Class14: RNASeq mini project

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Background

The data for today 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.

Data Import

Readding in the counts and the metadata

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

Tidy and verefy data

Q. How many genes are in this dataset?

```
nrow(counts)
```

```
[1] 19808
```

Q. How many control and kd experiments are there?

```
table( metadata$condition )
```

```
control_sirna      hoxa1_kd
            3             3
```

Q. Does the `metadata` match the `countdata`

```
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
```

```
colnames(counts)
```

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

```
metadata$id
```

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

Fix countdata to match coldata/metadata

```
newcounts <- counts[,-1]
dim(newcounts)
```

```
[1] 19808     6
```

```
colnames(newcounts) == metadata$id
```

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

Remove zero count genes

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

PCA quality control

We can use `prcomp()` function.

```
pc <- prcomp( t(countData), scale=T)
summary(pc)
```

```
Importance of components:
                           PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 7.373e-13
Proportion of Variance  0.4817  0.3365  0.06774  0.06074  0.05332 0.000e+00
Cumulative Proportion   0.4817  0.8182  0.88594  0.94668  1.00000 1.000e+00
```

Color by "control" (blue) or "kd" (red)

```
metadata$condition
```

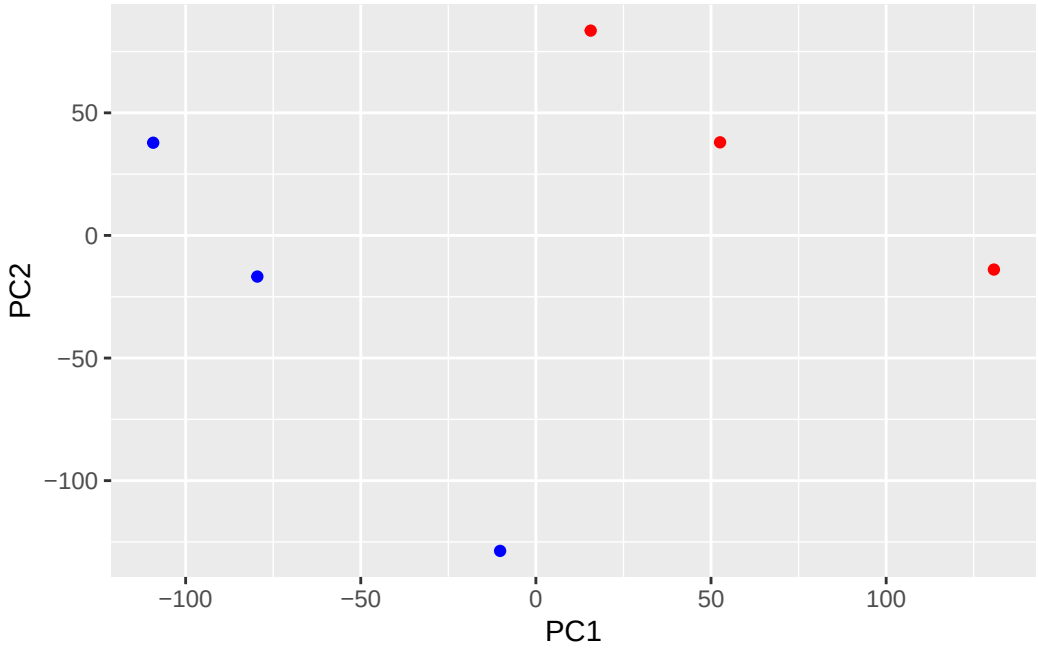
```
[1] "control_sirna" "control_sirna" "control_sirna" "hoxa1_kd"
[5] "hoxa1_kd"      "hoxa1_kd"
```

```
mycols <- c( rep("blue",3), rep("red",3) )
mycols
```

```
[1] "blue" "blue" "blue" "red"  "red"  "red"
```

```
library(ggplot2)

ggplot(pc$x) +
  aes(PC1, PC2) +
  geom_point(col=mycols)
```



