

Class14: RNA-Seq analysis mini-project

Sion Kang (PID: A17052234)

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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

```
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, 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

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

Inspect and tidy data

Does the counts columns match 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

```

```
colData$id
```

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

```
colnames(counts)
```

```

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

```

The fix here looks to be removing the first “length” column from counts

```

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?

```
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?

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

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

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

```
[1] 15975
```

Setup for DESeq

```
library(DESeq2)
```

Setup input object 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				

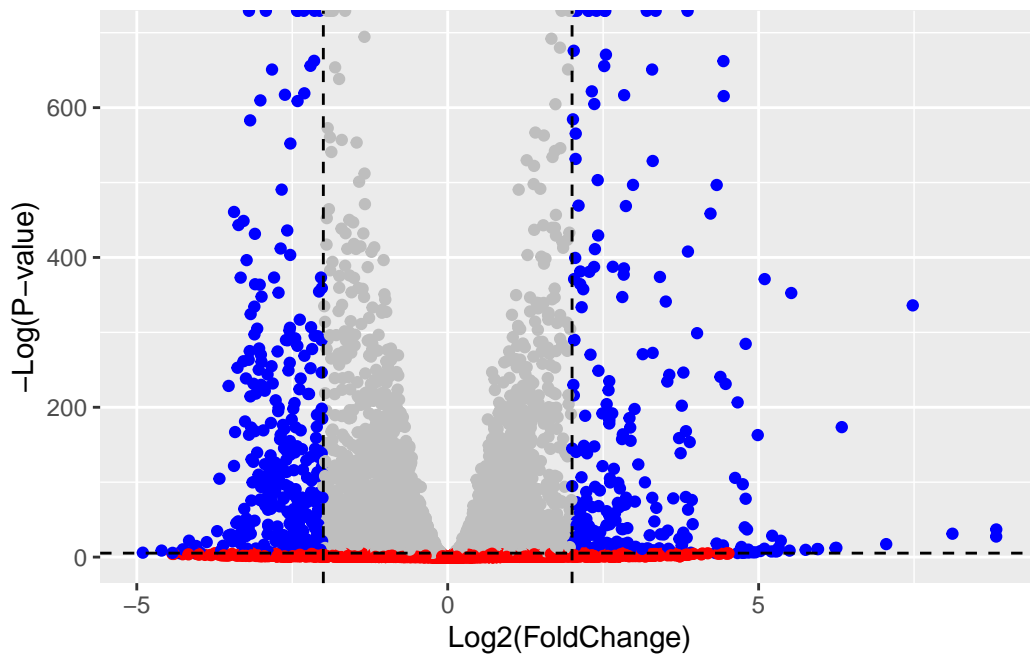
Volcano plot of results

```
library(ggplot2)
```

```
mycols <- rep("gray", nrow(res))  
mycols[abs (res$log2FoldChange) >= 2] <- "blue"  
mycols[res$padj > 0.005] <- "red"
```

```
ggplot(res) +
  aes(log2FoldChange, -log(padj)) +
  geom_point(col=mycols) +
  xlab("Log2(FoldChange)") + ylab("-Log(P-value)") +
  geom_vline(xintercept = c(-2,2), linetype="dashed", col="black")+
  geom_hline(yintercept= -log(0.005), 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"
```

```
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

```
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 8 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	entrez		
	<numeric>	<character>	<character>		
ENSG00000279457	6.86555e-01	NA	NA		

ENSG00000187634	5.15718e-03	SAMD11	148398
ENSG00000188976	1.76549e-35	NOC2L	26155
ENSG00000187961	1.13413e-07	KLHL17	339451
ENSG00000187583	9.19031e-01	PLEKHN1	84069
ENSG00000187642	4.03379e-01	PERM1	84808

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).
```

```
#####
```

Input vector for `gage()`

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
```

