# Class 14: RNAseq Project

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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

## Data import

Reading in the counts and metadata

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

Q. How many genes are in the dataset?

```
nrow(counts)
```

```
[1] 19808
```

Q. How any control and knockdown experiment are there?

```
head(metadata)
```

```
         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
```

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

```
control_sirna      hoxa1_kd
            3             3
```

## Tidy and verify data

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
```

The length column is a problem

```
colnames(counts)
```

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

```
metadata$id
```

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

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

```
[1] 19808     7
```

Make sure the column names of the countsdata matches the id's listed in the metadata

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

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

### Remove 0 count genes

```
#Sum each row and select the rows that don't sum to zero (!= is the opposite of ++)
to.keep <- rowSums(newcounts) != 0

# Keep the gene rows that don't sum to 0
countData <- newcounts[to.keep, ]
```

## PCA quality control

We can use ur `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 7.387e-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 knockdown(red)

```
metadata$condition
```

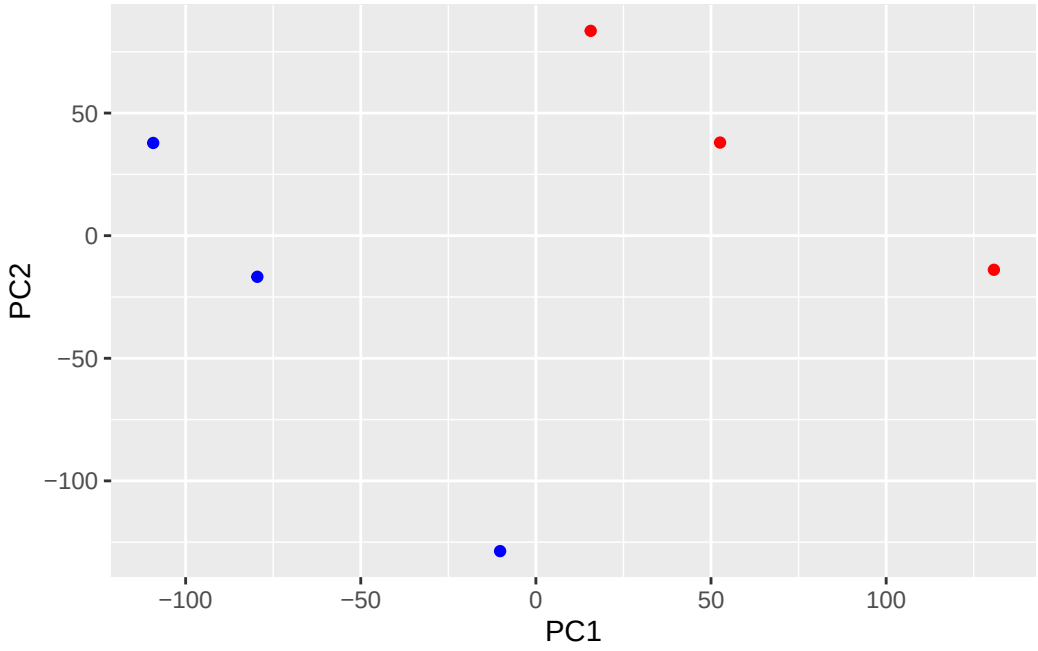
```
[1] "control_sirna" "control_sirna" "control_sirna" "hoxa1_kd"
[5] "hoxa1_kd"      "hoxa1_kd"
```

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

Plot the pca

```
library(ggplot2)