Q. How many genes do we have left after filtering?

```
nrow(countData)
```

```
[1] 15975
```

DESeq analysis

```
library(DESeq2)
```

Setup the DESeq input object

```
dds <- DESeqDataSetFromMatrix(countData = countData,
                              colData = metadata,
                              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
```

Extract results

```
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 1.43990e-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
```

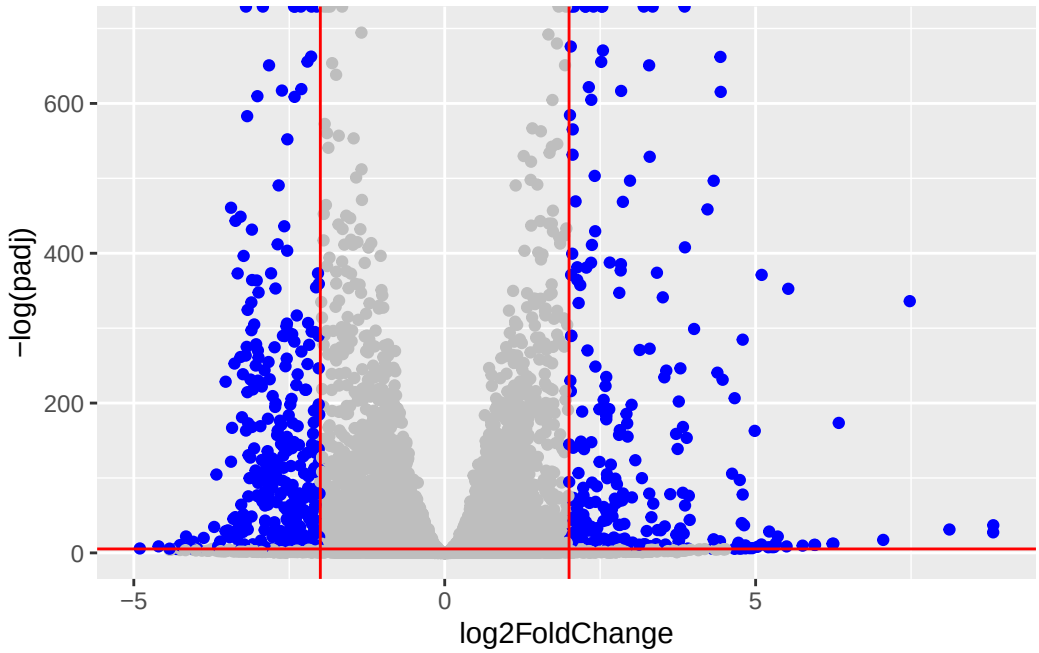
Volcano plot

A plot of log2 Fold-change vs -log of adjusted P-value with custom colors

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

```
ggplot(res) +
  aes(log2FoldChange, -log(padj)) +
  geom_point(col=mycols) +
  geom_vline(xintercept = c(-2,2), col="red") +
  geom_hline(yintercept = -log(0.005), col="red")
```

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



Add 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
```

Save results

```
write.csv(res, file="myresults.csv")
```

Pathway analysis

```
#/ message: false
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).
##############################################################################
```

KEGG

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

```
head(kegg.sets.hs, 1)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"
```

input vector for `gage()` called `foldchanges()` that has `names()` attribute set to ENTREZIDs.

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

```
# Get the results
keggres <- gage(foldchanges, gsets=kegg.sets.hs)
```

```
attributes(keggres)
```

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