Load up the KEGG genesets

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

Run pathway analysis

```
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	1.246882e-03	-3.059466
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	1.246882e-03	0.065461279
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	1.246882e-03
hsa03440 Homologous recombination	28	3.066756e-03
hsa04114 Oocyte meiosis	102	3.784520e-03

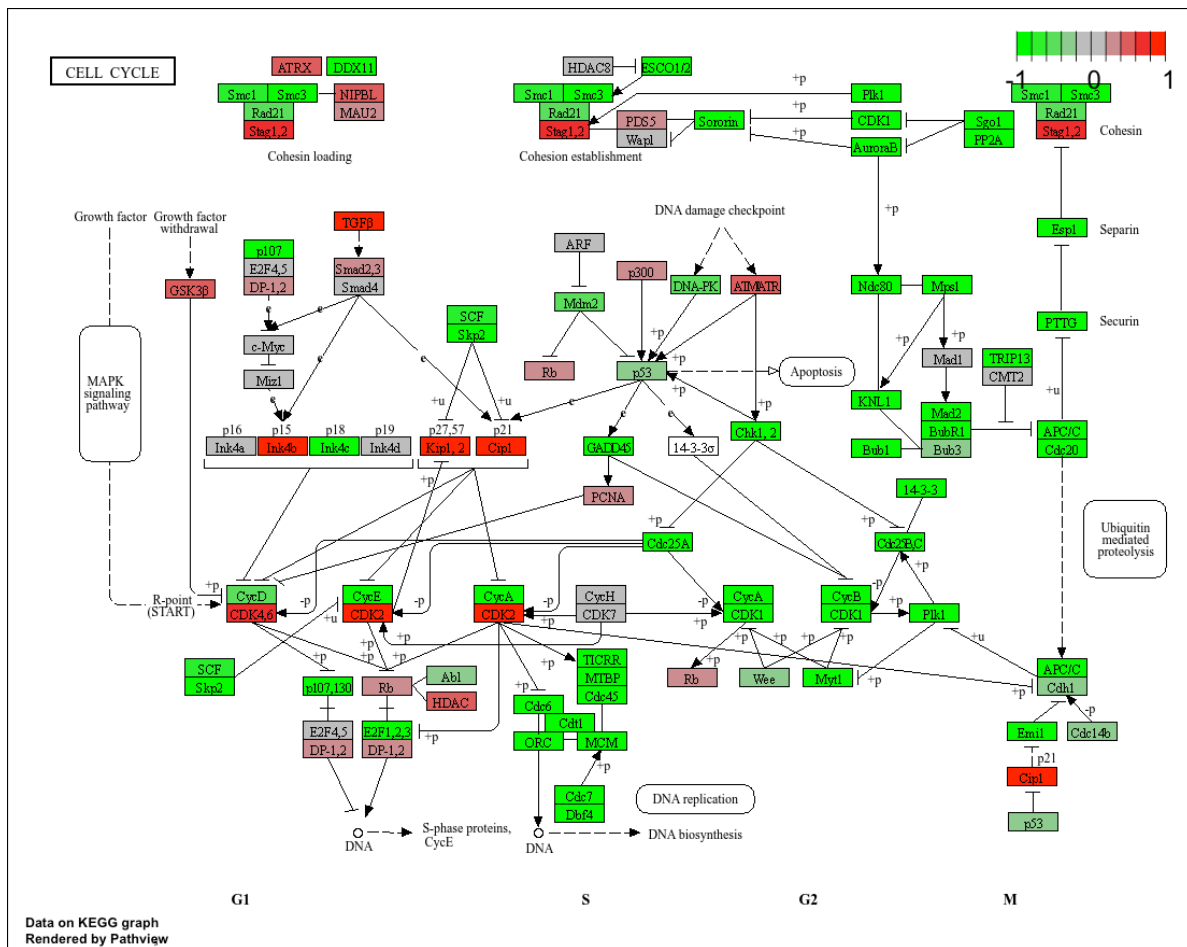
Cell cycle figure

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

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

Info: Working in directory /Users/shannonkang/Documents/bimm143/class14

Info: Writing image file hsa04110.pathview.png

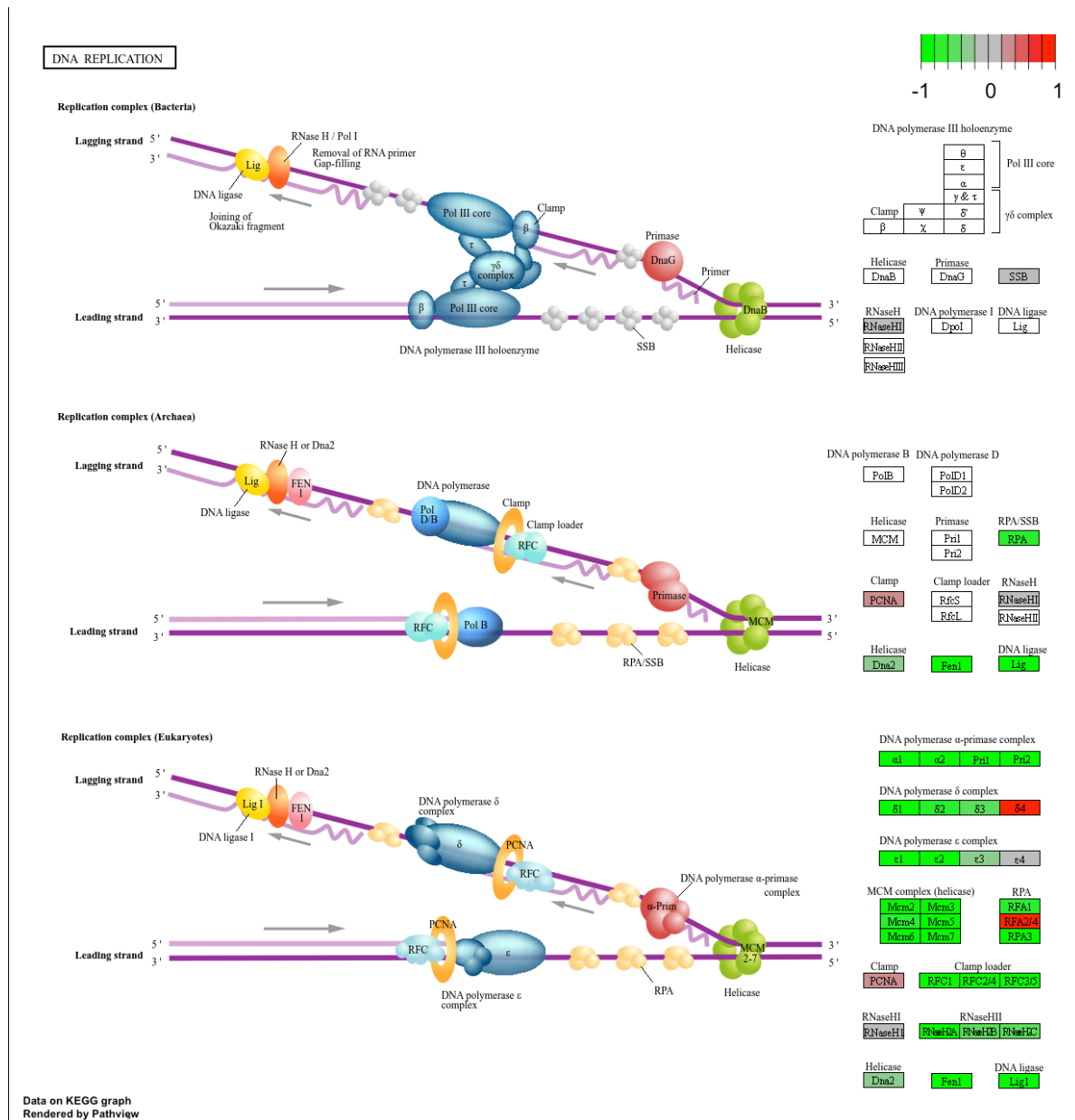


