Class 14: RNASeq mini-project

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Here we will perform a complete RNASeq analysis from counts to pathways and biological interpretation.

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.

Required packages

```
library(DESeq2)
library(AnnotationDbi)
library(org.Hs.eg.db)
library(pathview)
library(gage)
library(gageData)
library(ggplot2)
library(ggrepel)
```

Data import

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

```
head(countData)
```

```
                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)
```

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

Tidy counts to match metadata

Check the correspondence of colData rows and countData columns

```
rownames(colData)
```

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

```
colnames(countData)
```

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

Remove the first column so we can match the metadata

```
counts <- countData[, rownames(colData)]
head(counts)
```

```
                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
```

```
all(rownames(colData) == colnames(counts))
```

```
[1] TRUE
```

Remove zero count genes

We will have rows in `counts` for genes that we cannot say anything about because they have zero expression in the particular tissue we are looking at.

If `rowSums` is zero for a given gene, then it has no count data and we should exclude that gene from further consideration.

```
to.keep <- rowSums(counts) != 0

cleancounts <- counts[to.keep, ]
head(cleancounts)
```

```
                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
```

> Q: How many genes do we have left?

```
nrow(cleancounts)
```

```
[1] 15975
```

DESeq

Set up DESeq object for analysis

```r
dds <- DESeqDataSetFromMatrix(countData = cleancounts,
                              colData = colData,
                              design = ~condition)
```

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

Run DESeq analysis

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

Extract results

```r
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
```

Add gene annotations

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

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

```r
res$name <- mapIds(x = org.Hs.eg.db,
                   keys = rownames(res),
                   keytype = "ENSEMBL",
                   column = "GENENAME")
```

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

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

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

```r
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 1.43990e-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      symbol                   name      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
```

Save results to CSV file

```r
write.csv(res, file = "results.csv")
```

Result visualization

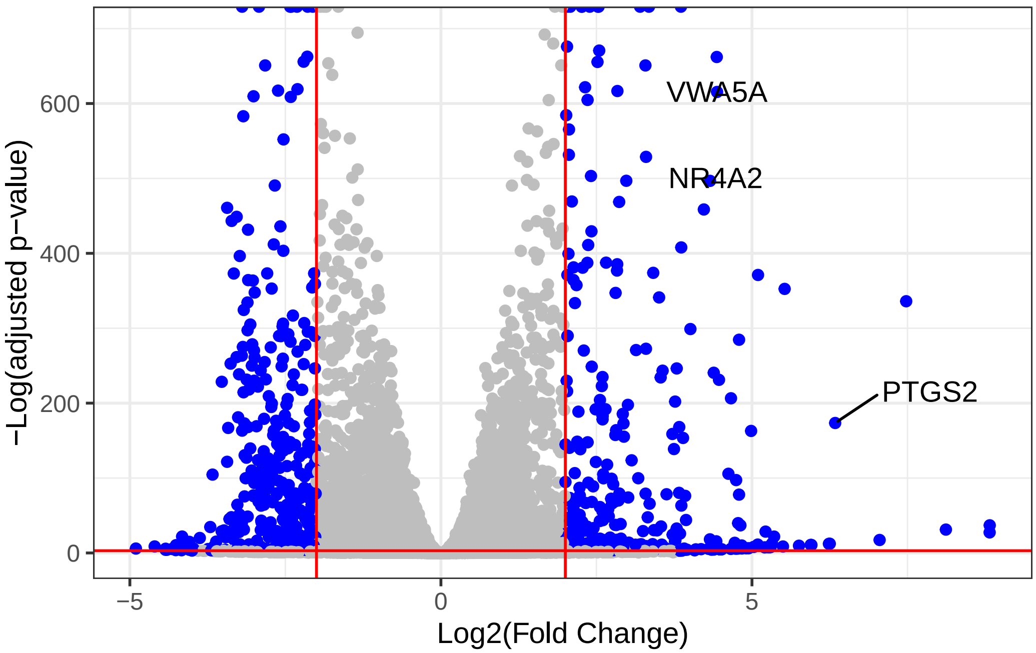
```r
mycols <- rep("gray", nrow(res))
mycols[res$log2FoldChange <= -2] <- "blue"
mycols[res$log2FoldChange >= 2] <- "blue"
mycols[res$padj >= 0.05] <- "gray"
```

```r
ggplot(res) +
  aes(x = log2FoldChange, y = -log(padj), label = symbol) +
  geom_point(col = mycols) +
  geom_vline(xintercept = c(-2, 2), col = "red") +
  geom_hline(yintercept = -log(0.05), col = "red") +
  labs(x = "Log2(Fold Change)", y = "-Log(adjusted p-value)") +
  geom_text_repel() +
  theme_bw()
```