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




Q. How many genes are left after filtering out the 0's?

```
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 the DESeq

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

### Extract the results

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

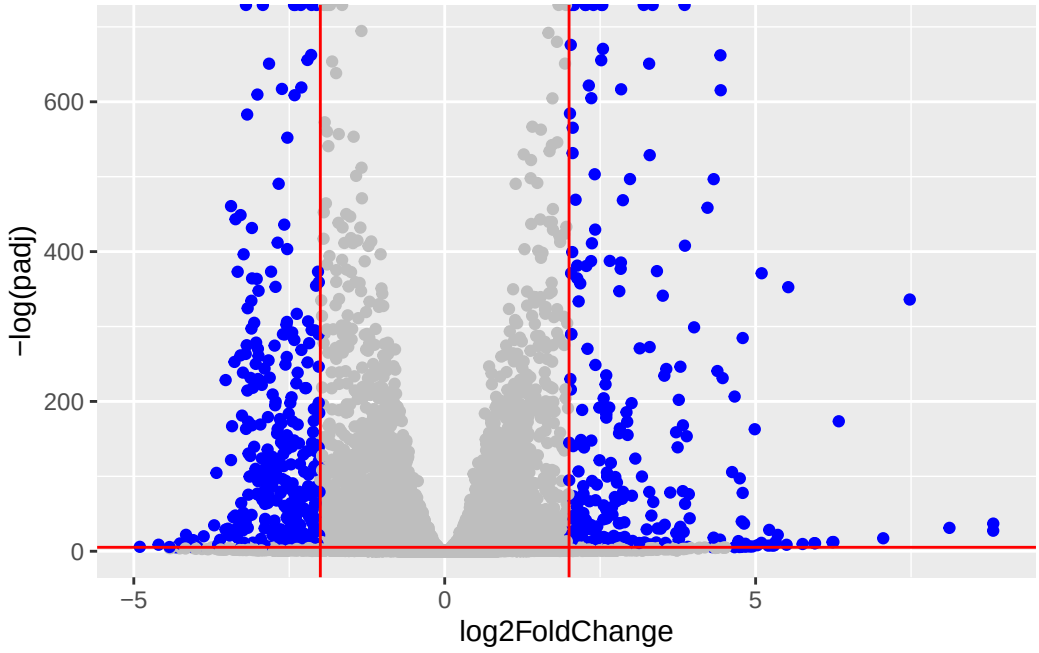
## Volcano Plot

Plot log2 Fold-Change vs -log of adjusted p value with custom colors

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

```
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

We want to add SYMBOL and ENTREZID values to our results object

```
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"
```

```
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
```

our res dataset gene names are in ENSEMBL format

```
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

```
#|mmessage: 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)
```

These are the ENTREZID nammes of genes involved in caffeine metabolism pathway

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

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

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

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

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

```
attributes(keggres)
```

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

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

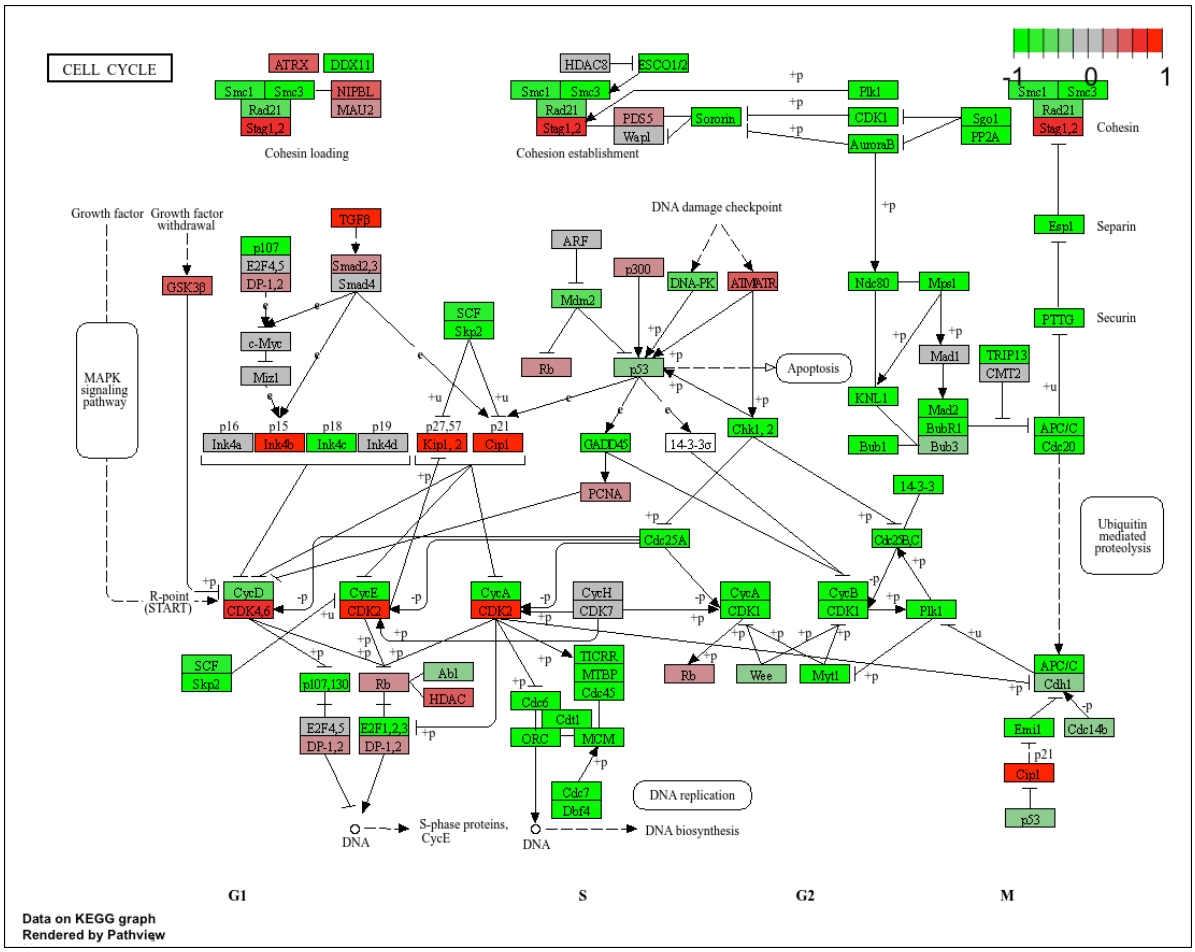
```
                          p.geomean stat.mean        p.val       q.val
hsa04110 Cell cycle      8.995727e-06 -4.378644 8.995727e-06 0.001889103
hsa03030 DNA replication 9.424076e-05 -3.951803 9.424076e-05 0.009841047
                         set.size         exp1
hsa04110 Cell cycle           121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