```
# Look at the first few down (less) pathways
head(keggres$less, 2)
```

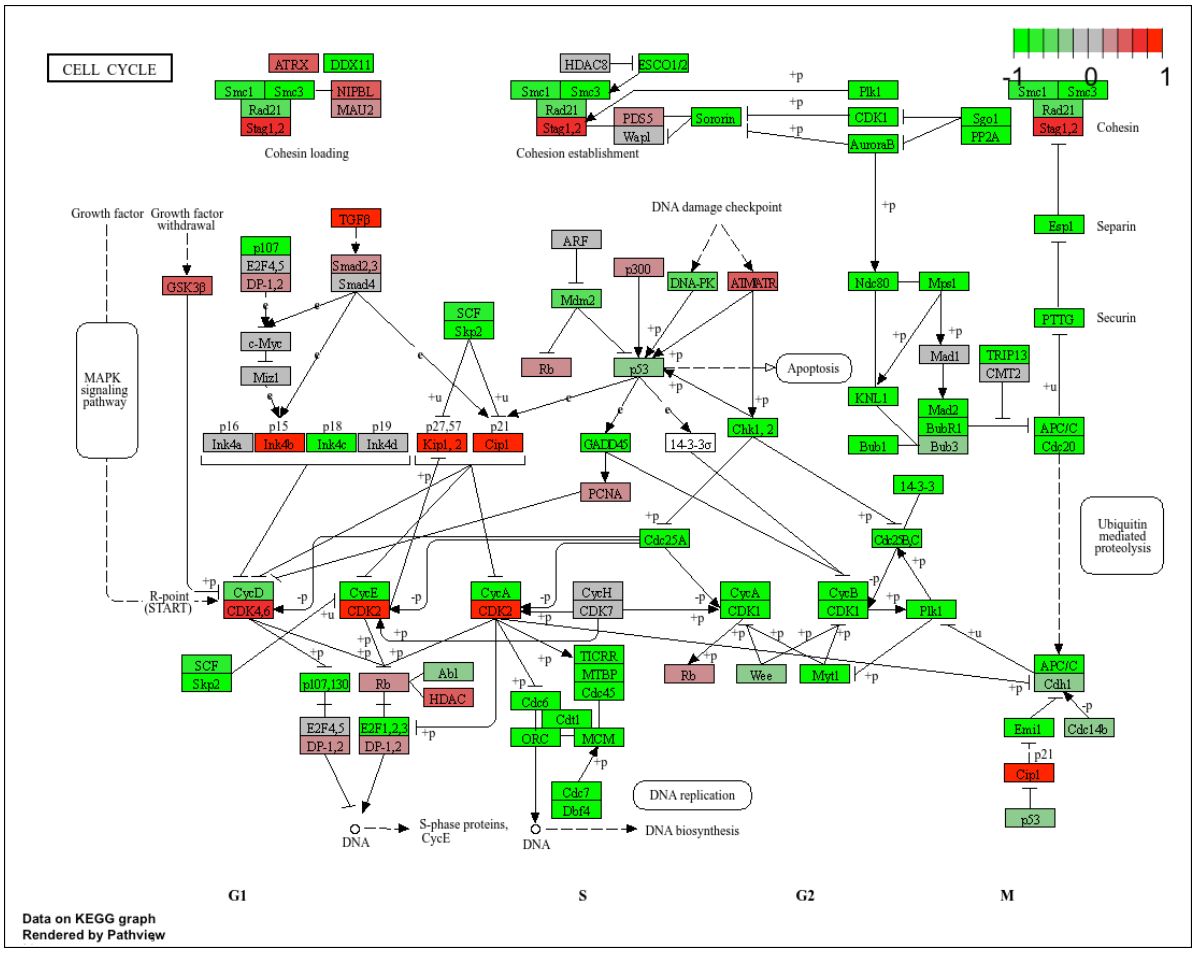
```
                           p.geomean stat.mean        p.val       q.val
hsa04110 Cell cycle      8.995727e-06 -4.378644 8.995727e-06 0.001889103
hsa03030 DNA replication 9.424076e-05 -3.951803 9.424076e-05 0.009841047
                         set.size         exp1
hsa04110 Cell cycle           121 8.995727e-06
hsa03030 DNA replication       36 9.424076e-05
```

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

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

