

Class 14: RNA-seq analysis mini-project

Snehita Vallumchetla (PID: A16853399)

Table of contents

Background:	1
Data Import:	2
Inspect and tidy data:	2
Setup for DESeq:	4
Run DESeq:	6
Volcano plot of results:	8
Gene annotation:	9
Pathway analysis:	10

Run gene ontology	22
--------------------------	-----------

Background:

The data for for hands-on session comes from GEO entry: GSE37704, which is associated with the following publication:

- Trapnell C, Hendrickson DG, Sauvageau M, Goff L et al. “Differential analysis of gene regulation at transcript resolution with RNA-seq”. Nat Biotechnol 2013 Jan;31(1):46-53. PMID: 23222703

The authors report on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1. Their results and others indicate that HOXA1 is required for lung fibroblast and HeLa cell cycle progression. In particular their analysis show that “loss of HOXA1 results in significant expression level changes in thousands of individual transcripts, along with isoform switching events in key regulators of the cell cycle”. For our session we have used their Sailfish gene-level estimated counts and hence are restricted to protein-coding genes only.

Data Import:

Load our files:

```
counts <- read.csv('GSE37704_featurecounts.csv', row.names = 1)
colData <- read.csv('GSE37704_metadata.csv')
```

Inspect and tidy data:

Q. Complete the code below to remove the troublesome first column from count-Data. Does the `counts` column match with the `colData` rows?

```
head(counts)
```

	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					

```
head(colData)
```

	id	condition
1	SRR493366	control_sirna
2	SRR493367	control_sirna
3	SRR493368	control_sirna
4	SRR493369	hoxa1_kd
5	SRR493370	hoxa1_kd
6	SRR493371	hoxa1_kd

Since it does not match we need to transform the data frame so that they align, since there is an extra `length` column in the counts data, which needs to be removed.

```
colnames(counts)
```

```
[1] "length"      "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370"
[7] "SRR493371"
```

```
countData <- counts[,-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

Check for matching countData and colData:

```
colnames(countData) == colData$id
```

```
[1] TRUE TRUE TRUE TRUE TRUE TRUE
```

Q1. How many genes in total?

19808 genes total in our dataset

```
nrow(countData)
```

```
[1] 19808
```

Q2. Filter to remove zero count genes (rows where there are zero counts in all columns). How many genes are left?

There are 15975 genes that are not zero count genes, within this data set.

```
to.keep.inds <- rowSums(countData) > 0
```

```
new.counts <- countData[to.keep.indxs,]
```

```
nrow(new.counts)
```

```
[1] 15975
```

Setup for DESeq:

```
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, saveRDS, setdiff,
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, 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

Setup input objects for DESeq:

```
dds <- DESeqDataSetFromMatrix(countData = new.counts,  
                              colData = colData,  
                              design = ~condition)
```

Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
design formula are characters, converting to factors

Run DESeq:

```
dds <- DESeq(dds)
```

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

```
res <- results(dds)
```

```
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	1.43989e-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	0.5215599	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				

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

Volcano plot of results:

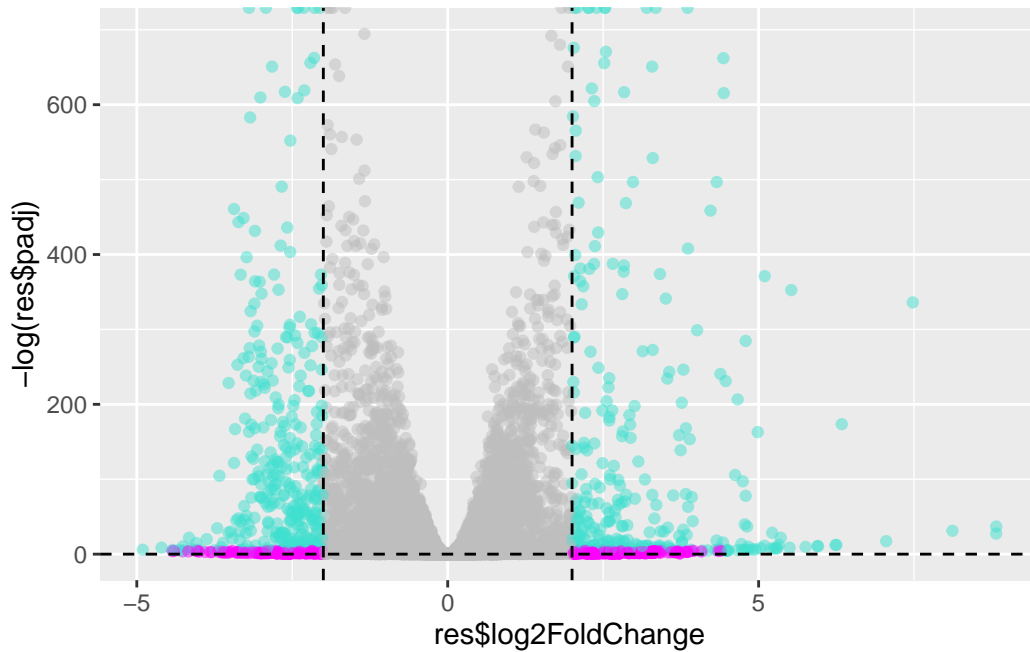
```
library(ggplot2)
```

```
mycols <- rep('gray', nrow(res))  
mycols[ abs(res$log2FoldChange) > 2 ] <- "magenta"
```

```
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )  
mycols[ inds ] <- "turquoise"
```

```
ggplot(res) +  
  aes(res$log2FoldChange, -log(res$padj)) +  
  geom_point(col = mycols, alpha = 0.5) +  
  geom_vline(xintercept = c(-2,2), linetype = 'dashed', color = 'black') +  
  geom_hline(yintercept = 0.01, linetype = 'dashed', color = 'black')
```

Warning: Removed 1237 rows containing missing values or values outside the scale range (`geom_point()`).



Gene annotation:

```
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"				

Q4. Use the `mapIds()` function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

Add gene SYMBOL, GENENAME, and ENTREZID:

```
res$symbol <- mapIds(org.Hs.eg.db,
  keys = rownames(res),
  keytype = 'ENSEMBL',
  column = 'SYMBOL')
```

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

```
res$genename <- mapIds(org.Hs.eg.db,
  keys = rownames(res),
  keytype = 'ENSEMBL',
  column = 'GENENAME')
```

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

```
res$entrez <- mapIds(org.Hs.eg.db,
  keys = rownames(res),
  keytype = 'ENSEMBL',
  column = 'ENTREZID')
```

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

```
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 9 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	1.43989e-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	0.5215599	1.040744	2.97994e-01
	padj	symbol	genename	entrez	
	<numeric>	<character>	<character>	<character>	
ENSG00000279457	6.86555e-01	NA	NA	NA	
ENSG00000187634	5.15718e-03	SAMD11	sterile alpha motif ..	148398	
ENSG00000188976	1.76549e-35	NOC2L	NOC2 like nucleolar ..	26155	
ENSG00000187961	1.13413e-07	KLHL17	kelch like family me..	339451	
ENSG00000187583	9.19031e-01	PLEKHN1	pleckstrin homology ..	84069	
ENSG00000187642	4.03379e-01	PERM1	PPARGC1 and ESRR ind..	84808	

Q5. 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")
```

Pathway analysis:

```
library(gage)
```

```
library(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>).