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

```
Warning: Removed 1409 rows containing missing values or values outside the scale range
(`geom_text_repel()`).
```

```
Warning: ggrepel: 14563 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



Pathway analysis

```
data("kegg.sets.hs")
data(sigmet.idx.hs)

kegg.sets.hs <- kegg.sets.hs[sigmet.idx.hs]

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

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

Upregulated pathways

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

```
[1] "hsa04640 Hematopoietic cell lineage"
[2] "hsa04630 Jak-STAT signaling pathway"
[3] "hsa00140 Steroid hormone biosynthesis"
[4] "hsa04142 Lysosome"
[5] "hsa04330 Notch signaling pathway"
```

```
keggresids_up <- substr(keggrespathways_up, start = 1, stop = 8)
keggresids_up
```

```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

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

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

```
Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14
```

```
Info: Writing image file hsa04640.pathview.png
```

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa04330.pathview.png
```

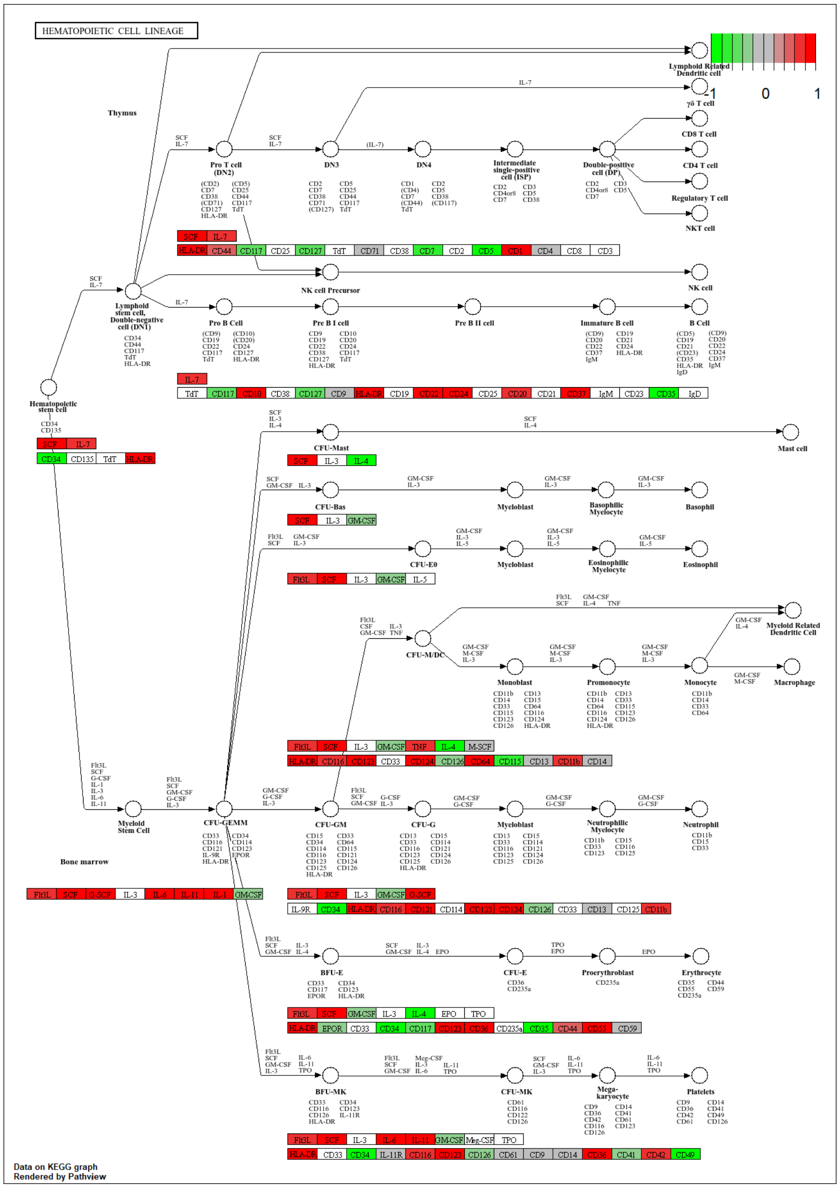

HEMATOPOIETIC CELL LINEAGE
-1
0
1
Thymus
Bone marrow
Hematopoietic stem cell
Lymphoid stem cell, Double-negative cell (DN1)
Pro T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
Lymphoid Related Dendritic cell
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
NK cell Precursor
NK cell
Pro B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-E0
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Myeloid Related Dendritic Cell
Monoblast
Promonocyte
Monocyte
Macrophage
Myeloid Stem Cell
CFU-GEMM
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
BFU-E
CFU-E
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
SCF IL-7
HLA-DR CD44 CD117 CD25 CD127 TdT CD71 CD38 CD7 CD2 CD5 CD1 CD4 CD8 CD3
IL-7
TdT CD117 CD10 CD38 CD127 CD9 HLA-DR CD19 CD22 CD24 CD25 CD20 CD21 CD37 IgM CD23 CD35 IgD
SCF IL-7
CD34 CD135 TdT HLA-DR
SCF IL-3 IL-4
SCF IL-3 GM-CSF
Flt3L SCF IL-3 GM-CSF IL-5
Flt3L SCF IL-3 GM-CSF TNF IL-4 M-SCF
HLA-DR CD116 CD123 CD33 CD124 CD126 CD64 CD115 CD13 CD11b CD14
Flt3L SCF G-SCF IL-3 IL-6 IL-11 IL-1 GM-CSF
Flt3L SCF IL-3 GM-CSF G-SCF
IL-9R CD34 HLA-DR CD116 CD121 CD114 CD123 CD124 CD126 CD33 CD13 CD125 CD11b
Flt3L SCF GM-CSF IL-3 IL-4 EPO TPO
HLA-DR EPOR CD33 CD34 CD117 CD123 CD36 CD235a CD35 CD44 CD55 CD59
Flt3L SCF IL-3 IL-6 IL-11 GM-CSF Meg-CSF TPO
HLA-DR CD33 CD34 IL-11R CD116 CD123 CD126 CD61 CD9 CD14 CD36 CD41 CD42 CD49
Data on KEGG graph
Rendered by Pathview