```
Info: Working in directory /Users/shivanilakkaraju/Desktop/bggn 213/class 14

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

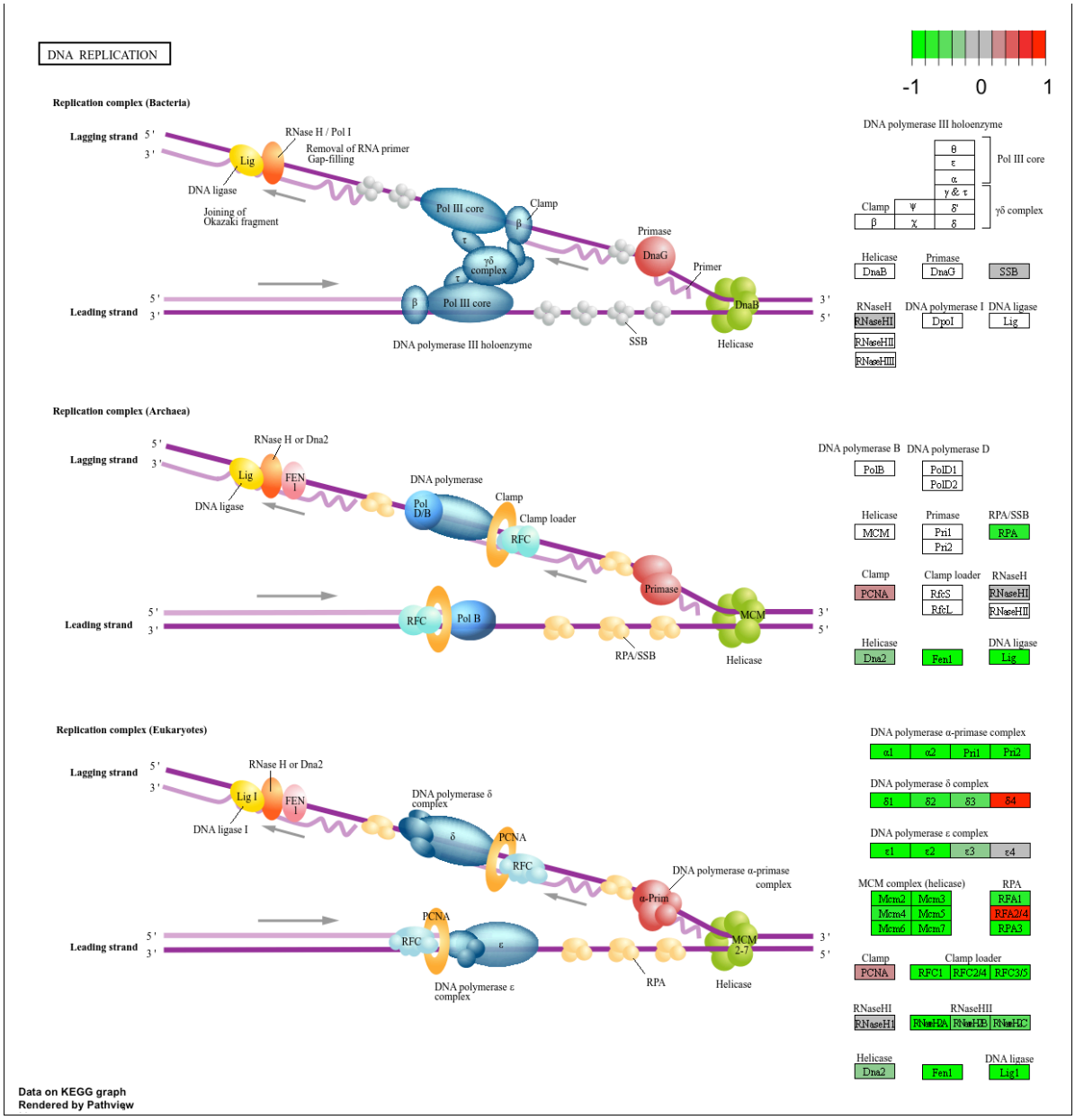



```
pathview(gene.data=foldchanges, pathway.id="hsa03030")
```