```
#####
```

Load up the kegg gene sets:

```
data(kegg.sets.hs)
```

```
head(kegg.sets.hs, 2)
```

```
$`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"
```

```
foldchanges <- res$log2FoldChange
names(foldchanges) <- res$entrez
```

```
keggres <- gage(foldchanges, gsets = kegg.sets.hs)
```

```
head(keggres$less)
```

	p.geomean	stat.mean
hsa04110 Cell cycle	8.995727e-06	-4.378644
hsa03030 DNA replication	9.424076e-05	-3.951803
hsa05130 Pathogenic Escherichia coli infection	1.405864e-04	-3.765330

hsa03013	RNA transport	1.375901e-03	-3.028500
hsa03440	Homologous recombination	3.066756e-03	-2.852899
hsa04114	Oocyte meiosis	3.784520e-03	-2.698128
		p.val	q.val
hsa04110	Cell cycle	8.995727e-06	0.001889103
hsa03030	DNA replication	9.424076e-05	0.009841047
hsa05130	Pathogenic Escherichia coli infection	1.405864e-04	0.009841047
hsa03013	RNA transport	1.375901e-03	0.072234819
hsa03440	Homologous recombination	3.066756e-03	0.128803765
hsa04114	Oocyte meiosis	3.784520e-03	0.132458191
		set.size	exp1
hsa04110	Cell cycle	121	8.995727e-06
hsa03030	DNA replication	36	9.424076e-05
hsa05130	Pathogenic Escherichia coli infection	53	1.405864e-04
hsa03013	RNA transport	144	1.375901e-03
hsa03440	Homologous recombination	28	3.066756e-03
hsa04114	Oocyte meiosis	102	3.784520e-03

Cell cycle figure:

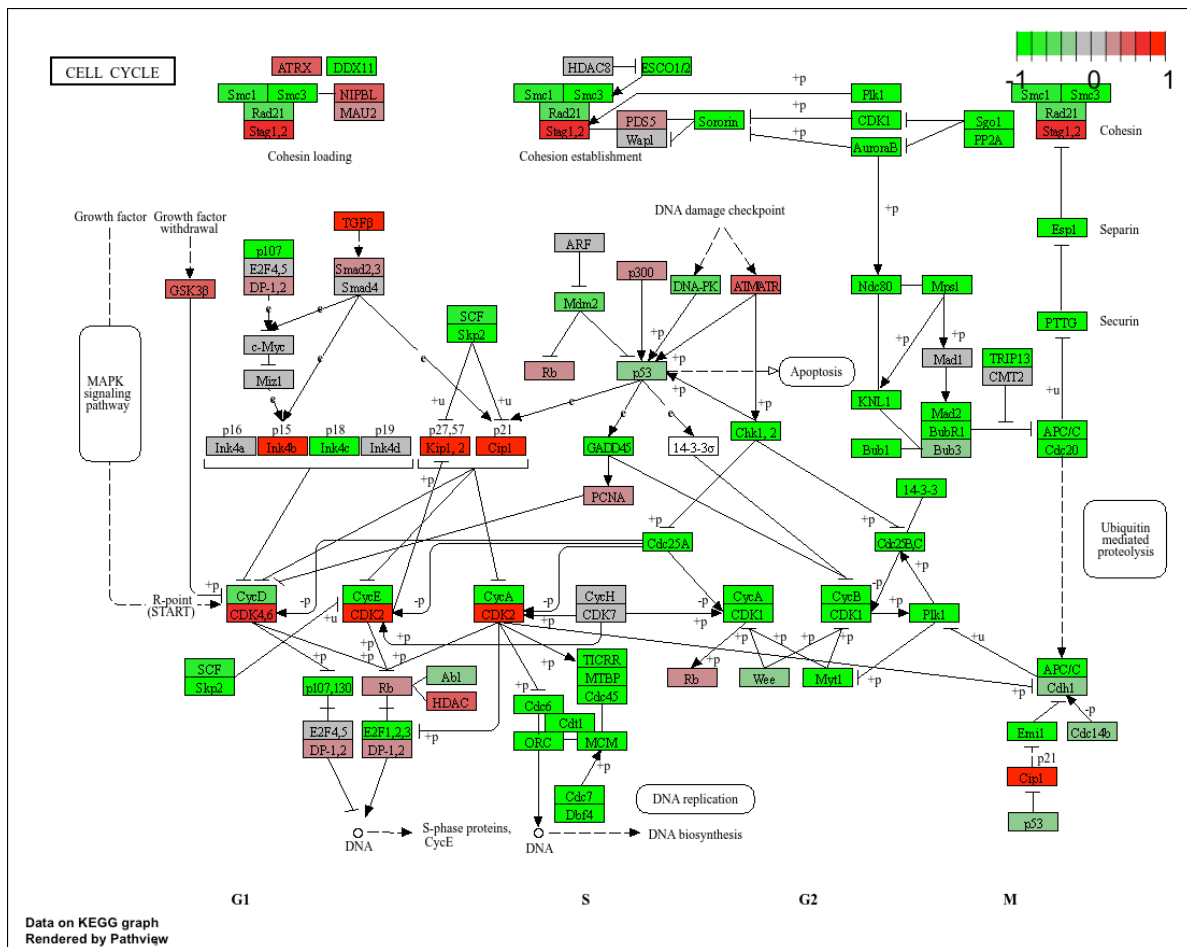
```
pathview(foldchanges, pathway.id = 'hsa04110')
```

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

Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa04110.pathview.png

Insert this figure in my report



We can look at the top 5 upregulated genes:

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

```
keggresids = substr(keggrespathways, start=1, stop=8)
```

```
keggresids
```

```
[1] "hsa04060" "hsa05323" "hsa05146" "hsa05332" "hsa04640"
```

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

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

Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa04060.pathview.png

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

Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa05323.pathview.png

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

Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa05146.pathview.png

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

Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa05332.pathview.png

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

Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa04640.pathview.png

Below are illustrations of the top 5 upregulated genes:

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

Let us also observe the top 5 down-regulated genes:

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