```
pathview(foldchanges, pathway.id= "hsa03030")
```

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

Info: Working in directory /Users/shannonkang/Documents/bimm143/class14

Info: Writing image file hsa03030.pathview.png



`head(keggres$greater)`

	p.geomean	stat.mean
hsa04060 Cytokine-cytokine receptor interaction	9.131044e-06	4.358967
hsa05323 Rheumatoid arthritis	1.809824e-04	3.666793
hsa05146 Amoebiasis	1.313400e-03	3.052596
hsa05332 Graft-versus-host disease	2.605234e-03	2.948229

hsa04640	Hematopoietic cell lineage	2.822776e-03	2.833362
hsa04630	Jak-STAT signaling pathway	5.202070e-03	2.585673
		p.val	q.val
hsa04060	Cytokine-cytokine receptor interaction	9.131044e-06	0.001917519
hsa05323	Rheumatoid arthritis	1.809824e-04	0.019003147
hsa05146	Amoebiasis	1.313400e-03	0.091937999
hsa05332	Graft-versus-host disease	2.605234e-03	0.118556573
hsa04640	Hematopoietic cell lineage	2.822776e-03	0.118556573
hsa04630	Jak-STAT signaling pathway	5.202070e-03	0.182072434
		set.size	exp1
hsa04060	Cytokine-cytokine receptor interaction	177	9.131044e-06
hsa05323	Rheumatoid arthritis	72	1.809824e-04
hsa05146	Amoebiasis	94	1.313400e-03
hsa05332	Graft-versus-host disease	22	2.605234e-03
hsa04640	Hematopoietic cell lineage	55	2.822776e-03
hsa04630	Jak-STAT signaling pathway	109	5.202070e-03

