Class 14

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Data Import

```r
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 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


Warning: package 'GenomeInfoDb' was built under R version 4.3.2


Loading required package: SummarizedExperiment


Warning: package 'SummarizedExperiment' was built under R version 4.3.2


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
```

```
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"

# Import metadata and take a peak
metadata = read.csv(metaFile, row.names=1)
head(metadata)
```

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

Have a wee peak at these objects

```
# Import countdata
counts = read.csv(countFile, row.names=1)
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
```

Data Tidying

I need to get rid of the first `length` column in `counts`:

```
counts <- counts[,-1]
```

Remove any genes with zero counts in all samples/columns

> Q. How many genes do we have to start with?

```
nrow(counts)
```

```
[1] 19808
```

- FInd the rowSums() this will be zero for any genes with no data
- Find the zero sum genes
- Remove them before doing our DESeq

```
to.rm.ind <- rowSums(counts) == 0
counts <- counts[!to.rm.ind,]
nrow(counts)
```

```
[1] 15975
```

DESeq setup and analysis

```
library(DESeq2)
```

```
dds <- DESeqDataSetFromMatrix(countData = counts,
                              colData = metadata,
                              design = ~condition)
```

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

Now run our main DESeq analysis

```
#1 message: false
dds <- DESeq(dds)
```

```
estimating size factors
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```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 1.43989e-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
```

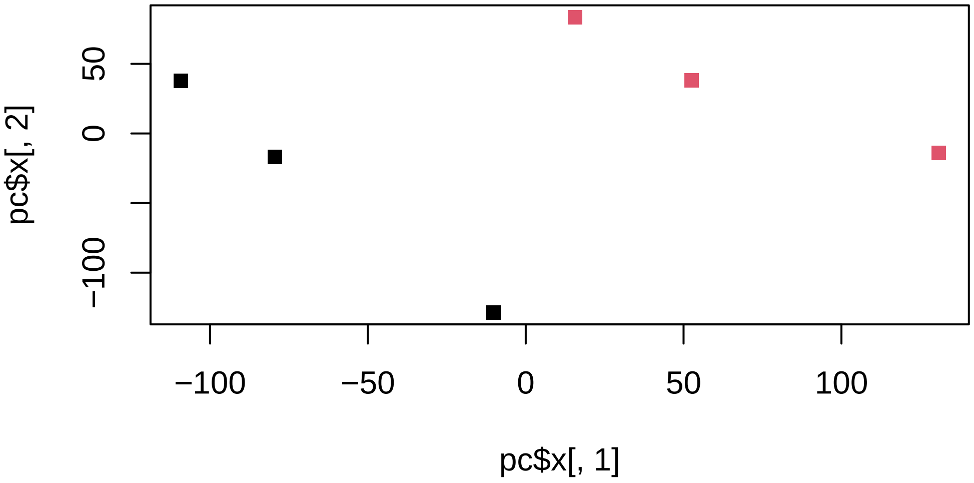
Side-note: QC with PCA

```r
pc <- prcomp(t(counts),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
```

```
plot(pc$x[,1],pc$x[,2], col=as.factor(metadata$condition), pch=15)
```

