

Class 13: RNA-Seq analysis mini-project

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Data Import

The data for the 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.

```
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 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
```

```
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 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
countDataTmp = read.csv(countFile, row.names=1)
head(countDataTmp)
```

	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 count-Data

```
# Note we need to remove the odd first $length col
countData <- as.matrix(countDataTmp[, -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 that my metadata and count data match

```
rownames(colData) == colnames(countData)
```

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

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).

```
#head(countData)

to.keep <- rowSums(countData) != 0
countData <- countData[to.keep,]

nrow(countData)
```

```
[1] 15975
```

```
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

```
#DESeq analysis
```

```
library(DESeq2)
```

```
head(colData)
```

```
          condition
SRR493366 control_sirna
SRR493367 control_sirna
SRR493368 control_sirna
SRR493369      hoxa1_kd
SRR493370      hoxa1_kd
SRR493371      hoxa1_kd
```

Setup the object that DESeq needs for analysis with the lovely long -winded function:

```
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

Run the analysis

```
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)
```

```
res
```

log2 fold change (MLE): condition hoxa1 kd vs control sirna

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

DataFrame with 15975 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
...
ENSG00000273748	35.30265	0.674387	0.303666	2.220817	2.63633e-02
ENSG00000278817	2.42302	-0.388988	1.130394	-0.344117	7.30758e-01
ENSG00000278384	1.10180	0.332991	1.660261	0.200565	8.41039e-01
ENSG00000276345	73.64496	-0.356181	0.207716	-1.714752	8.63908e-02
ENSG00000271254	181.59590	-0.609667	0.141320	-4.314071	1.60276e-05
	padj				
	<numeric>				
ENSG00000279457	6.86555e-01				
ENSG00000187634	5.15718e-03				
ENSG00000188976	1.76549e-35				
ENSG00000187961	1.13413e-07				
ENSG00000187583	9.19031e-01				
...	...				
ENSG00000273748	4.79091e-02				
ENSG00000278817	8.09772e-01				
ENSG00000278384	8.92654e-01				
ENSG00000276345	1.39762e-01				
ENSG00000271254	4.53648e-05				

log2 fold change (MLE): condiditon hoxa1 kd vs control sirna

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

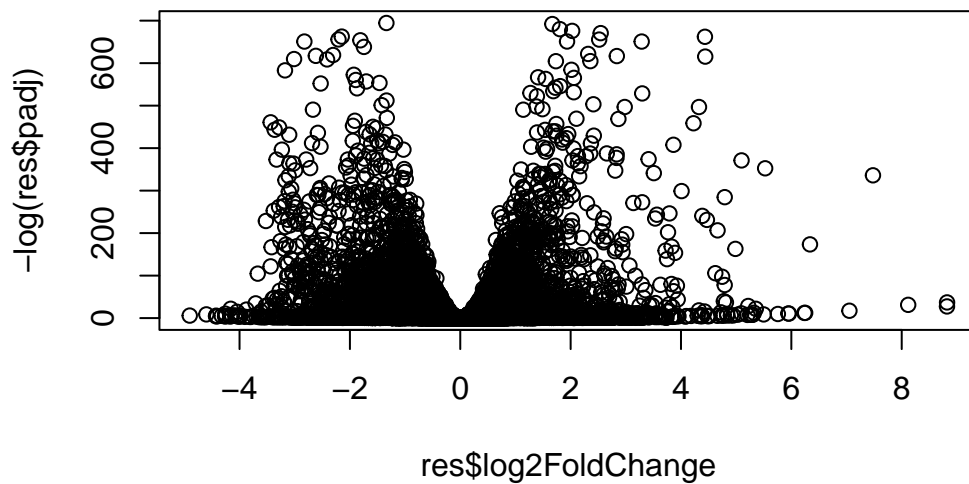
Q3. Call the `summary()` function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
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
```