Figure 1: hsa04640

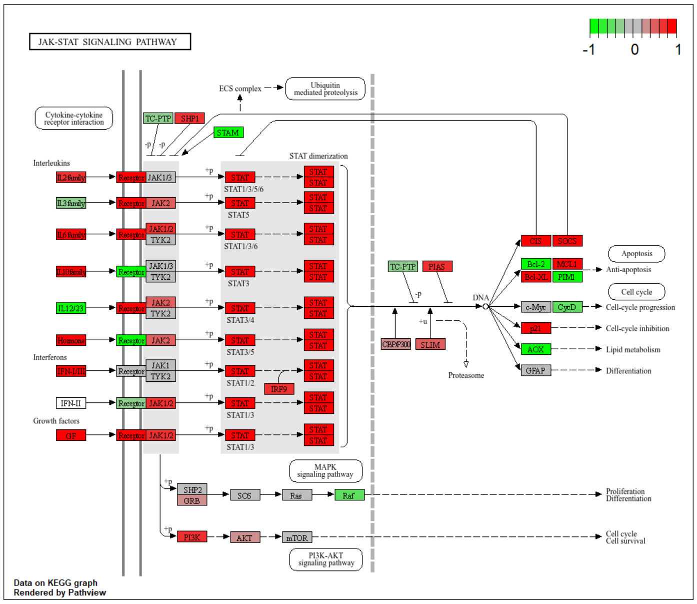


Figure 2: hsa04630

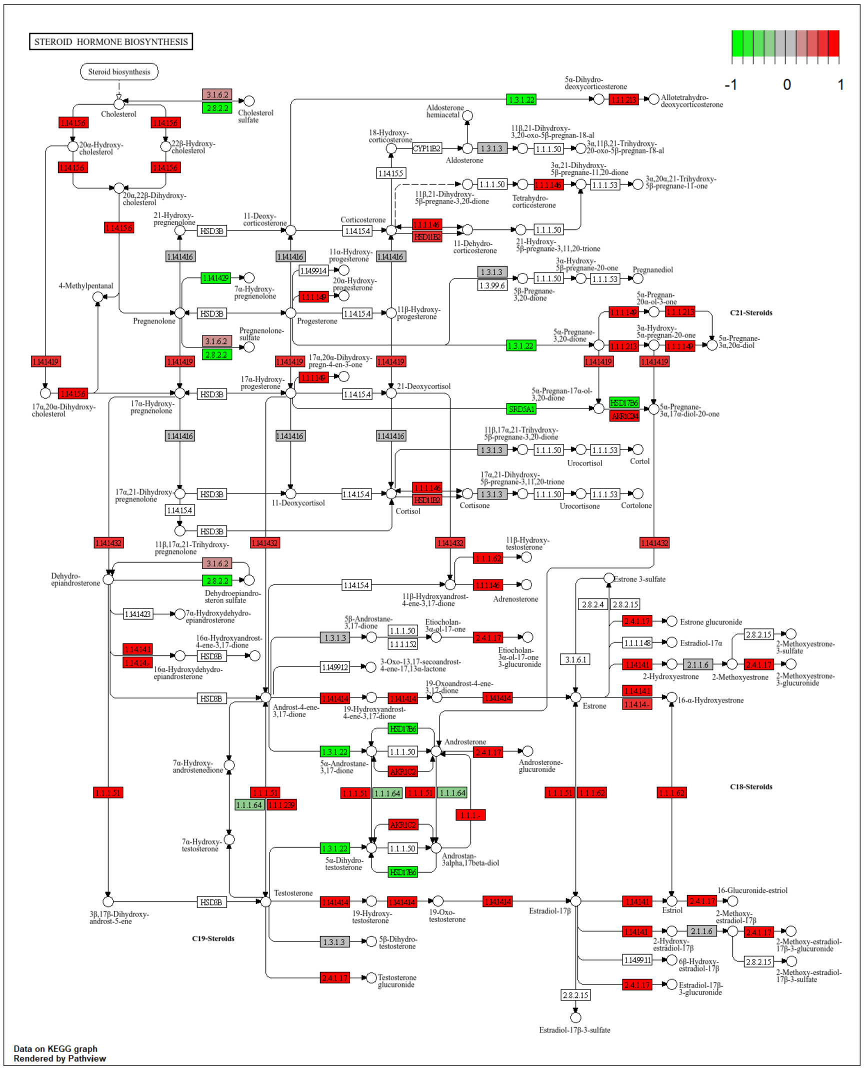


Figure 3: hsa00140

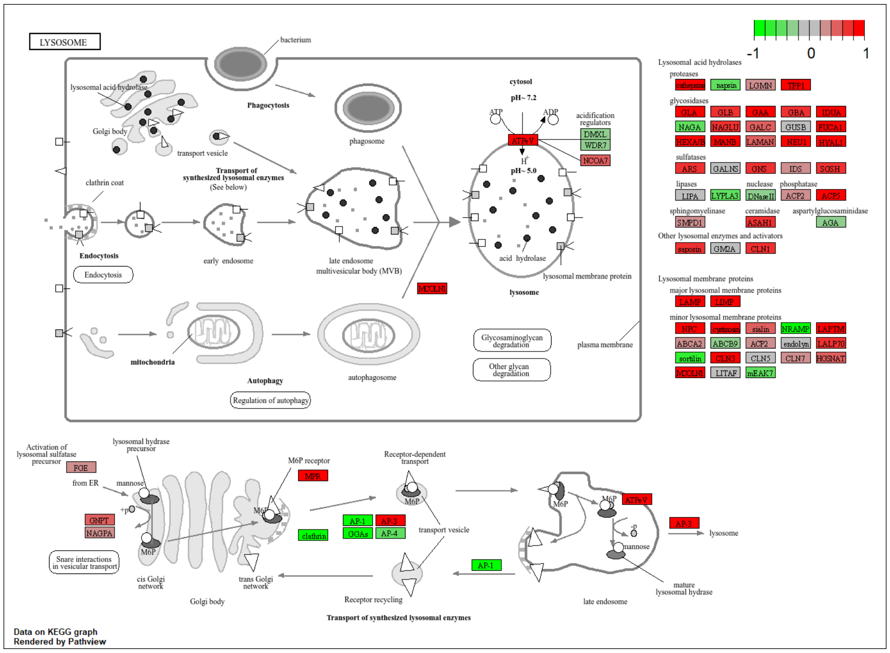


Figure 4: hsa04142

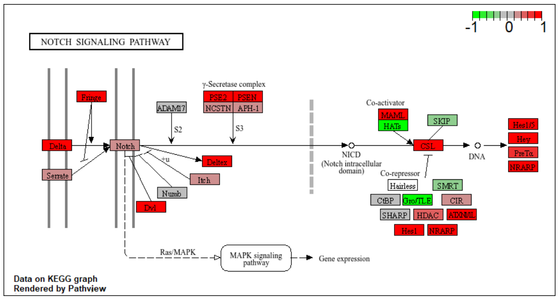


Figure 5: hsa04330

Downregulated pathways

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

```
[1] "hsa04110 Cell cycle"              "hsa03030 DNA replication"
[3] "hsa03013 RNA transport"           "hsa03440 Homologous recombination"
[5] "hsa04114 Oocyte meiosis"
```

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

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

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

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory C:/Users/grace/Desktop/UCSD/4/Spring/BIMM 143/Labs/Class14