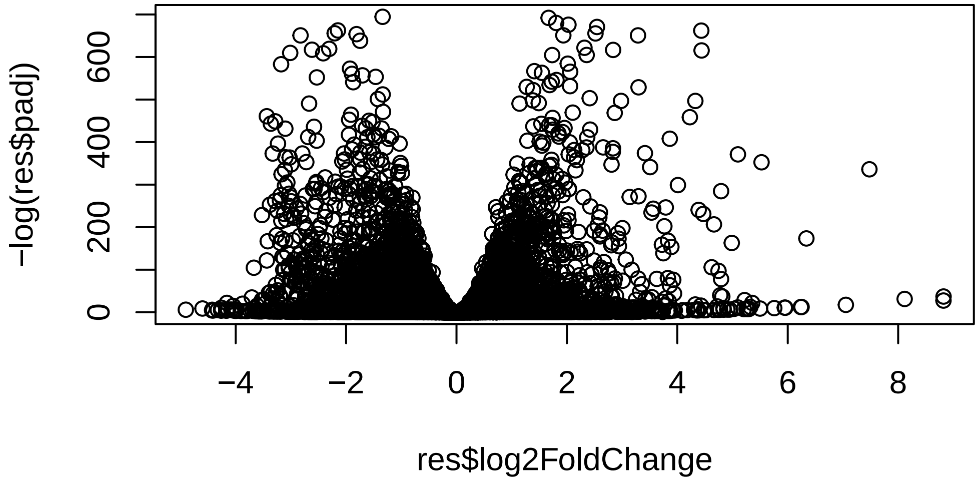


Add annotation data

Save my results

Visualization

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

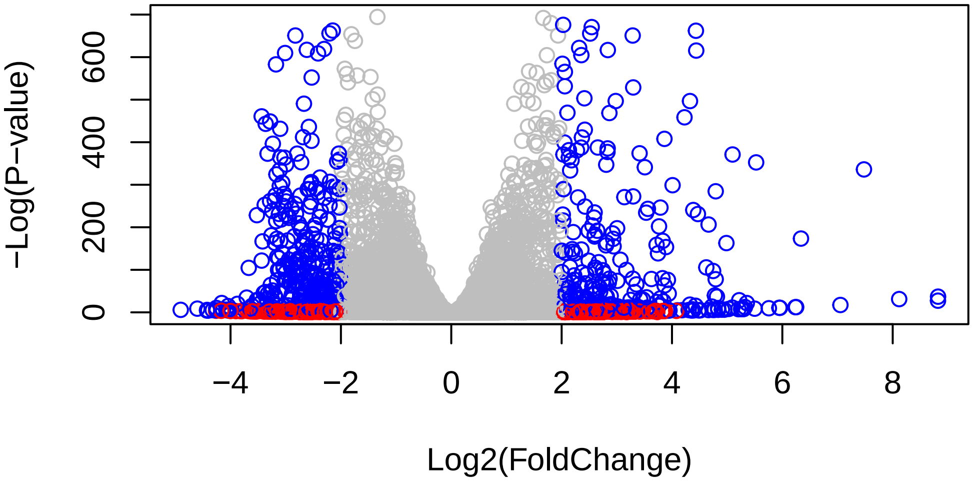


```r
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

# Color red the genes with absolute fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

# Color blue those with adjusted p-value less than 0.01
#  and absolute fold change more than 2
inds <- (res$pvalue<0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"
```

```
plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log2(FoldChange)", ylab="-Log(
```



Let's add some color and annotation data to this plot.

```
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(row.names(counts))
```

```
[1] "ENSG00000279457" "ENSG00000187634" "ENSG00000188976" "ENSG00000187961"
[5] "ENSG00000187583" "ENSG00000187642"
```

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(counts),
                     keytype = "ENSEMBL",
                     column = "SYMBOL",
                     multiVals="first")
```

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

```
res$entrez <- mapIds(org.Hs.eg.db,
                     keys=row.names(counts),
                     keytype = "ENSEMBL",
                     column = "ENTREZID",
                     multiVals="first")
```

```
'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
```

Geneset enrichmen/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).
##############################################################################
```

The `gage()` function wants a "vector of importance" in our case here it will be fold-change values with associated entrez gene names.

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

```
data(kegg.sets.hs)
# Get the results
keggres = gage(foldchange, 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
```

hsa04110 cell cycle

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

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

Info: Working in directory /Users/sophialu1999/Desktop/UCSD Biological Sciences Ph.D./BGGN21