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

Info: Working in directory /Users/shivanilakkaraju/Desktop/bggn 213/class 14

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




```
head(keggres$greater, 2)
```

```
                                                    p.geomean stat.mean
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06  4.358967
hsa05323 Rheumatoid arthritis                   1.809824e-04  3.666793
                                                        p.val       q.val
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06 0.001917519
```

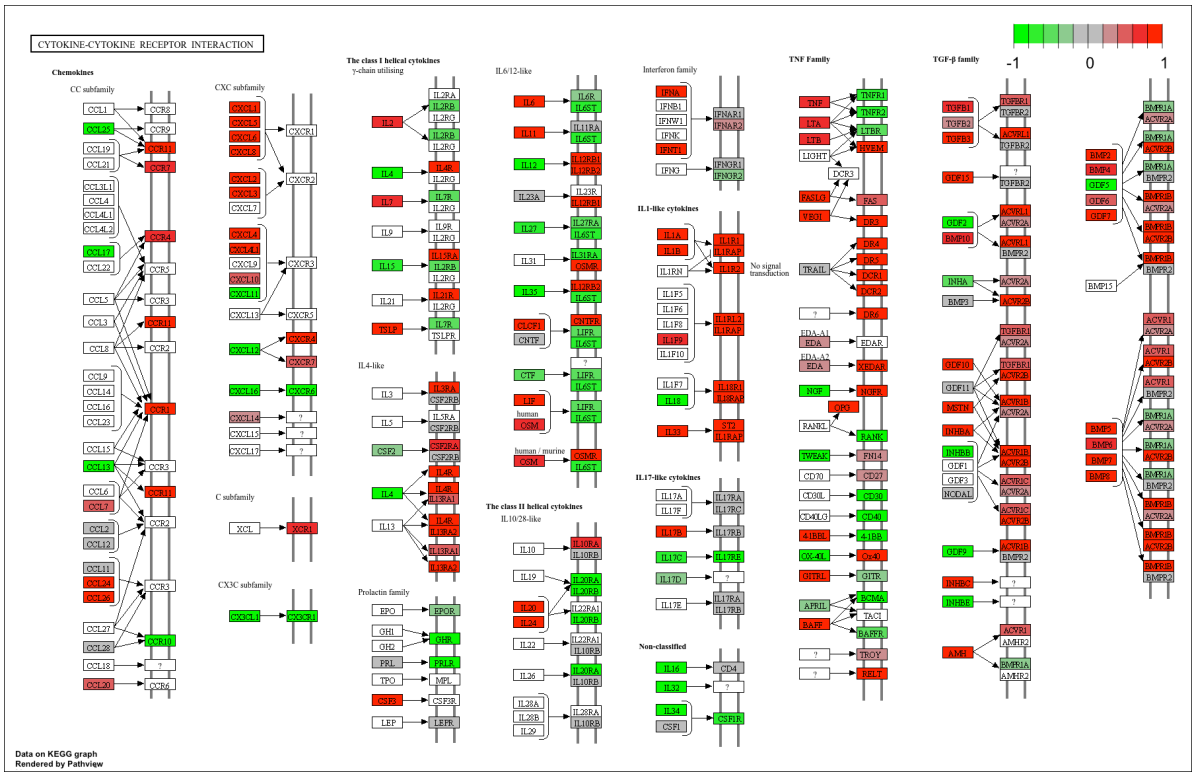
```
hsa05323 Rheumatoid arthritis                    1.809824e-04 0.019003147
                                                 set.size         exp1
hsa04060 Cytokine-cytokine receptor interaction      177 9.131044e-06
hsa05323 Rheumatoid arthritis                         72 1.809824e-04
```

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

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

```
Info: Working in directory /Users/shivanilakkaraju/Desktop/bggn 213/class 14
```

```
Info: Writing image file hsa04060.pathview.png
```

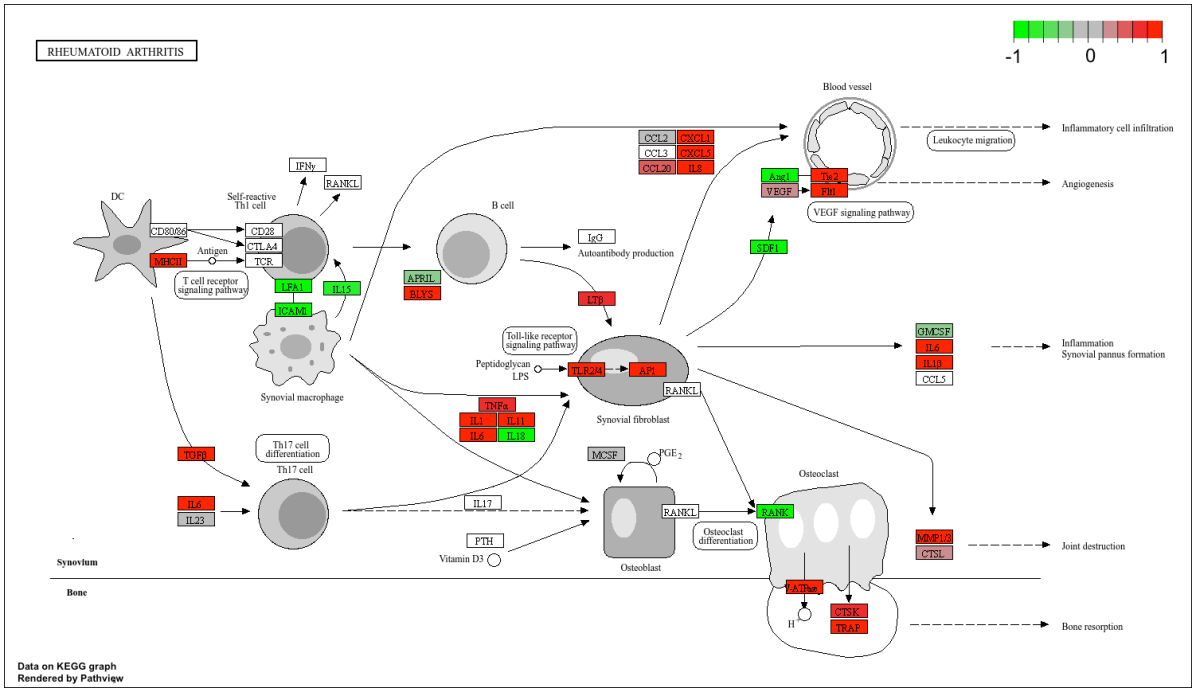



```
pathview(gene.data=foldchanges, pathway.id="hsa05323")
```

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

```
Info: Working in directory /Users/shivanilakkaraju/Desktop/bggn 213/class 14
```

```
Info: Writing image file hsa05323.pathview.png
```



GO gene ontology

```
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
GO:0048285 organelle fission            1.536227e-15 -8.063910 1.536227e-15
GO:0000280 nuclear division             4.286961e-15 -7.939217 4.286961e-15
GO:0007067 mitosis                      4.286961e-15 -7.939217 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
```

```
GO:0007059 chromosome segregation        2.028624e-11 -6.878340 2.028624e-11
GO:0000236 mitotic prometaphase          1.729553e-10 -6.695966 1.729553e-10
                                                q.val set.size         exp1
GO:0048285 organelle fission             5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division              5.841698e-12      352 4.286961e-15
GO:0007067 mitosis                       5.841698e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.658603e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase          1.178402e-07       84 1.729553e-10
```

Reactome

```
inds <- abs(res$log2FoldChange) >= 2 & res$padj <= 0.05
top.genes <- res$entrez[inds]
```

```
write.table(top.genes, file="top_genes.txt", row.names=FALSE, col.names=FALSE, quote=FALSE)
```