Info: Writing image file hsa04114.pathview.png
```

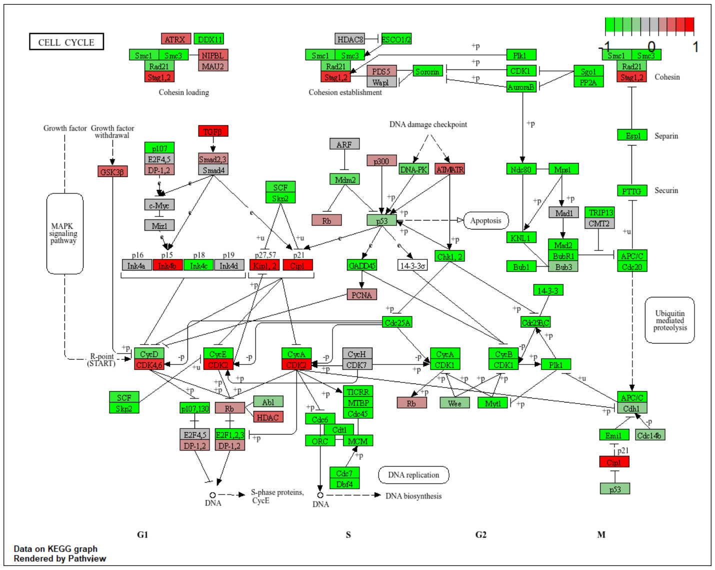


Figure 6: hsa04110

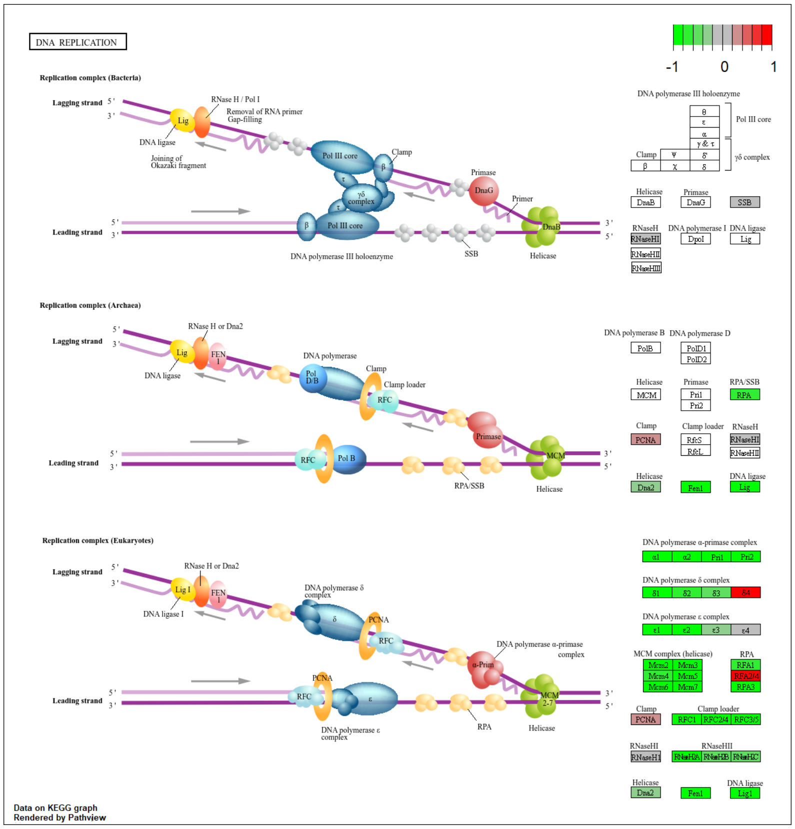


Figure 7: hsa03030

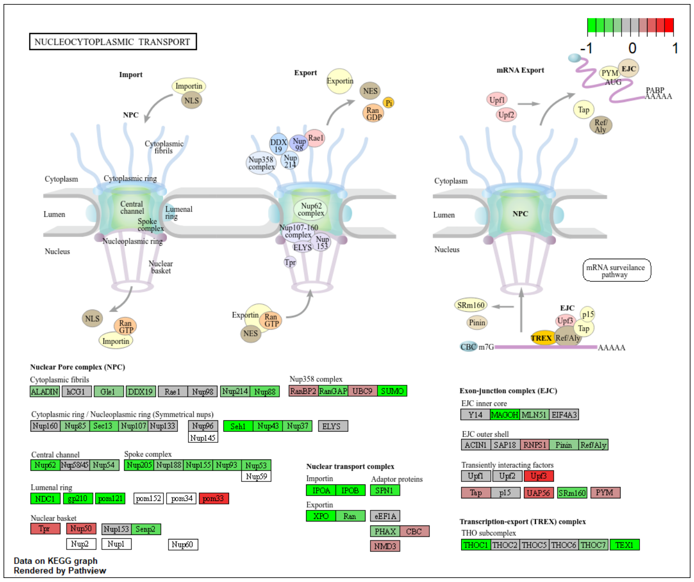


Figure 8: hsa03013

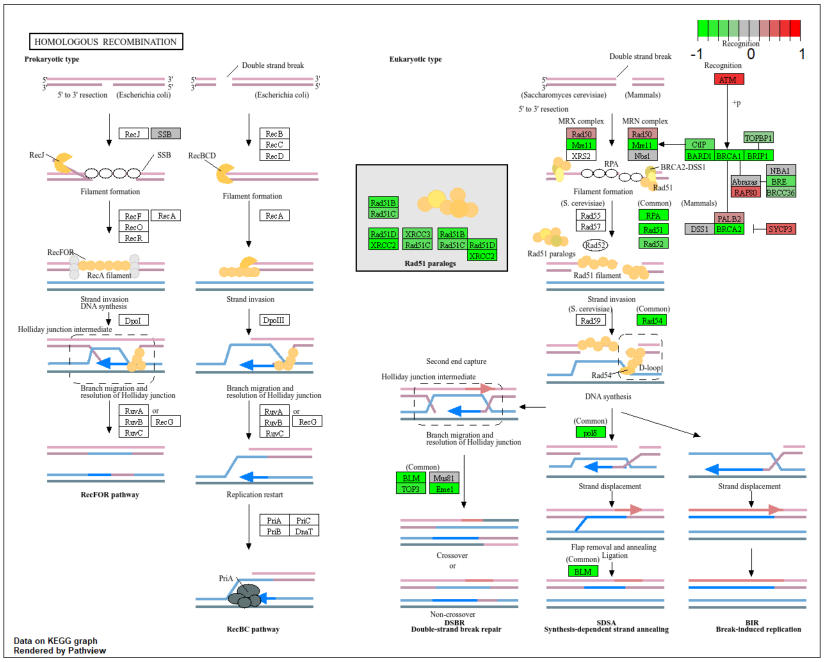


Figure 9: hsa03440

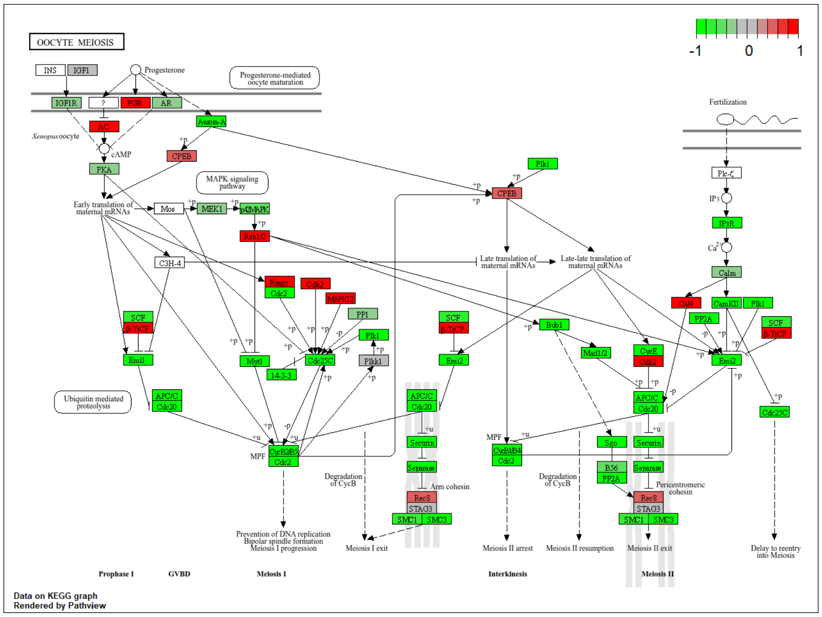


Figure 10: hsa04114

Gene ontology

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

gobpsets = go.sets.hs[go.subs.hs$BP]

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

lapply(gobpres, head)
```

```
$greater
                                            p.geomean stat.mean        p.val
GO:0007156 homophilic cell adhesion       8.519724e-05  3.824205 8.519724e-05
GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
GO:0048729 tissue morphogenesis           1.432451e-04  3.643242 1.432451e-04
GO:0007610 behavior                       1.925222e-04  3.565432 1.925222e-04
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                               q.val set.size         exp1
```

```
GO:0007156 homophilic cell adhesion       0.1951953      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1951953      424 1.432451e-04
GO:0007610 behavior                       0.1967577      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3565320      257 5.932837e-04
GO:0035295 tube development               0.3565320      391 5.953254e-04

$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

$stats
                                          stat.mean     exp1
GO:0007156 homophilic cell adhesion        3.824205 3.824205
GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
GO:0048729 tissue morphogenesis            3.643242 3.643242
GO:0007610 behavior                        3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Reactome analysis

Reactome analysis online

We need to make a file of our significant genes that we can upload to the reactome webpage (https://reactome.org/PathwayBrowser/#TOOL=AT).