```
keggresids_down = substr(keggrespathways_down, start=1, stop=8)
```

```
keggresids_down
```

```
[1] "hsa04110" "hsa03030" "hsa05130" "hsa03013" "hsa03440"
```

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

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

```
Info: Working in directory /Users/snehita/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/snehita/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/snehita/Desktop/bimm 143/class14
```

```
Info: Writing image file hsa05130.pathview.png
```

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

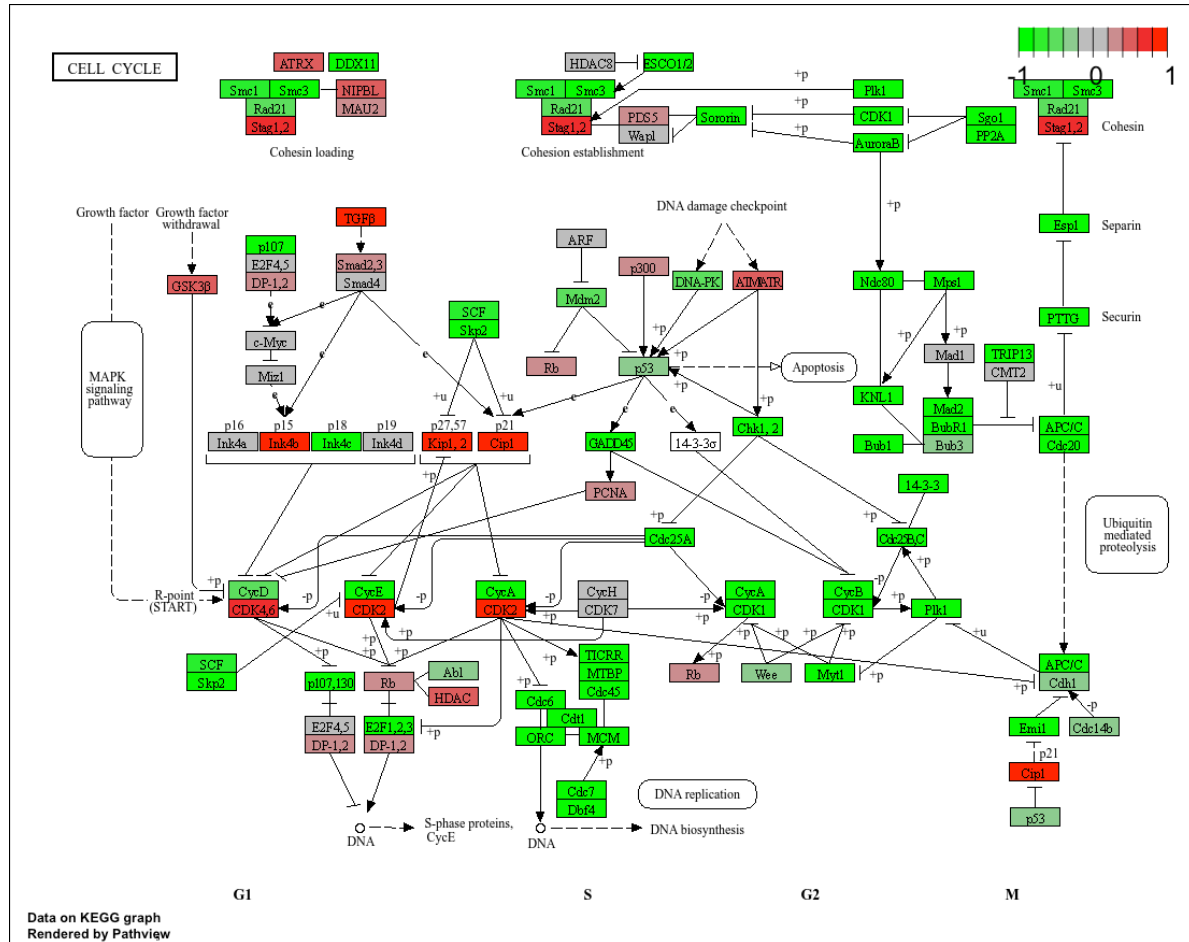
```
Info: Working in directory /Users/snehita/Desktop/bimm 143/class14
```

Info: Writing image file hsa03013.pathview.png

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

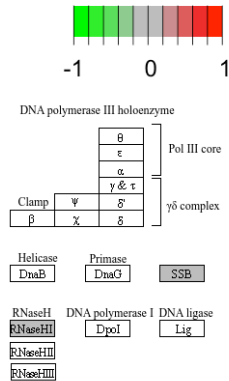
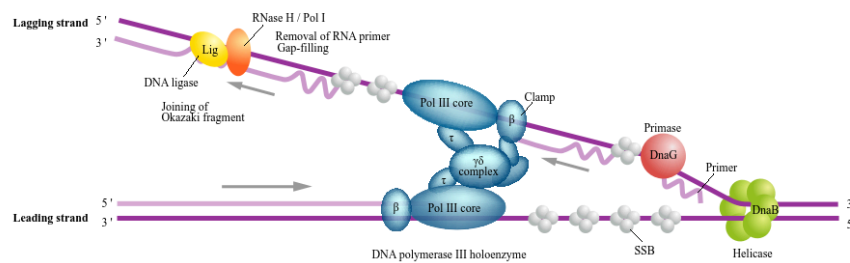
Info: Working in directory /Users/snehita/Desktop/bimm 143/class14

Info: Writing image file hsa03440.pathview.png

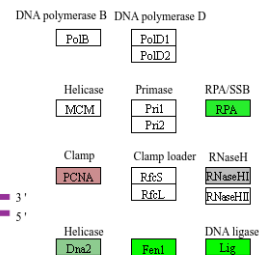
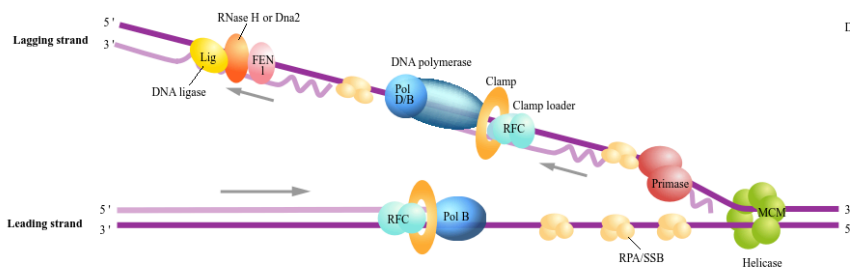


DNA REPLICATION

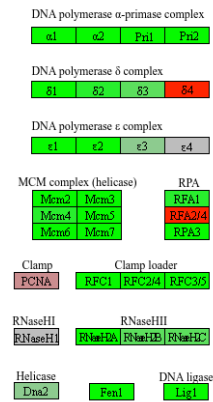
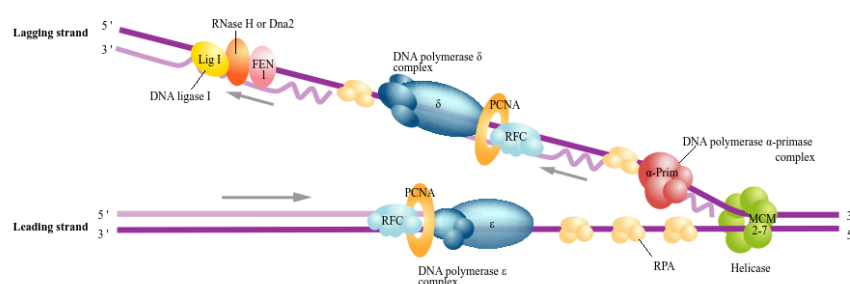
Replication complex (Bacteria)



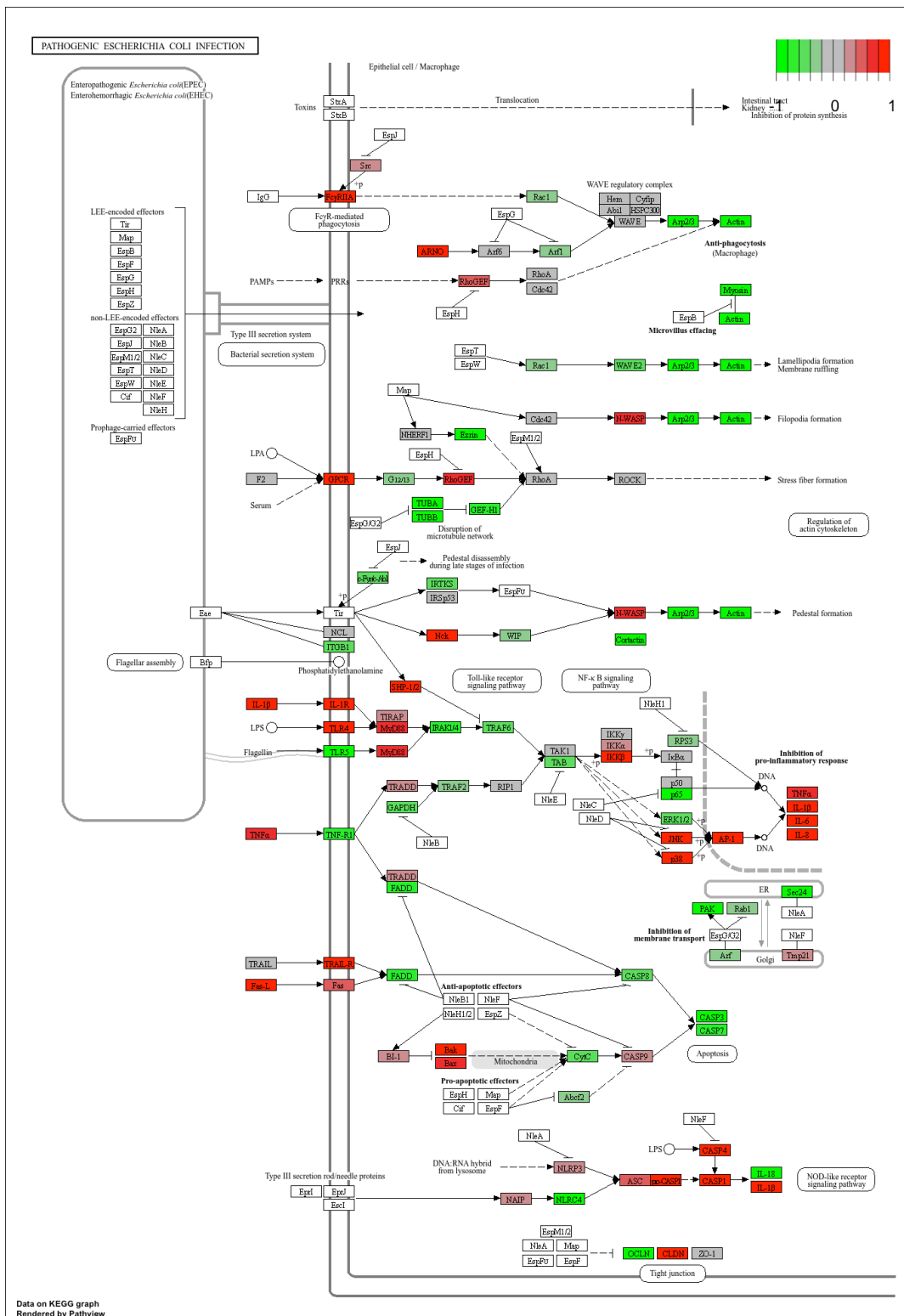
Replication complex (Archaea)



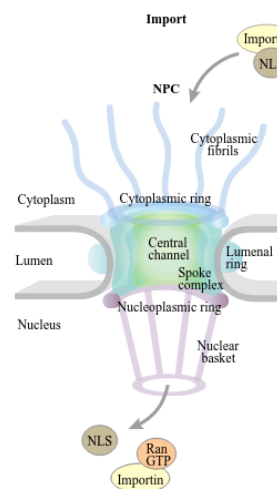
Replication complex (Eukaryotes)



Data on KEGG graph
Rendered by Pathview

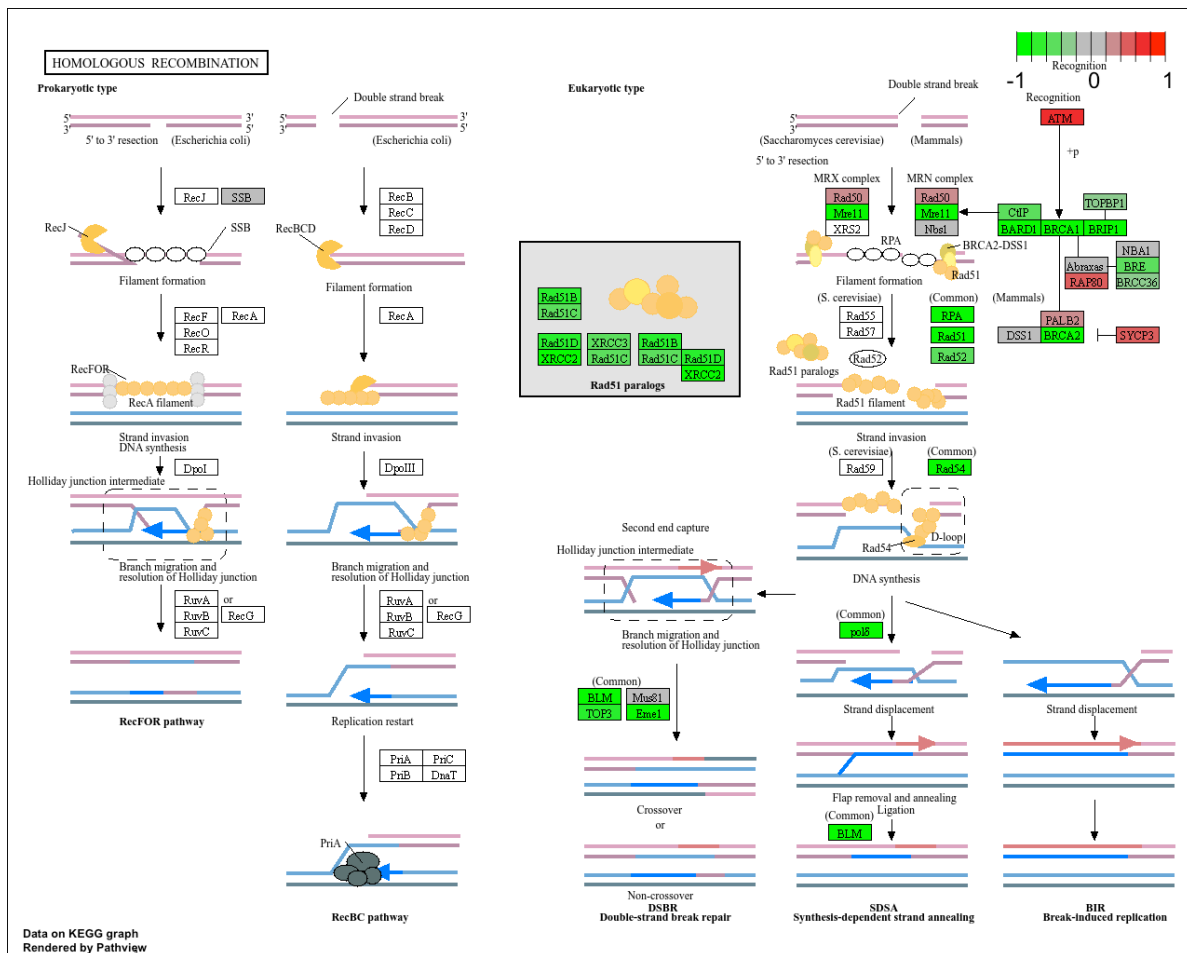


NUCLEOCYTOPLASMIC TRANSPORT



Nuclear Pore complex (NPC)

Cytoplasmic fibrils
ALADIN, ICG1, Gle1, DDX19, Rae1, Nup160, Nup85, Sec13, Nup107, Nup133, Nup153, Nup205, Nup208, Nup210, Nup214, Nup216, Nup220, Nup229, Nup231, Nup235, Nup236, Nup241, Nup242, Nup243, Nup244, Nup245, Nup246, Nup247, Nup248, Nup249, Nup250, Nup251, Nup252, Nup253, Nup254, Nup255, Nup256, Nup257, Nup258, Nup259, Nup260, Nup261, Nup262, Nup263, Nup264, Nup265, Nup266, Nup267, Nup268, Nup269, Nup270, Nup271, Nup272, Nup273, Nup274, Nup275, Nup276, Nup277, Nup278, Nup279, Nup280, Nup281, Nup282, Nup283, Nup284, Nup285, Nup286, Nup287, Nup288, Nup289, Nup290, Nup291, Nup292, Nup293, Nup294, Nup295, Nup296, Nup297, Nup298, Nup299, Nup300, Nup301, Nup302, Nup303, Nup304, Nup305, Nup306, Nup307, Nup308, Nup309, Nup310, Nup311, Nup312, Nup313, Nup314, Nup315, Nup316, Nup317, Nup318, Nup319, Nup320, Nup321, Nup322, Nup323, Nup324, Nup325, Nup326, Nup327, Nup328, Nup329, Nup330, Nup331, Nup332, Nup333, Nup334, Nup335, Nup336, Nup337, Nup338, Nup339, Nup340, Nup341, Nup342, Nup343, Nup344, Nup345, Nup346, Nup347, Nup348, Nup349, Nup350, Nup351, Nup352, Nup353, Nup354, Nup355, Nup356, Nup357, Nup358, Nup359, Nup360, Nup361, Nup362, Nup363, Nup364, Nup365, Nup366, Nup367, Nup368, Nup369, Nup370, Nup371, Nup372, Nup373, Nup374, Nup375, Nup376, Nup377, Nup378, Nup379, Nup380, Nup