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

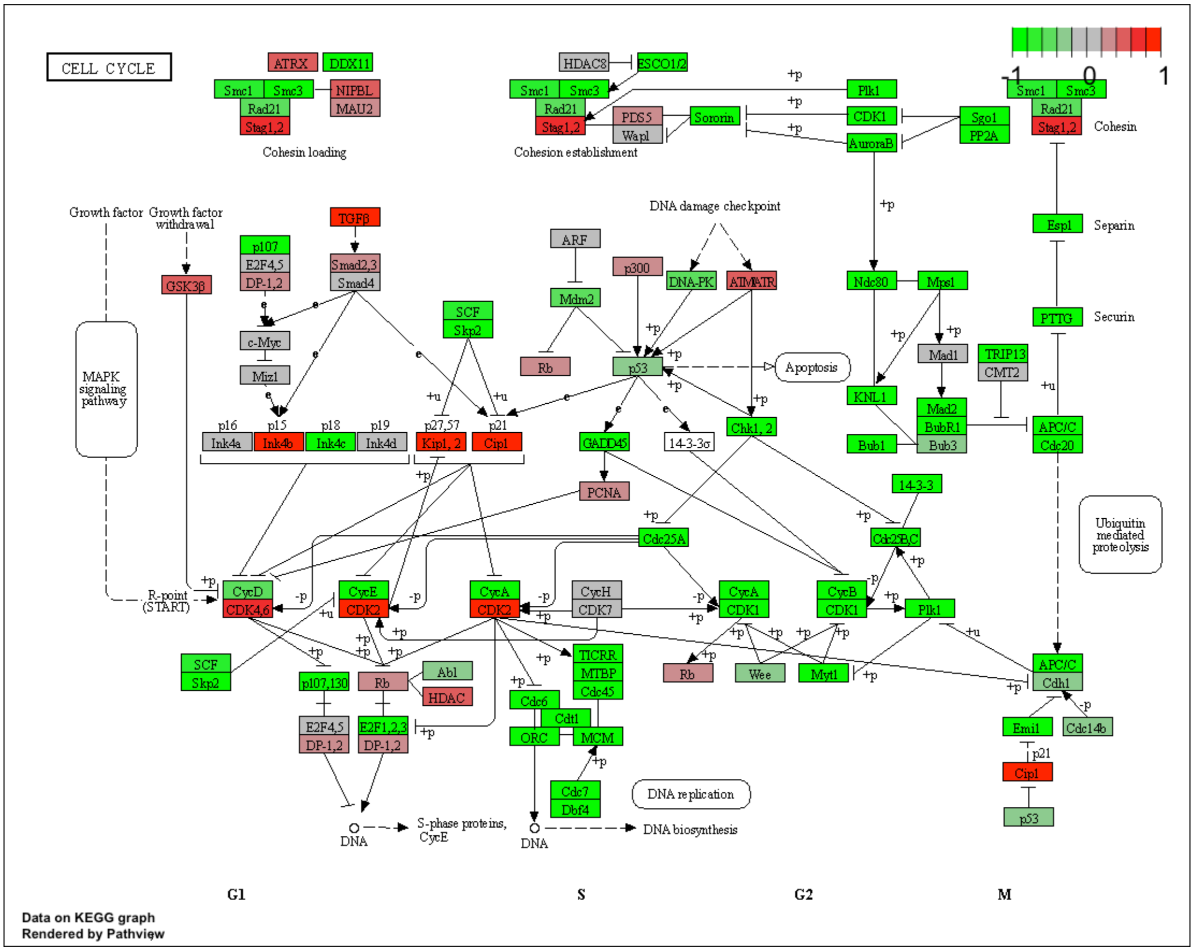
Have a look at my figure (Figure 1)

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(foldchange, gsets=gobpsets, same.dir=TRUE)
```

```
head(gobpres$less)
```



Data on KEGG graph
Rendered by Pathview

Figure 1: Cell Cycle hsa04110