```
pathview(foldchanges, pathway.id= "hsa04060")
```

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

Info: Working in directory /Users/shannonkang/Documents/bimm143/class14

Info: Writing image file hsa04060.pathview.png

```
pathview(foldchanges, pathway.id= "hsa05323")
```

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

Info: Working in directory /Users/shannonkang/Documents/bimm143/class14

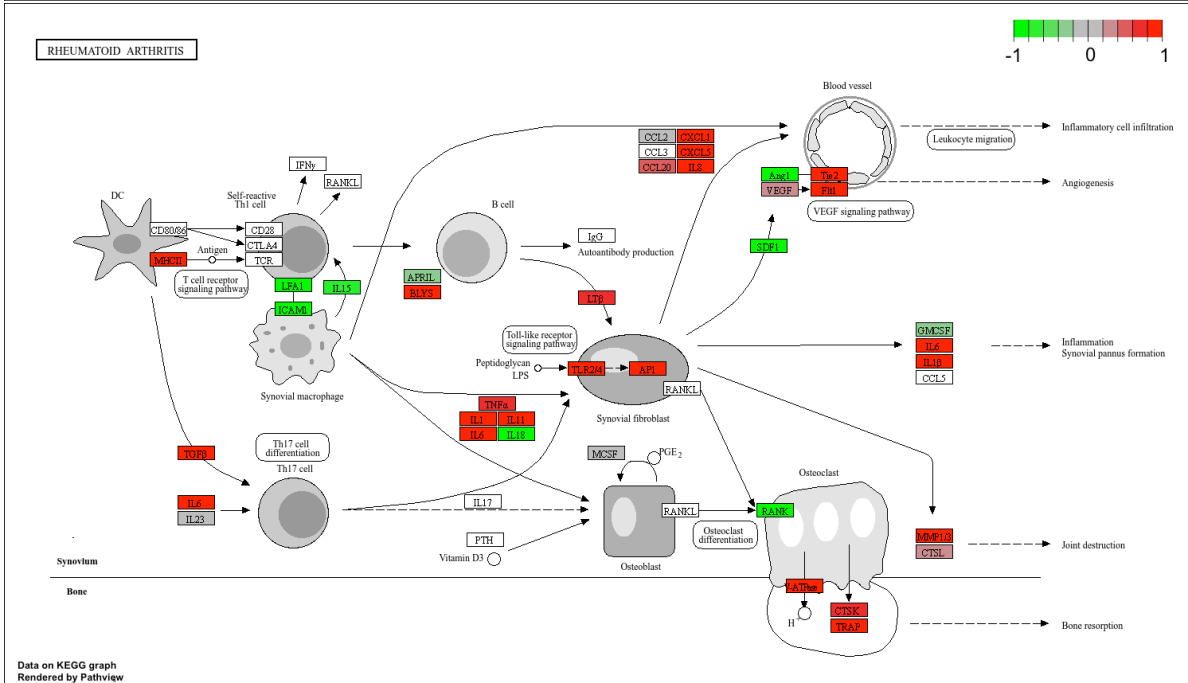
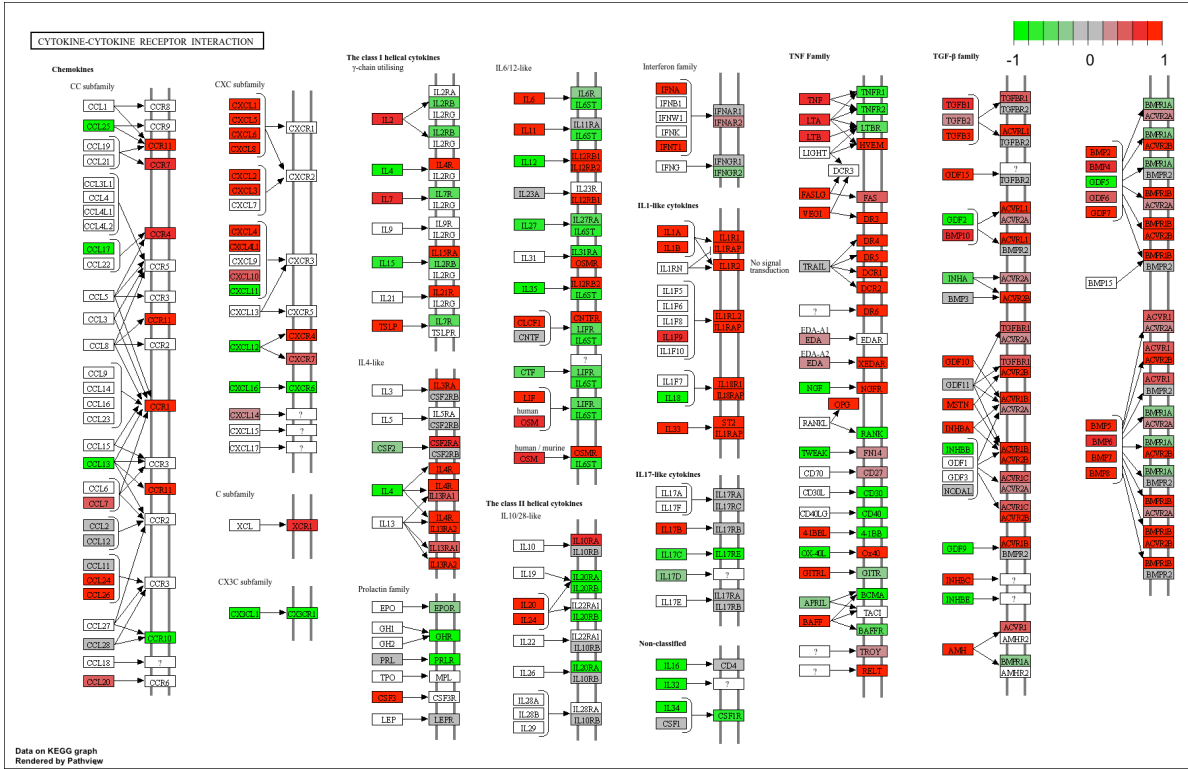
Info: Writing image file hsa05323.pathview.png