381, Nup382, Nup383, Nup384, Nup385, Nup386, Nup387, Nup388, Nup389, Nup390, Nup391, Nup392, Nup393, Nup394, Nup395, Nup396, Nup397, Nup398, Nup399, Nup400, Nup401, Nup402, Nup403, Nup404, Nup405, Nup406, Nup407, Nup408, Nup409, Nup410, Nup411, Nup412, Nup413, Nup414, Nup415, Nup416, Nup417, Nup418, Nup419, Nup420, Nup421, Nup422, Nup423, Nup424, Nup425, Nup426, Nup427, Nup428, Nup429, Nup430, Nup431, Nup432, Nup433, Nup434, Nup435, Nup436, Nup437, Nup438, Nup439, Nup440, Nup441, Nup442, Nup443, Nup444, Nup445, Nup446, Nup447, Nup448, Nup449, Nup450, Nup451, Nup452, Nup453, Nup454, Nup455, Nup456, Nup457, Nup458, Nup459, Nup460, Nup461, Nup462, Nup463, Nup464, Nup465, Nup466, Nup467, Nup468, Nup469, Nup470, Nup471, Nup472, Nup473, Nup474, Nup475, Nup476, Nup477, Nup478, Nup479, Nup480, Nup481, Nup482, Nup483, Nup484, Nup485, Nup486, Nup487, Nup488, Nup489, Nup490, Nup491, Nup492, Nup493, Nup494, Nup495, Nup496, Nup497, Nup498, Nup499, Nup500, Nup501, Nup502, Nup503, Nup504, Nup505, Nup506, Nup507, Nup508, Nup509, Nup510, Nup511, Nup512, Nup513, Nup514, Nup515, Nup516, Nup517, Nup518, Nup519, Nup520, Nup521, Nup522, Nup523, Nup524, Nup525, Nup526, Nup527, Nup528, Nup529, Nup530, Nup531, Nup532, Nup533, Nup534, Nup535, Nup536, Nup537, Nup538, Nup539, Nup540, Nup541, Nup542, Nup543, Nup544, Nup545, Nup546, Nup547, Nup548, Nup549, Nup550, Nup551, Nup552, Nup553, Nup554, Nup555, Nup556, Nup557, Nup558, Nup559, Nup560, Nup561, Nup562, Nup563, Nup564, Nup565, Nup566, Nup567, Nup568, Nup569, Nup570, Nup571, Nup572, Nup573, Nup574, Nup575, Nup576, Nup577, Nup578, Nup579, Nup580, Nup581, Nup582, Nup583, Nup584, Nup585, Nup586, Nup587, Nup588, Nup589, Nup590, Nup591, Nup592, Nup593, Nup594, Nup595, Nup596, Nup597, Nup598, Nup599, Nup600, Nup601, Nup602, Nup603, Nup604, Nup605, Nup606, Nup607, Nup608, Nup609, Nup610, Nup611, Nup612, Nup613, Nup614, Nup615, Nup616, Nup617, Nup618, Nup619, Nup620, Nup621, Nup622, Nup623, Nup624, Nup625, Nup626, Nup627, Nup628, Nup629, Nup630, Nup631, Nup632, Nup633, Nup634, Nup635, Nup636, Nup637, Nup638, Nup639, Nup640, Nup641, Nup642, Nup643, Nup644, Nup645, Nup646, Nup647, Nup648, Nup649, Nup650, Nup651, Nup652, Nup653, Nup654, Nup655, Nup656, Nup657, Nup658, Nup659, Nup660, Nup661, Nup662, Nup663, Nup664, Nup665, Nup666, Nup667, Nup668, Nup669, Nup670, Nup671, Nup672, Nup673, Nup674, Nup675, Nup676, Nup677, Nup678, Nup679, Nup680, Nup681, Nup682, Nup683, Nup684, Nup685, Nup686, Nup687, Nup688, Nup689, Nup690, Nup691, Nup692, Nup693, Nup694, Nup695, Nup696, Nup697, Nup698, Nup699, Nup700, Nup701, Nup702, Nup703, Nup704, Nup705, Nup706, Nup707, Nup708, Nup709, Nup710, Nup711, Nup712, Nup713, Nup714, Nup715, Nup716, Nup717, Nup718, Nup719, Nup720, Nup721, Nup722, Nup723, Nup724, Nup725, Nup726, Nup727, Nup728, Nup729, Nup730, Nup731, Nup732, Nup