:** Ubiquitin-mediated proteolysis is involved in the degradation of APC/C, Cdh1, and p21. APC/C is involved in the degradation of p21 and p53. Cdh1 is involved in the degradation of p21 and p53.

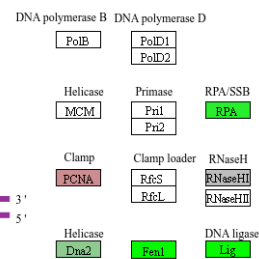
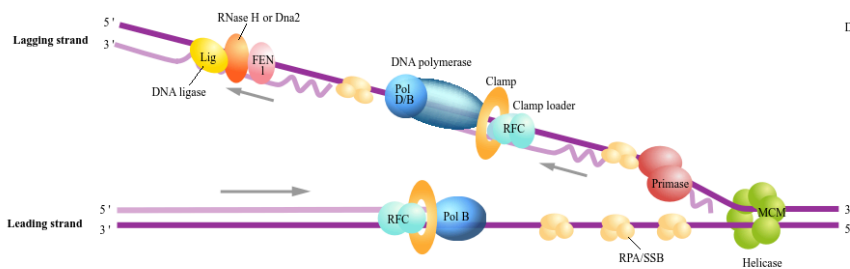
The diagram is color-coded and includes a legend for protein states (e.g., active, inactive, phosphorylated). The diagram is rendered by Pathview.

DNA REPLICATION

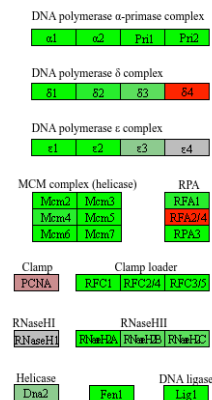
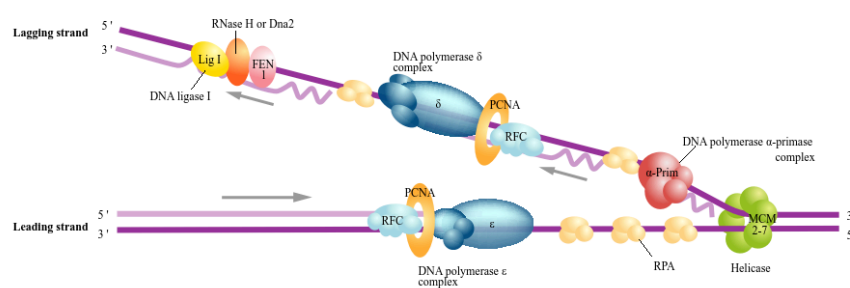
Replication complex (Bacteria)



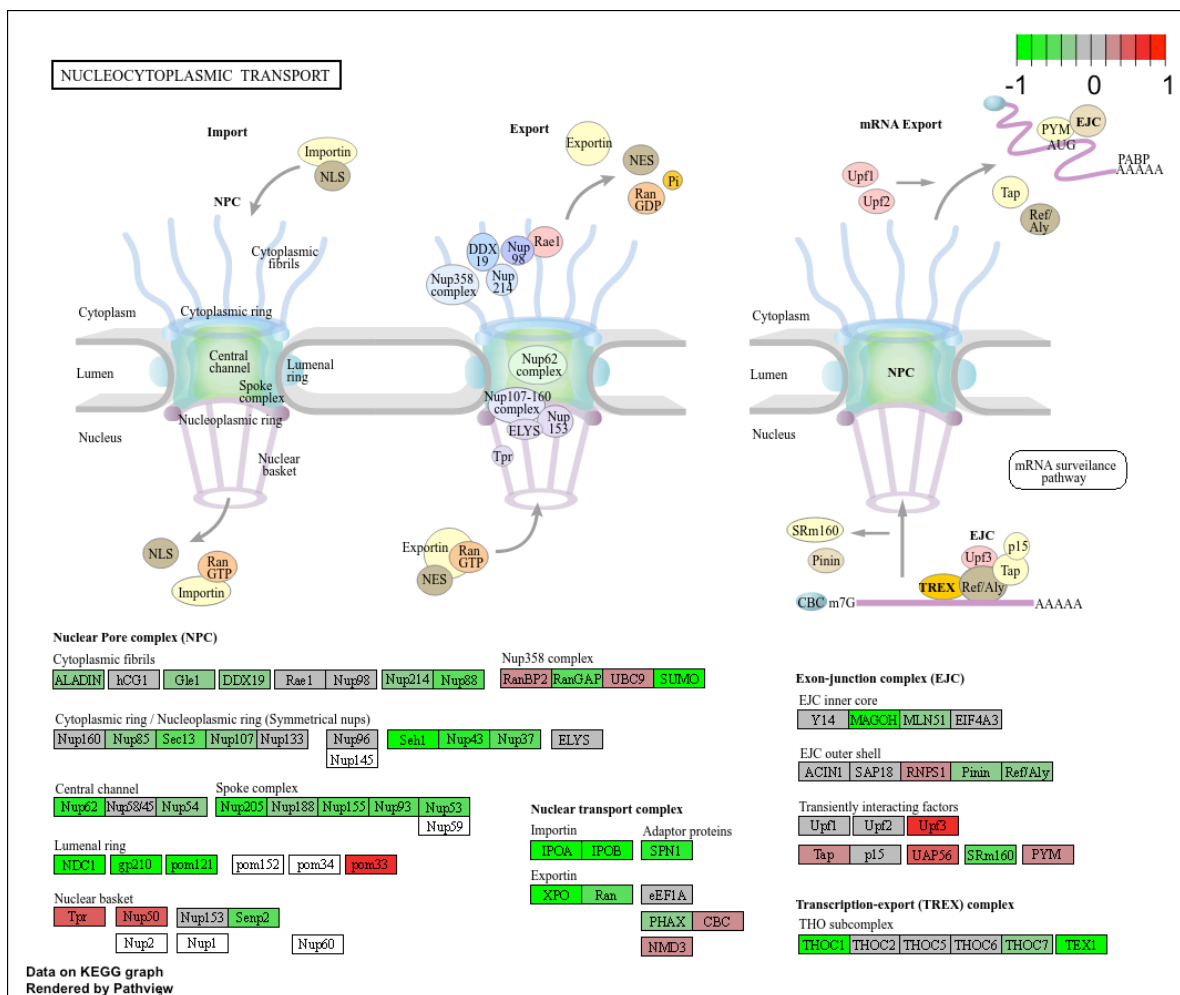
Replication complex (Archaea)