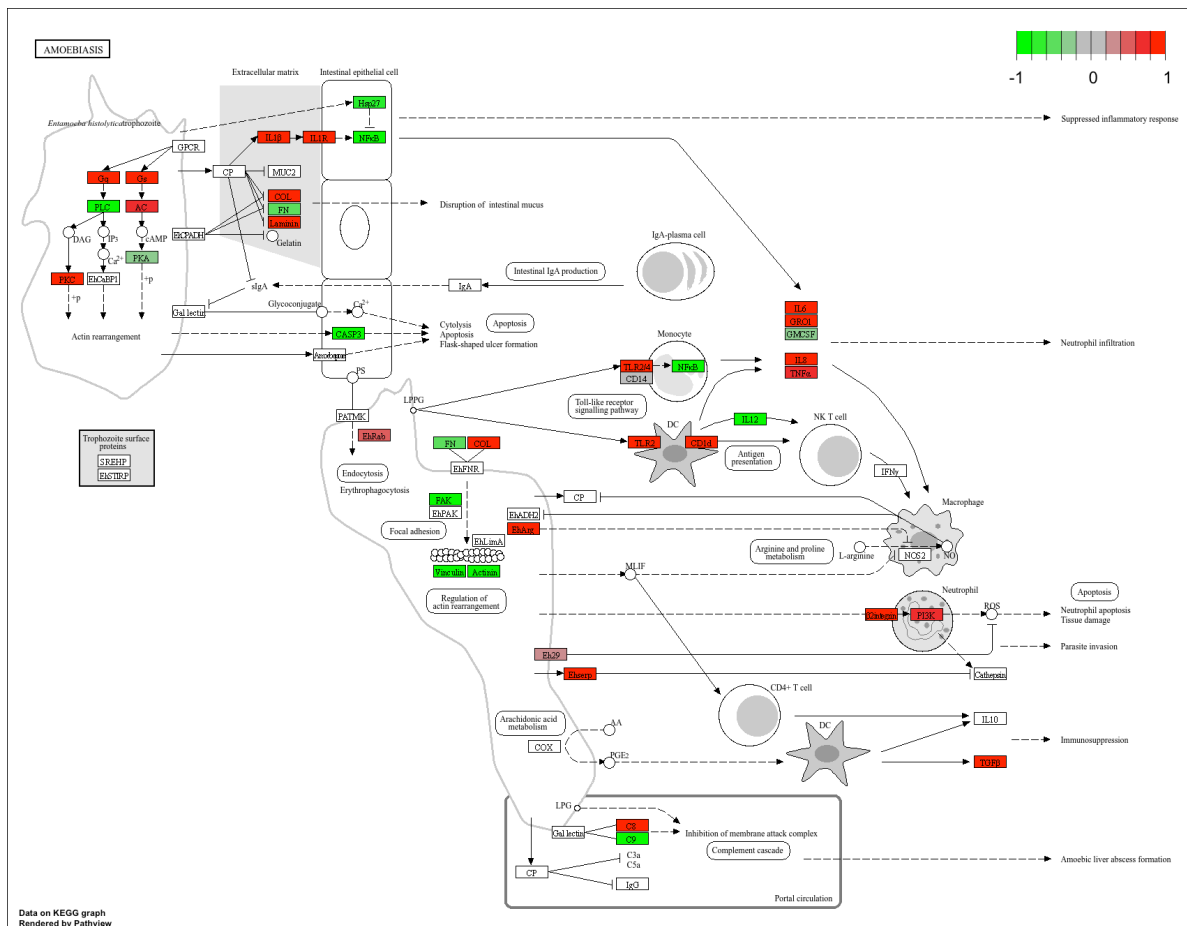
```
pathview(foldchanges, pathway.id= "hsa05146")
```

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

Info: Working in directory /Users/shannonkang/Documents/bimm143/class14

Info: Writing image file hsa05146.pathview.png





Gene Ontology analysis

Run pathway analysis with GO

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

# Focus on Biological Process subset 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	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