733, Nup734, Nup735, Nup736, Nup737, Nup738, Nup739, Nup740, Nup741, Nup742, Nup743, Nup744, Nup745, Nup746, Nup747, Nup748, Nup749, Nup750, Nup751, Nup752, Nup753, Nup754, Nup755, Nup756, Nup757, Nup758, Nup759, Nup760, Nup761, Nup762, Nup763, Nup764, Nup765, Nup766, Nup767, Nup768, Nup769, Nup770, Nup771, Nup772, Nup773, Nup774, Nup775, Nup776, Nup777, Nup778, Nup779, Nup780, Nup781, Nup782, Nup783, Nup784, Nup785, Nup786, Nup787, Nup788, Nup789, Nup790, Nup791, Nup792, Nup793, Nup794, Nup795, Nup796, Nup797, Nup798, Nup799, Nup800, Nup801, Nup802, Nup803, Nup804, Nup805, Nup806, Nup807, Nup808, Nup809, Nup810, Nup811, Nup812, Nup813, Nup814, Nup815, Nup816, Nup817, Nup818, Nup819, Nup820, Nup821, Nup822, Nup823, Nup824, Nup825, Nup826, Nup827, Nup828, Nup829, Nup830, Nup831, Nup832, Nup833, Nup834, Nup835, Nup836, Nup837, Nup838, Nup839, Nup840, Nup841, Nup842, Nup843, Nup844, Nup845, Nup846, Nup847, Nup848, Nup849, Nup850, Nup851, Nup852, Nup853, Nup854, Nup855, Nup856, Nup857, Nup858, Nup859, Nup860, Nup861, Nup862, Nup863, Nup864, Nup865, Nup866, Nup867, Nup868, Nup869, Nup870, Nup871, Nup872, Nup873, Nup874, Nup875, Nup876, Nup877, Nup878, Nup879, Nup880, Nup881, Nup882, Nup883, Nup884, Nup885, Nup886, Nup887, Nup888, Nup889, Nup890, Nup891, Nup892, Nup893, Nup894, Nup895, Nup896, Nup897, Nup898, Nup899, Nup900, Nup901, Nup902, Nup903, Nup904, Nup905, Nup906, Nup907, Nup908, Nup909, Nup910, Nup911, Nup912, Nup913, Nup914, Nup915, Nup916, Nup917, Nup918, Nup919, Nup920, Nup921, Nup922, Nup923, Nup924, Nup925, Nup926, Nup927, Nup928, Nup929, Nup930, Nup931, Nup932, Nup933, Nup934, Nup935, Nup936, Nup937, Nup938, Nup939, Nup940, Nup941, Nup942, Nup943, Nup944, Nup945, Nup946, Nup947, Nup948, Nup949, Nup950, Nup951, Nup952, Nup953, Nup954, Nup955, Nup956, Nup957, Nup958, Nup959, Nup960, Nup961, Nup962, Nup963, Nup964, Nup965, Nup966, Nup967, Nup968, Nup969, Nup970, Nup971, Nup972, Nup973, Nup974, Nup975, Nup976, Nup977, Nup978, Nup979, Nup980, Nup981, Nup982, Nup983, Nup984, Nup985, Nup986, Nup987, Nup988, Nup989, Nup990, Nup991, Nup992, Nup993, Nup994, Nup995, Nup996, Nup997, Nup998, Nup999, Nup1000, Nup1001, Nup1002, Nup1003, Nup1004, Nup1005, Nup1006, Nup1007, Nup1008, Nup1009, Nup1010, Nup1011, Nup1012, Nup1013, Nup1014, Nup1015, Nup1016, Nup1017, Nup1018, Nup1019, Nup1020, Nup1021, Nup1022, Nup1023, Nup1024, Nup1025, Nup1026, Nup1027, Nup1028, Nup1029, Nup1030, Nup1031, Nup1032, Nup1033, Nup1034, Nup1035, Nup1036, Nup1037, Nup1038, Nup1039, Nup1040, Nup1041, Nup1042, Nup1043, Nup1044, Nup1045, Nup1046, Nup1047, Nup1048, Nup1049, Nup1050, Nup1051, Nup1052, Nup1053, Nup1054, Nup1055, Nup1056, Nup1057, Nup1058, Nup1059, Nup1060, Nup1061, Nup1062, Nup1063, Nup1064, Nup1065, Nup1066, Nup1067, Nup1068, Nup1069, Nup1070, Nup1071, Nup1072, Nup1073, Nup1074, Nup1075, Nup1076, Nup1077, Nup1078, Nup1079, Nup1080, Nup1081, Nup1082, Nup1083, Nup1084, Nup1085, Nup1086, Nup1087, Nup1088, Nup1089, Nup1090, Nup1091, Nup1092, Nup1093, Nup1094, Nup1095, Nup1096, Nup1097, Nup1098, Nup1099, Nup1100, Nup1101, Nup1102, Nup1103, Nup1104, Nup1105, Nup1106, Nup1107, Nup1108, Nup1109, Nup1110, Nup1111, Nup1112, Nup1113, Nup1114, Nup1115, Nup1116, Nup1117, Nup1118, Nup1119, Nup1120, Nup1121, Nup1122, Nup1123, Nup1124, Nup1125, Nup1126, Nup1127, Nup1128, Nup1129, Nup1130, Nup1131, Nup1132, Nup1133, Nup1134, Nup1135, Nup1136, Nup1137, Nup1138, Nup1139, Nup1140, Nup1141, Nup1142, Nup1143, Nup1144, Nup1145, Nup1146, Nup1147, Nup1148, Nup1149, Nup1150, Nup1151, Nup1152, Nup1153, Nup1154, Nup1155, Nup1156, Nup1157, Nup1158, Nup1159, Nup1160, Nup1161, Nup1162, Nup1163, Nup1164, Nup1165, Nup1166, Nup1167, Nup1168, Nup1169, Nup1170, Nup1171, Nup1172, Nup1173, Nup1174, Nup1175, Nup1176, Nup1177, Nup1178, Nup1179, Nup1180, Nup1181, Nup1182, Nup1183, Nup1184, Nup1185, Nup1186, Nup1187, Nup1188, Nup1189, Nup1190, Nup1191, Nup1192, Nup1193, Nup1194, Nup1195, Nup1196, Nup1197, Nup1198, Nup1199, Nup1200, Nup1201, Nup1202, Nup1203, Nup1204, Nup1205, Nup1206, Nup1207, Nup1208, Nup1209, Nup1210, Nup1211, Nup1212, Nup1213, Nup1214, Nup1215, Nup1216, Nup1217, Nup1218, Nup1219, Nup1220, Nup1221, Nup1222, Nup1223, Nup1224, Nup1225, Nup1226, Nup1227, Nup1228, Nup1229, Nup1230, Nup1231, Nup1232, Nup1233, Nup1234, Nup1235, Nup1236, Nup1237, Nup1238, Nup1239, Nup1240, Nup1241, Nup1242, Nup1243, Nup1244, Nup1245, Nup1246, Nup1247, Nup1248, Nup1249, Nup1250, Nup1251, Nup1252, Nup1253, Nup1254, Nup1255, Nup1256, Nup1257, Nup1258, Nup1259, Nup1260, Nup1261, Nup1262, Nup1263, Nup1264, Nup1265, Nup1266, Nup1267, Nup1268, Nup1269, Nup1270, Nup1271, Nup1272, Nup1273, Nup1274, Nup1275, Nup1276, Nup1277, Nup1278, Nup1279, Nup1280, Nup1281, Nup1282, Nup1283, Nup1284, Nup1285, Nup1286, Nup1287, Nup1288, Nup1289, Nup1290, Nup1291, Nup1292, Nup1293, Nup1294, Nup1295, Nup1296, Nup1297, Nup1298, Nup1299, Nup1300, Nup1301, Nup1302, Nup1303, Nup1304, Nup1305, Nup1306, Nup1307, Nup1308, Nup1309, Nup1310, Nup1311, Nup1312, Nup1313, Nup1314, Nup1315, Nup1316, Nup1317, Nup1318, Nup1319, Nup1320, Nup1321, Nup1322, Nup1323, Nup1324, Nup1325, Nup1326, Nup1327, Nup1328, Nup1329, Nup1330, Nup1331, Nup1332, Nup1333, Nup1334, Nup1335, Nup1336, Nup1337, Nup1338, Nup1339, Nup1340, Nup1341, Nup1342, Nup1343, Nup1344, Nup1345, Nup1346, Nup1347, Nup1348, Nup1349, Nup1350, Nup1351, Nup1352, Nup1353, Nup1354, Nup1355, Nup1356, Nup1357, Nup1358, Nup1359, Nup1360, Nup1361, Nup1362, Nup1363, Nup1364, Nup1365, Nup1366, Nup1367, Nup1368, Nup1369, Nup1370, Nup1371, Nup1372, Nup1373, Nup1374, Nup1375, Nup1376, Nup1377, Nup1378, Nup1379, Nup1380, Nup1381, Nup1382, Nup1383, Nup1384, Nup1385, Nup1386, Nup1387, Nup1388, Nup1389, Nup1390, Nup1391, Nup1392, Nup1393, Nup1394, Nup1395, Nup1396, Nup1397, Nup1398, Nup1399, Nup1400, Nup1401, Nup1402, Nup1403, Nup1404, Nup1405, Nup1406, Nup1407, Nup1408, Nup1409, Nup1410, Nup1411, Nup1412, Nup1413, Nup1414, Nup1415, Nup1416, Nup1417, Nup1418, Nup1419, Nup1420, Nup1421, Nup1422, Nup1423, Nup1424, Nup1425, Nup1426, Nup1427, Nup1428, Nup1429, Nup1430, Nup1431, Nup1432, Nup1433, Nup1434, Nup1435, Nup1436, Nup1437, Nup1438, Nup1439, Nup1440, Nup1441, Nup1442, Nup1443, Nup1444, Nup1445, Nup1446, Nup1447, Nup1448, Nup1449, Nup1450, Nup1451, Nup1452, Nup1453, Nup1454, Nup1455, Nup1456, Nup1457, Nup1458, Nup1459, Nup1460, Nup1461, Nup1462, Nup1463, Nup1464, Nup1465, Nup1466, Nup1467, Nup1468, Nup1469, Nup1470, Nup1471, Nup1472, Nup1473, Nup1474, Nup1475, Nup1476, Nup1477, Nup1478, Nup1479, Nup1480, Nup1481, Nup1482, Nup1483, Nup1484, Nup1485, Nup1486, Nup1487, Nup1488, Nup1489, Nup1490, Nup1491, Nup1492, Nup1493, Nup1494, Nup1495, Nup1496, Nup1497, Nup1498, Nup1499, Nup1500, Nup1501, Nup1502, Nup1503, Nup1504, Nup1505, Nup1506, Nup1507, Nup1508, Nup1509, Nup1510, Nup1511, Nup1512, Nup1513, Nup1514, Nup1515, Nup1516, Nup1517, Nup1518, Nup1519, Nup1520, Nup1521, Nup1522, Nup1523, Nup1524, Nup1525, Nup1526, Nup1527, Nup1528, Nup1529, Nup1530, Nup1531, Nup1532, Nup1533, Nup1534, Nup1535, Nup1536, Nup1537, Nup1538, Nup1539, Nup1540, Nup1541, Nup1542, Nup1543, Nup1544, Nup1545, Nup1546, Nup1547, Nup1548, Nup1549, Nup1550, Nup1551, Nup1552, Nup1553, Nup1554, Nup1555, Nup1556, Nup1557, Nup1558, Nup1559, Nup1560, Nup1561, Nup1562, Nup1563, Nup1564, Nup1565, Nup1566, Nup1567, Nup1568, Nup1569, Nup1570, Nup1571, Nup1572, Nup1573, Nup1574, Nup1575, Nup1576, Nup1577, Nup1578, Nup1579, Nup1580, Nup1581, Nup1582, Nup1583, Nup1584, Nup1585, Nup1586, Nup1587, Nup1588, Nup1589, Nup1590, Nup1591, Nup1592, Nup1593, Nup1594, Nup1595, Nup1596, Nup1597, Nup1598, Nup1599, Nup1600, Nup1601, Nup1602, Nup1603, Nup1604, Nup1605, Nup1606, Nup1607, Nup1608, Nup1609, Nup1610, Nup1611, Nup1612, Nup1613, Nup1614, Nup1615, Nup1616, Nup1617, Nup1618, Nup1619, Nup1620, Nup1621, Nup1622, Nup1623, Nup1624, Nup1625, Nup1626, Nup1627, Nup1628, Nup1629, Nup1630, Nup1631, Nup1632, Nup1633, Nup1634, Nup1635, Nup1636, Nup1637, Nup1638, Nup1639, Nup1640, Nup1641, Nup1642, Nup1643, Nup1644, Nup1645, Nup1646, Nup1647, Nup1648, Nup1649, Nup1650, Nup1651, Nup1652, Nup1653, Nup1654, Nup1655, Nup1656, Nup1657, Nup1658, Nup1659, Nup1660, Nup1661, Nup1662, Nup1663, Nup1664, Nup1665, Nup1666, Nup1667, Nup1668, Nup1669, Nup1670, Nup1671, Nup1672, Nup1673, Nup1674, Nup1675, Nup1676, Nup1677, Nup1678, Nup1679, Nup1680, Nup1681, Nup1682, Nup1683, Nup1684, Nup1685, Nup1686, Nup1687, Nup1688, Nup1689, Nup1690, Nup1691, Nup1692, Nup1693, Nup1694, Nup1695, Nup1696, Nup1697, Nup1698, Nup1699, Nup1700, Nup1701