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

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

```
Info: Working in directory /Users/katiemostoller/Desktop/Class 14
```

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

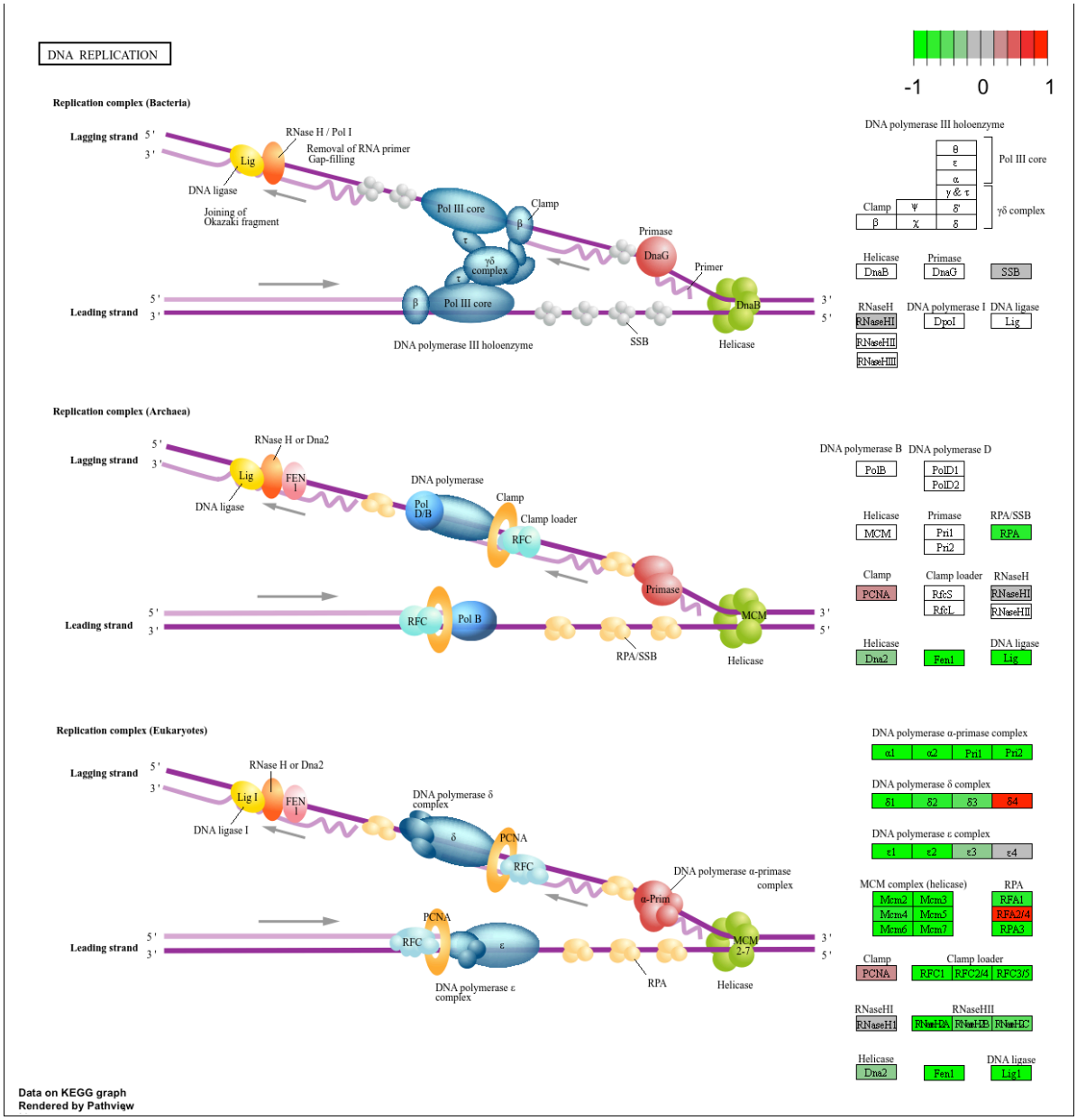



```
pathview(foldchanges, pathway.id = "hsa03030")
```