```
                                            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             5.843127e-12      376 1.536227e-15
GO:0000280 nuclear division              5.843127e-12      352 4.286961e-15
GO:0007067 mitosis                       5.843127e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195965e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.659009e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase          1.178690e-07       84 1.729553e-10
```

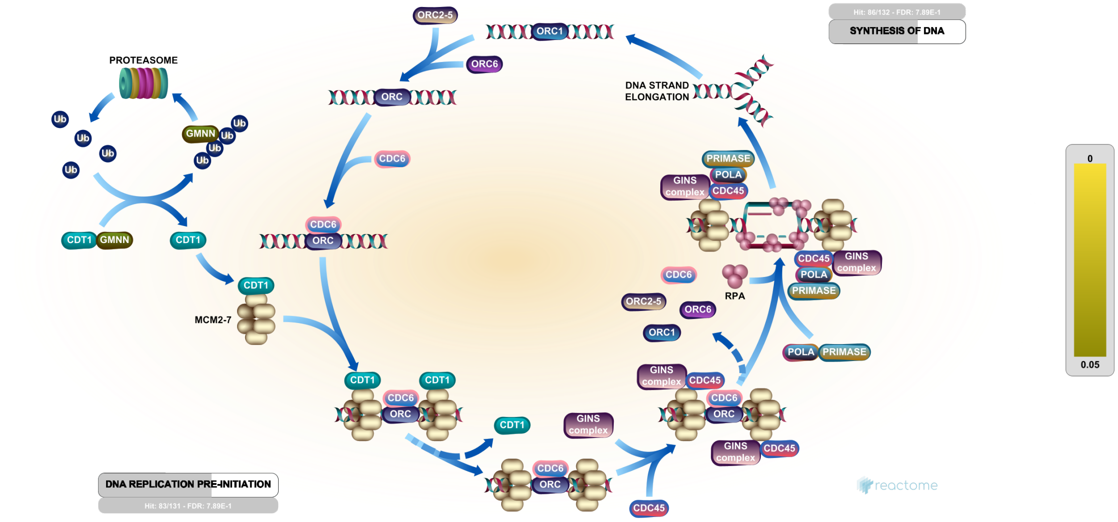
Reactome

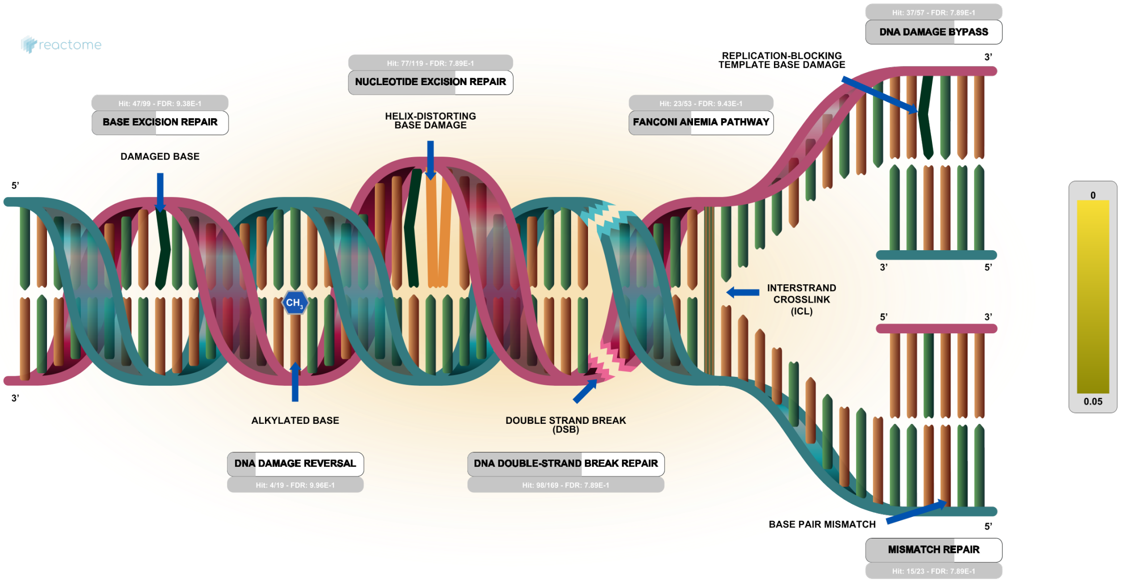
We will use the online version of Reactome. It wants a list of your genes. We will write ths out from R here:

```r
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]

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

Reactome website: https://reactome.org/PathwayBrowser/#TOOL=AT


Hit: 86/132 - FDR: 7.89E-1
SYNTHESIS OF DNA
ORC2-5
ORC1
ORC6
ORC
DNA STRAND
ELONGATION
PROTEASOME
Ub
GMNN
CDC6
PRIMASE
POLA
GINS
complex
CDC45
CDT1
RPA
MCM2-7
DNA REPLICATION PRE-INITIATION
Hit: 83/131 - FDR: 7.89E-1
reactome
0
0.05


reactome
Hit: 37/57 - FDR: 7.89E-1
DNA DAMAGE BYPASS
Hit: 77/119 - FDR: 7.89E-1
NUCLEOTIDE EXCISION REPAIR
REPLICATION-BLOCKING
TEMPLATE BASE DAMAGE
3'
Hit: 47/99 - FDR: 9.38E-1
BASE EXCISION REPAIR
Hit: 23/53 - FDR: 9.43E-1
FANCONI ANEMIA PATHWAY
HELIX-DISTORTING
BASE DAMAGE
DAMAGED BASE
5'
3'
CH_3
INTERSTRAND
CROSSLINK
(ICL)
3'
5'
5'
3'
ALKYLATED BASE
DOUBLE STRAND BREAK
(DSB)
DNA DAMAGE REVERSAL
Hit: 4/19 - FDR: 9.96E-1
DNA DOUBLE-STRAND BREAK REPAIR
Hit: 98/169 - FDR: 7.89E-1
BASE PAIR MISMATCH
5'
MISMATCH REPAIR
Hit: 15/23 - FDR: 7.89E-1
0
0.05