Volcano plot

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

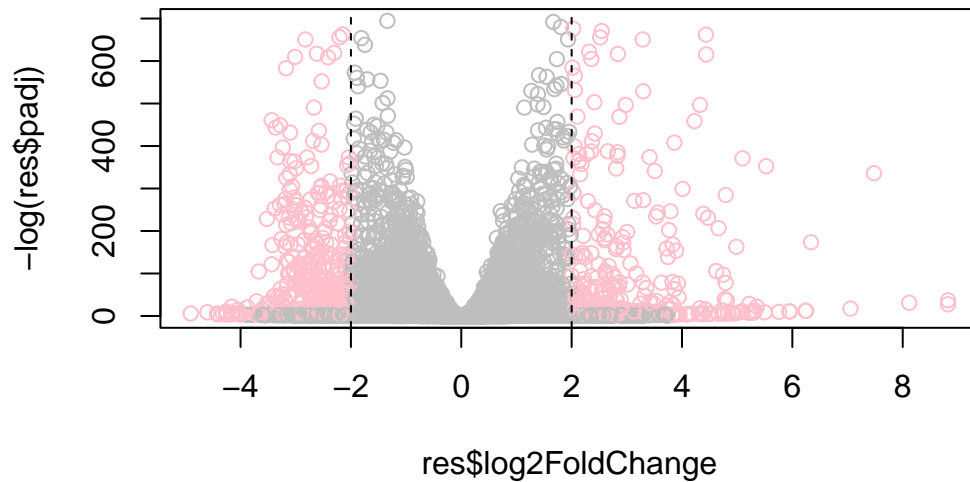


Make some colors to highlight the subset of genes with high fold change values

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

```
mycols <- rep("grey", nrow(res))
mycols[abs(res$log2FoldChange)>2] <- "pink"
mycols[res$padj>0.05]="grey"

plot(res$log2FoldChange, -log(res$padj), col=mycols)
abline(v=c(-2,2), lty=2)
```



Q5. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

Add gene annotation data

We will load up AnnotationDbi and our Human data package to add gene symbols and entrez IDs to our results objects.

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

Genset enrichment analysis (pathway analysis)

```
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=rownames(res),  
  keytype="ENSEMBL",  
  column="SYMBOL"  
)
```

'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

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

'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")
```

KEGG Pathway

```
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"

```

```
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>).

```
#####
```

```
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"
```

```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$less)[1:5]
```

```
# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

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

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

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

```
Info: Working in directory /Users/danikapagaduan/Desktop/BIMM 143 R Studio/class13
```

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

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

```
Info: Working in directory /Users/danikapagaduan/Desktop/BIMM 143 R Studio/class13
```

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

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