, Nup1702, Nup1703, Nup1704, Nup1705, Nup1706, Nup1707, Nup1708, Nup1709, Nup1710, Nup1711, Nup1712, Nup1713, Nup1714, Nup1715, Nup1716, Nup1717, Nup1718, Nup1719, Nup1720, Nup1721, Nup1722, Nup1723, Nup1724, Nup1725, Nup1726, Nup1727, Nup1728, Nup1729, Nup1730, Nup1731, Nup1732, Nup1733, Nup1734, Nup1735, Nup1736, Nup1737, Nup1738, Nup1739, Nup1740, Nup1741, Nup1742, Nup1743, Nup1744, Nup1745, Nup1746, Nup1747, Nup1748, Nup1749, Nup1750, Nup1751, Nup1752, Nup1753, Nup1754, Nup1755, Nup1756, Nup1757, Nup1758, Nup1759, Nup1760, Nup1761, Nup1762, Nup1763, Nup1764, Nup1765, Nup1766, Nup1767, Nup1768, Nup1769, Nup1770, Nup1771, Nup1772, Nup1773, Nup1774, Nup1775, Nup1776, Nup1777, Nup1778, Nup1779, Nup1780, Nup1781, Nup1782, Nup1783, Nup1784, Nup1785, Nup1786, Nup1787, Nup1788, Nup1789, Nup1790, Nup1791, Nup1792, Nup1793, Nup1794, Nup1795, Nup1796, Nup1797, Nup1798, Nup1799, Nup1800, Nup1801, Nup1802, Nup1803, Nup1804, Nup1805, Nup1806, Nup1807, Nup1808, Nup1809, Nup1810, Nup1811, Nup1812, Nup1813, Nup1814, Nup1815, Nup1816, Nup1817, Nup1818, Nup1819, Nup1820, Nup1821, Nup1822, Nup1823, Nup1824, Nup1825, Nup1826, Nup1827, Nup1828, Nup1829, Nup1830, Nup1831, Nup1832, Nup1833, Nup1834, Nup1835, Nup1836, Nup1837, Nup1838, Nup1839, Nup1840, Nup1841, Nup1842, Nup1843, Nup1844, Nup1845, Nup1846, Nup1847, Nup1848, Nup1849, Nup1850, Nup1851, Nup1852, Nup1853, Nup1854, Nup1855, Nup1856, Nup1857, Nup1858, Nup1859, Nup1860, Nup1861, Nup1862, Nup1863, Nup1864, Nup1865, Nup1866, Nup1867, Nup1868, Nup1869, Nup1870, Nup1871, Nup1872, Nup1873, Nup1874, Nup1875, Nup1876, Nup1877, Nup1878, Nup1879, Nup1880, Nup1881, Nup1882, Nup1883, Nup1884, Nup1885, Nup1886, Nup1887, Nup1888, Nup1889, Nup1890, Nup1891, Nup1892, Nup1893, Nup1894, Nup1895, Nup1896, Nup1897, Nup1898, Nup1899, Nup1900, Nup1901, Nup1902, Nup1903, Nup1904, Nup1905, Nup1906, Nup1907, Nup1908, Nup1909, Nup1910, Nup1911, Nup1912, Nup1913, Nup1914, Nup1915, Nup1916, Nup1917, Nup1918, Nup1919, Nup1920, Nup1921, Nup1922, Nup1923, Nup1924, Nup1925, Nup1926, Nup1927, Nup1928, Nup1929, Nup1930, Nup1931, Nup1932, Nup1933, Nup1934, Nup1935, Nup1936, Nup1937, Nup1938, Nup1939, Nup1940, Nup1941, Nup1942, Nup1943, Nup1944, Nup1945



Run gene ontology

```
data(go.sets.hs)
data(go.subs.hs)

# Let us focus on just the biological processes of GO
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets, same.dir=TRUE)

head(gobpres$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	expl
G0:0048285	organelle fission	5.841698e-12	376	1.536227e-15
G0:0000280	nuclear division	5.841698e-12	352	4.286961e-15
G0:0007067	mitosis	5.841698e-12	352	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.195672e-11	362	1.169934e-14
G0:0007059	chromosome segregation	1.658603e-08	142	2.028624e-11
G0:0000236	mitotic prometaphase	1.178402e-07	84	1.729553e-10