```r
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
[1] "Total number of significant genes: 8147"
```

```r
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quote=
```

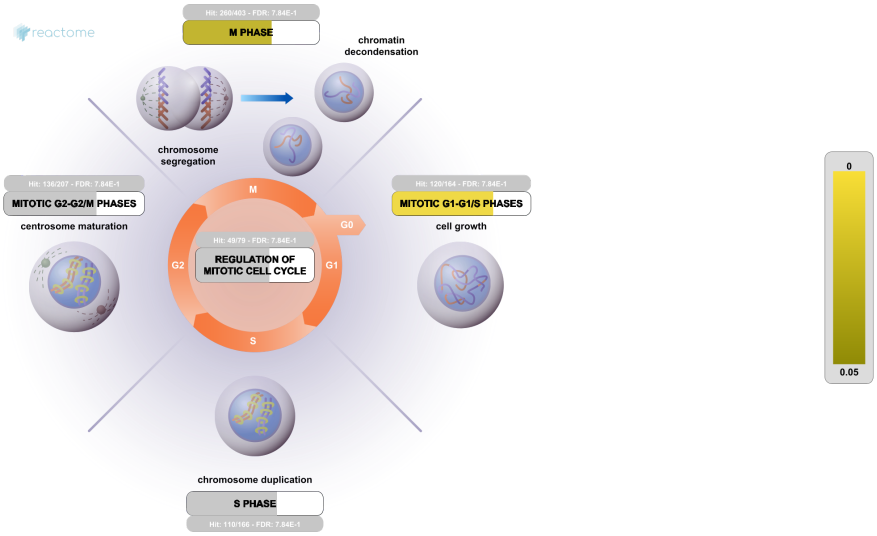


Figure 11: Reactome image - cell cycle, mitotic