```
Info: Working in directory /Users/danikapagaduan/Desktop/BIMM 143 R Studio/class13
```

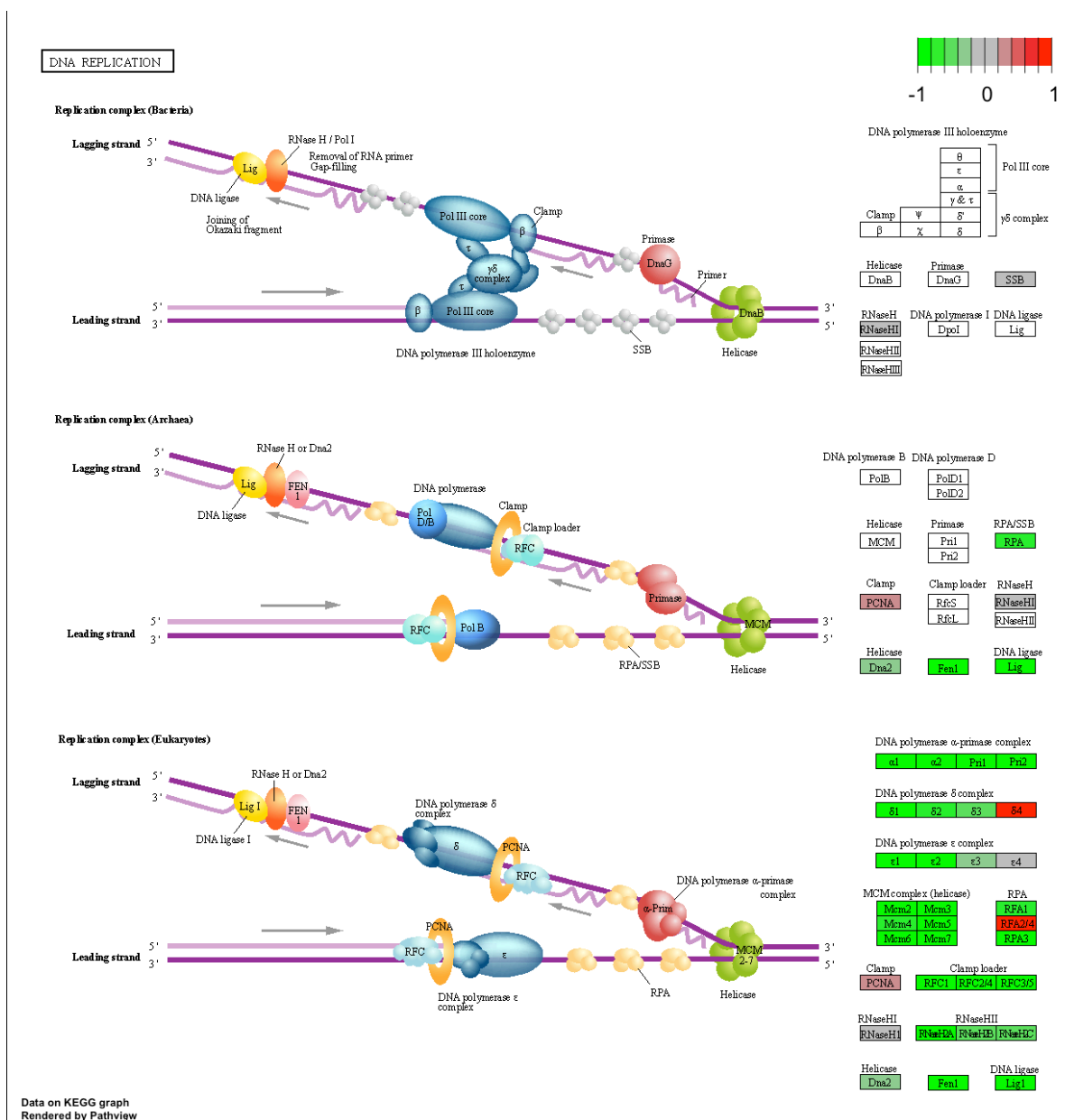
```
Info: Writing image file hsa03013.pathview.png
```

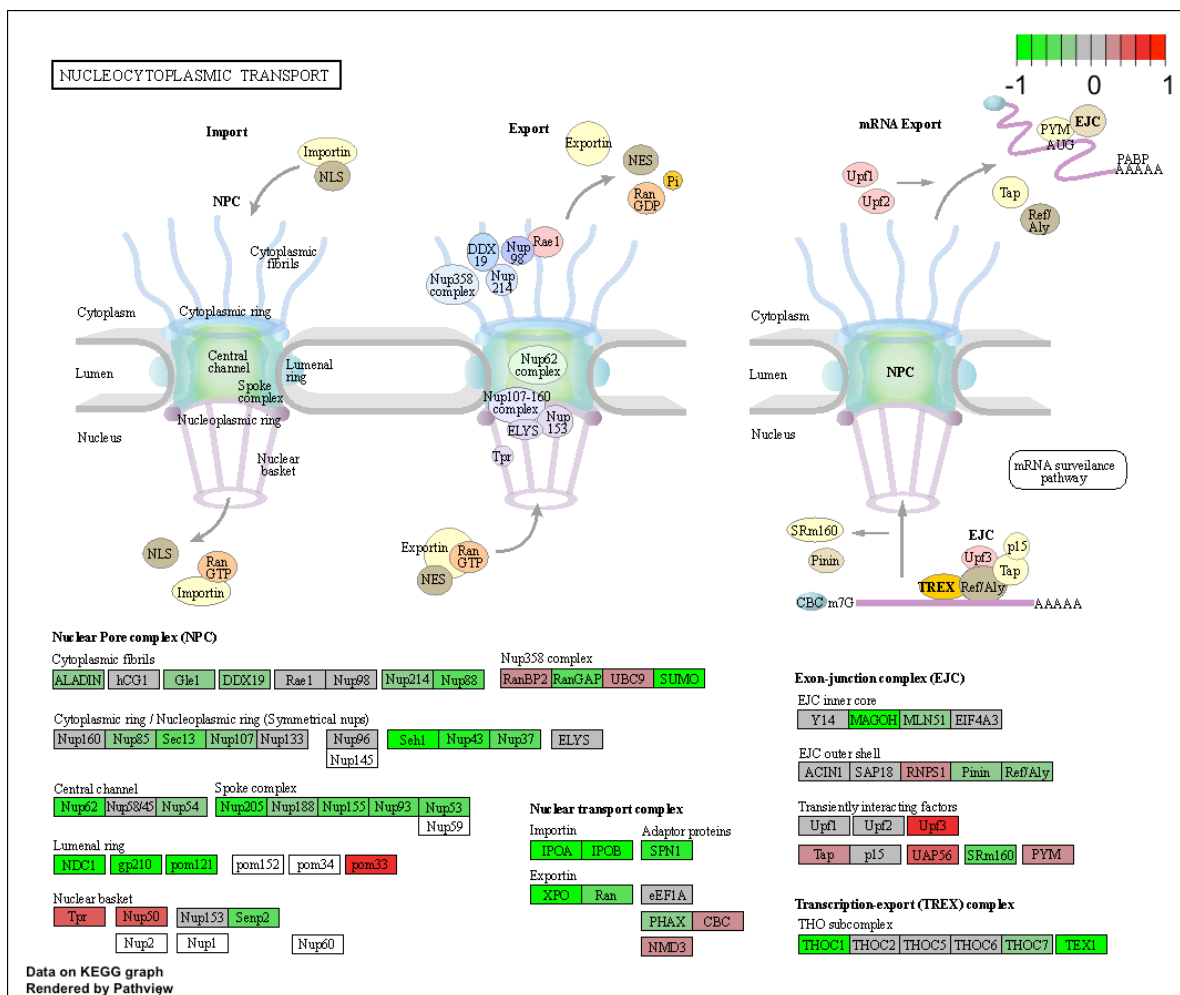
```
'select()' returned 1:1 mapping between keys and columns
```

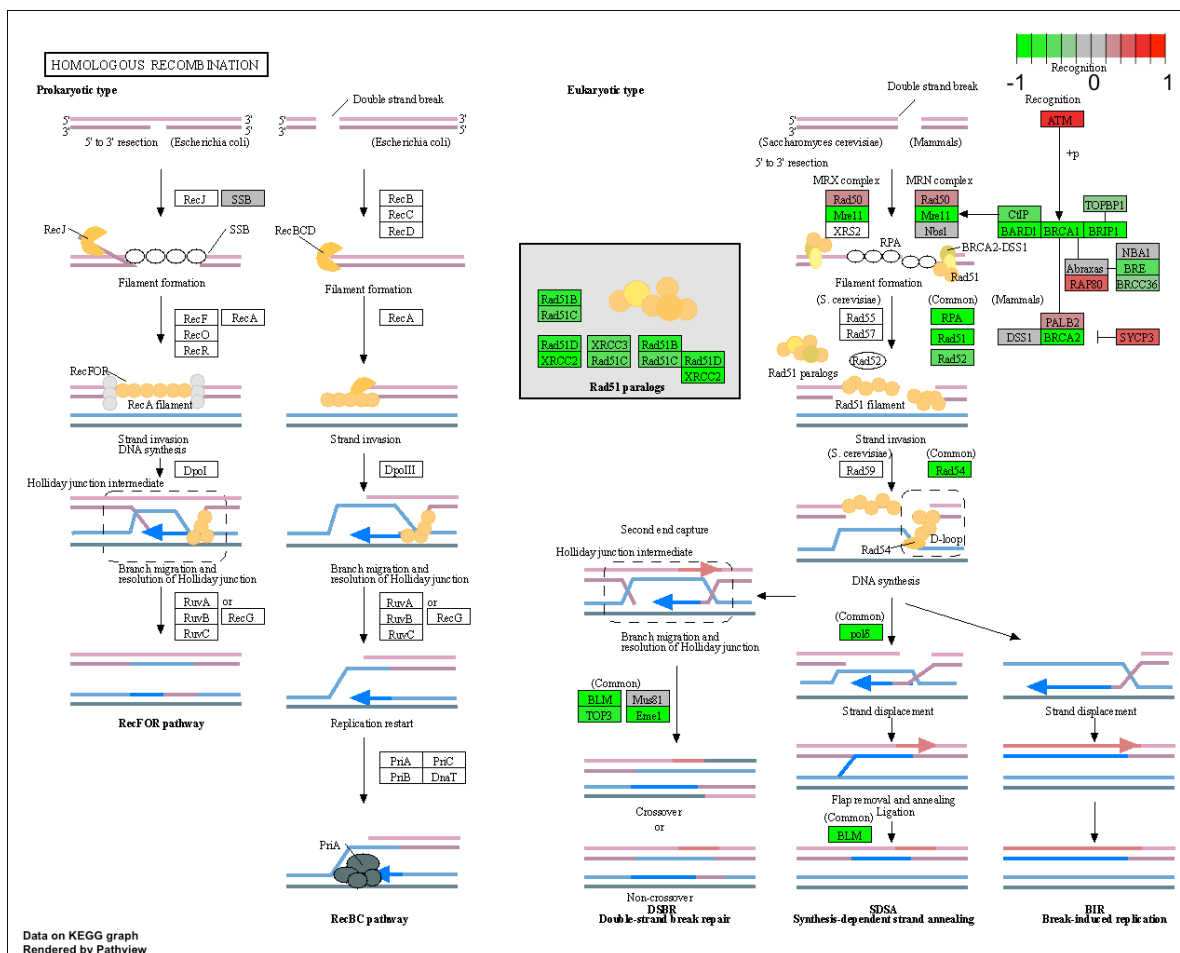
```
Info: Working in directory /Users/danikapagaduan/Desktop/BIMM 143 R Studio/class13
```

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

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





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	2.195494e-04	3.530241	2.195494e-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	0.1951953	113	8.519724e-05
G0:0002009	morphogenesis of an epithelium	0.1951953	339	1.396681e-04
G0:0048729	tissue morphogenesis	0.1951953	424	1.432451e-04
G0:0007610	behavior	0.2243795	427	2.195494e-04
G0:0060562	epithelial tube morphogenesis	0.3711390	257	5.932837e-04
G0:0035295	tube development	0.3711390	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	5.841698e-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

\$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	3.530241	3.530241
G0:0060562	epithelial tube morphogenesis	3.261376	3.261376
G0:0035295	tube development	3.253665	3.253665