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

```
Info: Working in directory /Users/katiemostoller/Desktop/Class 14
```

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




```
head(keggres$greater)
```

```
                                                      p.geomean stat.mean
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06  4.358967
```

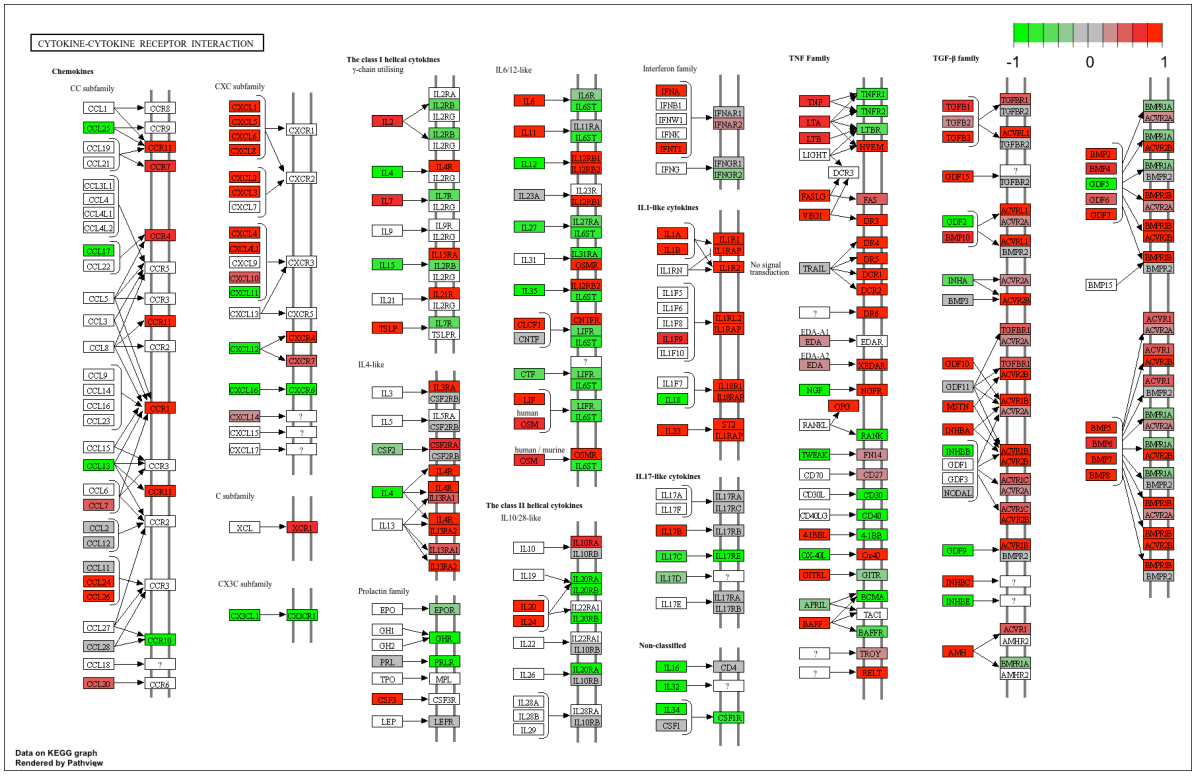
```
hsa05323 Rheumatoid arthritis                    1.809824e-04  3.666793
hsa05146 Amoebiasis                              1.313400e-03  3.052596
hsa05332 Graft-versus-host disease               2.605234e-03  2.948229
hsa04640 Hematopoietic cell lineage              2.822776e-03  2.833362
hsa04630 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/katiemostoller/Desktop/Class 14
```

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

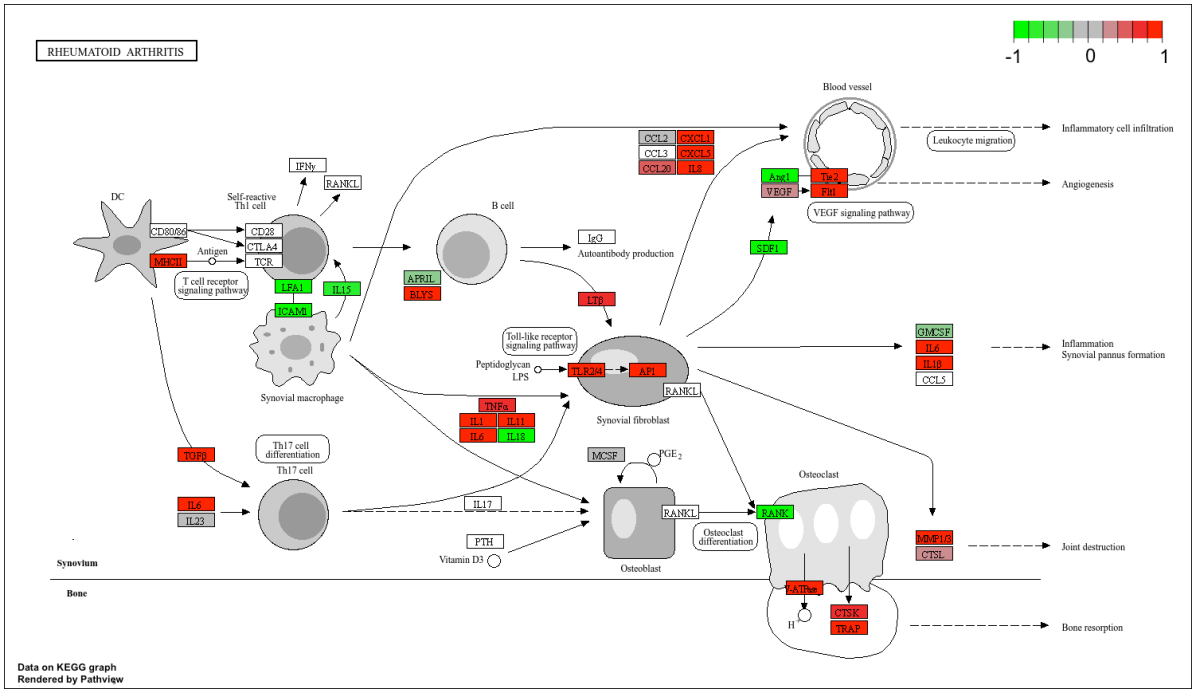



```
pathview(foldchanges, pathway.id = "hsa05323")
```

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

```
Info: Working in directory /Users/katiemostoller/Desktop/Class 14
```

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




## GO Gene Ontology

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

# Focus just on GO biological process (bp)
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets)
```

```
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

We can use reactome via R or via their fancy new website interface. The web interface wants a set of ENTREZID values for your genes of interest that we need to generate.

```
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)
```