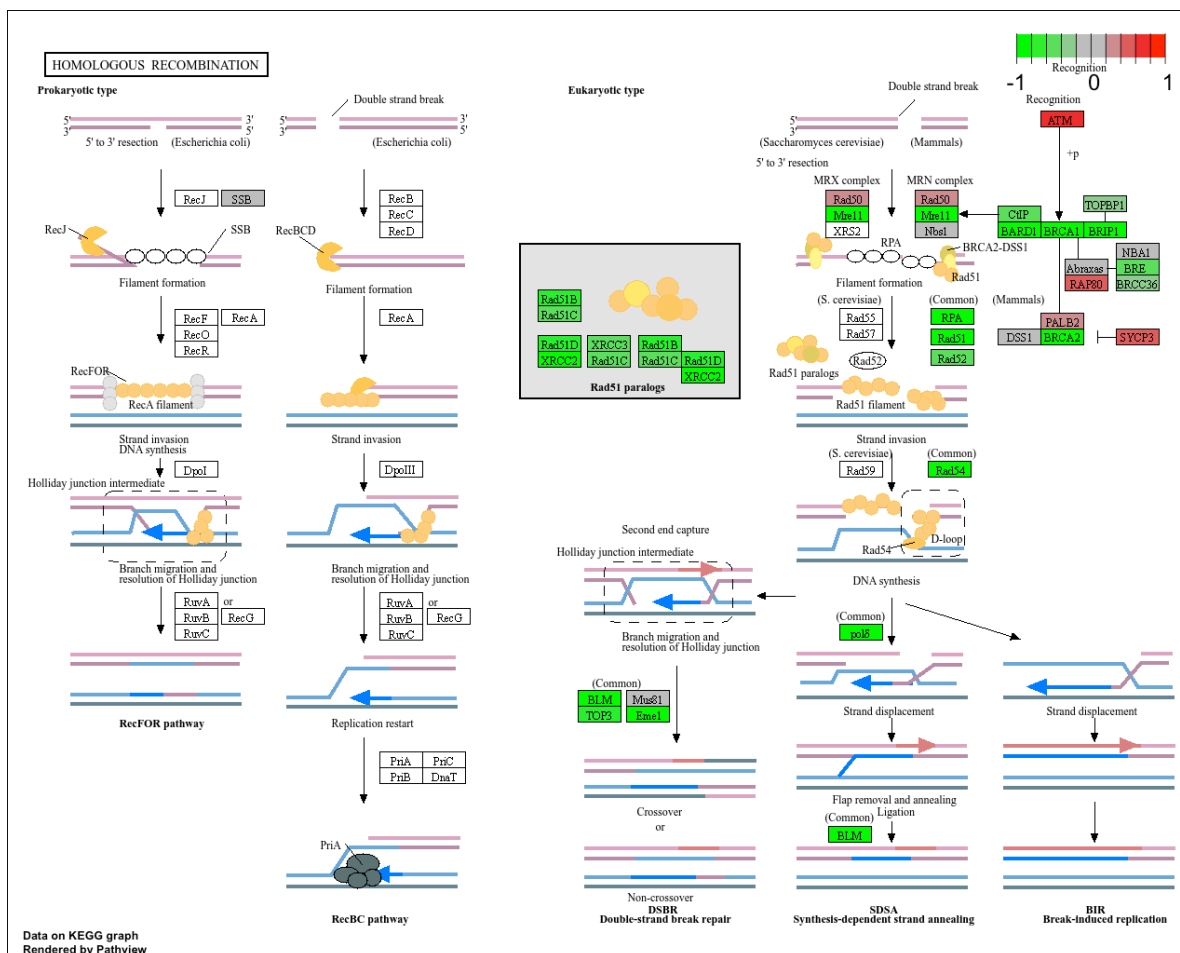


Replication complex (Eukaryotes)



Data on KEGG graph
Rendered by Pathview





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
G0:0002009	morphogenesis of an epithelium	3.653886
G0:0048729	tissue morphogenesis	3.643242
G0:0007610	behavior	3.565432
G0:0060562	epithelial tube morphogenesis	3.261376
G0:0035295	tube development	3.253665

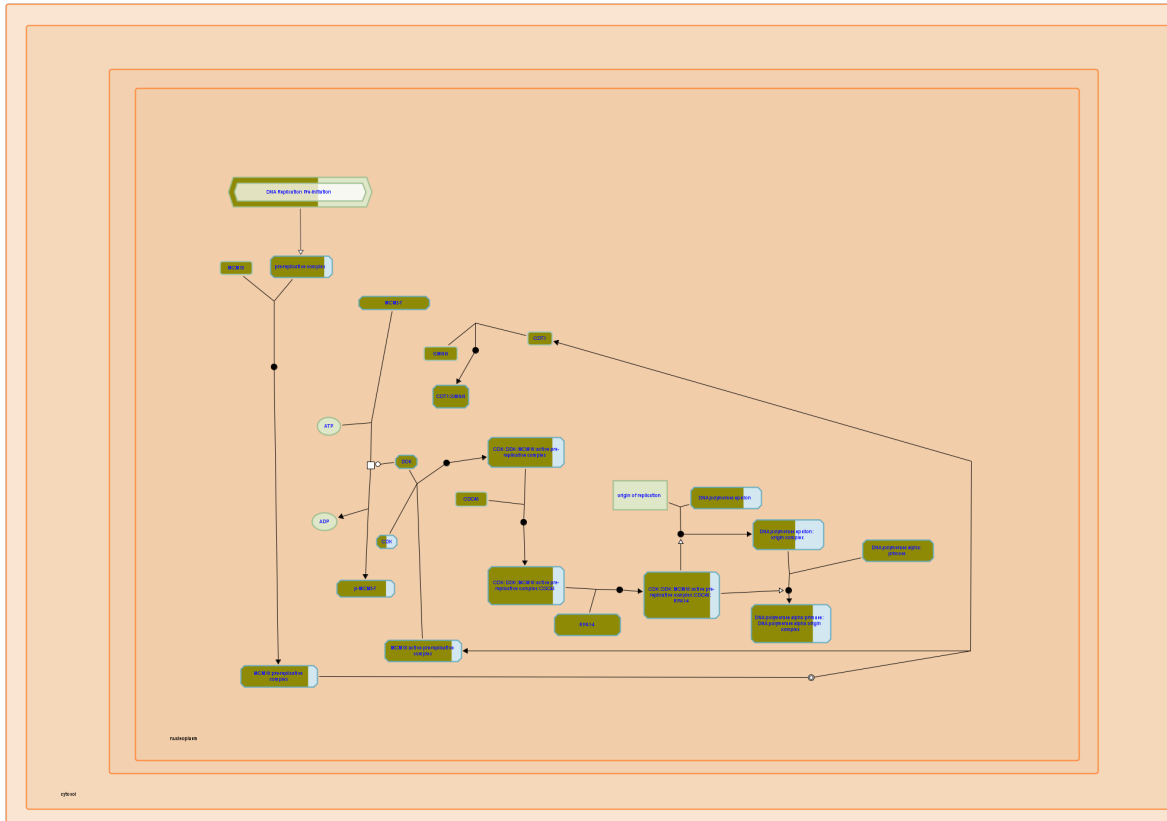
```
sig_genes = res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
[1] "Total number of significant genes: 8147"
```

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

This code block above created a table txt file which can be uploaded to the reactome website in order to visualize the pathway analysis in an online browser.

This shows that the “cell cycle” pathway has the most significant “entities P-value” at a value of 2.43E-4. This aligns with the KEGG results since the first diagram we obtained through the KEGG analysis was a cell cycle diagram (hsa04110). Different amounts of information existing in each database may cause these results to vary slightly.



Activation of the pre-replicative complex: