Class_14_RNA_seq_mini_project

Johann Tailor

Data Import and Reading

```
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

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
```

```r
metaFile <- read.csv("GSE37704_metadata.csv")
countFile <- read.csv("GSE37704_featurecounts.csv", row.names = 1)
```

Data Exploration

```r
head(metaFile)
```

```
        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
```

```r
head(countFile)
```

```
                 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
```

Check for similarity between column names:

```
colnames(countFile)
```

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

```
head(metaFile)
```

```
         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
```

Filter our the zeros

removing the length column

```
countdata <- countFile[,-1]
```

Checking for the sample exactness

```
#Using == to check if they are perfectly same
colnames(countdata) == metaFile$id
```

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

Some genes have no values, so we want to get rid of it.

```
#Only select for values which have a sum greater than 0 for each row/gene:
non.zero.inds <- rowSums(countdata) > 0

#Looks for that in all the rows/genes:
non.zero.counts <- countdata[non.zero.inds,]

head(non.zero.counts)
```

	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

```
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

Setup for DESeq

```
library(DESeq2)
```

#DESeq analysis

```
dds <- DESeqDataSetFromMatrix(countData = non.zero.counts,
                              colData = metaFile,
                              design = ~condition)
```

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

```
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)
```

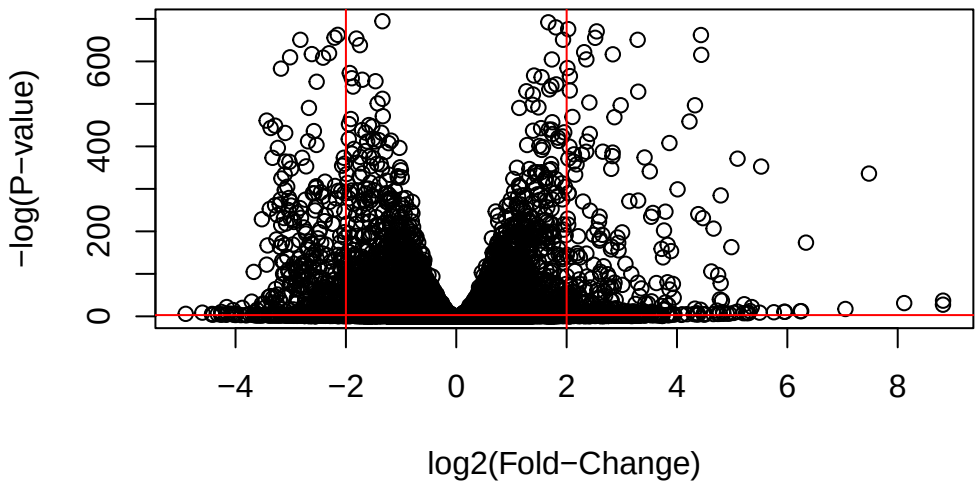
```
head(res)
```

```
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
```

##Making the Volcano Plot:

```
plot(res$log2FoldChange, -log(res$padj),
     ylab="-log(P-value)",
     xlab="log2(Fold-Change)")
abline(v=2, col= "red")
abline(v=-2, col= "red")
abline(h=-log(0.05), col= "red")
```



We can also use ggplot but first we have to make it as a data frame:

```
res_df <- as.data.frame(res)
```

```
library(ggplot2)

#Color all points gray:
mycols <- rep("gray", nrow(res_df))

#Setting limits:
mycols
```

```
  [1] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
 [11] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[141] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[151] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[161] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[171] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[181] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[191] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[201] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[211] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[221] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[231] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[241] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[251] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[261] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[271] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[281] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[291] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[301] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[311] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[321] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[331] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[341] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[351] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[361] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[371] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[381] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[391] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[401] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[441] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[651] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[661] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[681] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[811] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[821] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[1031] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1041] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1051] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[1071] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1081] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[1131] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1141] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[1221] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1231] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1241] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1251] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1261] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[1271] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[5521] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[5531] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[5541] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[5601] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[7751] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[7761] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[7771] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
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[14871] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14881] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14891] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14901] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14911] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14921] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14931] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14941] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14951] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14961] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14971] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14981] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[14991] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15001] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15011] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15021] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15031] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15041] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15051] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15061] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15071] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15081] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15091] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15101] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15111] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15121] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15131] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
```
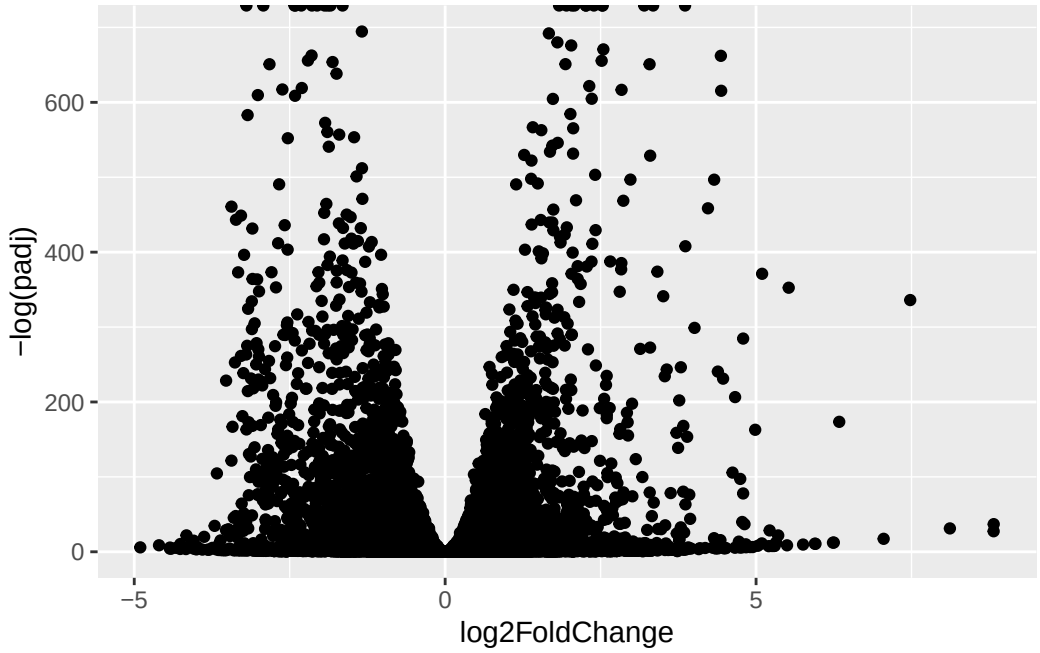

```
[15141] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15151] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15161] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15171] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15181] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15191] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15201] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15211] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15221] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15231] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15241] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15251] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15261] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15271] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15281] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15291] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15301] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15311] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15321] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15331] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15341] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15351] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15361] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15371] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15381] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15391] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15401] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15411] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15421] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15431] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15441] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15451] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15461] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15471] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15481] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15491] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15501] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15511] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15521] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15531] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15541] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15551] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15561] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
```

```
[15571] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15581] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15591] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15601] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15611] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15621] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15631] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15641] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15651] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15661] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15671] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15681] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15691] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15701] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15711] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15721] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15731] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15741] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15751] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15761] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15771] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15781] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15791] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15801] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15811] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15821] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15831] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15841] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15851] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15861] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15871] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15881] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15891] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15901] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15911] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15921] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15931] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15941] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15951] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15961] "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray" "gray"
[15971] "gray" "gray" "gray" "gray" "gray"
```

```
ggplot(res_df, aes(x = log2FoldChange, y = -log(padj))) +
  geom_point()
```

```
Warning: Removed 1237 rows containing missing values (`geom_point()`).
```



Annotating the genes

```
library(AnnotationDbi)
library(org.Hs.eg.db)
```

Let's see the databases that we can translate between

```
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"
```

Let's try to use different datbases and translate the IDs:

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

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

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

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

```
head(res_df$entrez)
```

```
[1] NA       "148398" "26155"  "339451" "84069"  "84808"
```

#Result extraction

KEGG and GO analysis

BiocManager::install(c("pathview", "gage", "gageData"))

```
library(gage)
library(gageData)
library(pathview)
```

```
foldchanges <- res_df$log2FoldChange
names(foldchanges) <- res_df$entrez
head(foldchanges)
```

```
      <NA>      148398       26155      339451       84069       84808
0.17925708  0.42645712 -0.69272046  0.72975561  0.04057653  0.54281049
```

Pathway Analysis

```
library(gage)
library(gageData)

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

# Focus on signaling and metabolic pathways only
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

# Examine the first 3 pathways
head(kegg.sets.hs, 3)
```

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

$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"  "54576"
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"  "54963"
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549"
[49] "8824"   "8833"   "9"      "978"

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"    "10714"
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"    "113"
 [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"    "159"
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"    "221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"  "270"
```

```
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"   "2984"
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"    "3251"
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"   "4831"
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"  "50940"
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"   "5140"
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"   "5148"
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"   "5169"
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"  "5422"
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"   "5433"
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"   "5441"
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"  "55821"
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"  "6240"
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"   "84172"
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"   "9060"
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"    "957"
[161] "9583"   "9615"
```

Let's look at what is inside:

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

```
[1]  0.17925708  0.42645712 -0.69272046  0.72975561  0.04057653  0.54281049
```

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

```
head(keggres$less)
```

```
                                      p.geomean stat.mean p.val q.val
hsa00232 Caffeine metabolism                 NA        NaN    NA    NA
hsa00983 Drug metabolism - other enzymes     NA        NaN    NA    NA
hsa00230 Purine metabolism                   NA        NaN    NA    NA
hsa04514 Cell adhesion molecules (CAMs)      NA        NaN    NA    NA
hsa04010 MAPK signaling pathway              NA        NaN    NA    NA
hsa04012 ErbB signaling pathway              NA        NaN    NA    NA
                                      set.size exp1
hsa00232 Caffeine metabolism                 0   NA
hsa00983 Drug metabolism - other enzymes     0   NA
```

```
hsa00230 Purine metabolism                      0   NA
hsa04514 Cell adhesion molecules (CAMs)         0   NA
hsa04010 MAPK signaling pathway                 0   NA
hsa04012 ErbB signaling pathway                 0   NA
```

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

```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.
```

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

```
Info: Working in directory /Users/johanntailor/Desktop/UCSD Courses/Winter 2024/Bioinformati
```

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

Gene Ontology (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)

lapply(gobpres, head)
```

```
$greater
                                           p.geomean stat.mean p.val q.val
GO:0000002 mitochondrial genome maintenance        NA       NaN    NA    NA
GO:0000003 reproduction                            NA       NaN    NA    NA
GO:0000012 single strand break repair              NA       NaN    NA    NA
GO:0000018 regulation of DNA recombination         NA       NaN    NA    NA
GO:0000019 regulation of mitotic recombination     NA       NaN    NA    NA
GO:0000022 mitotic spindle elongation              NA       NaN    NA    NA
```

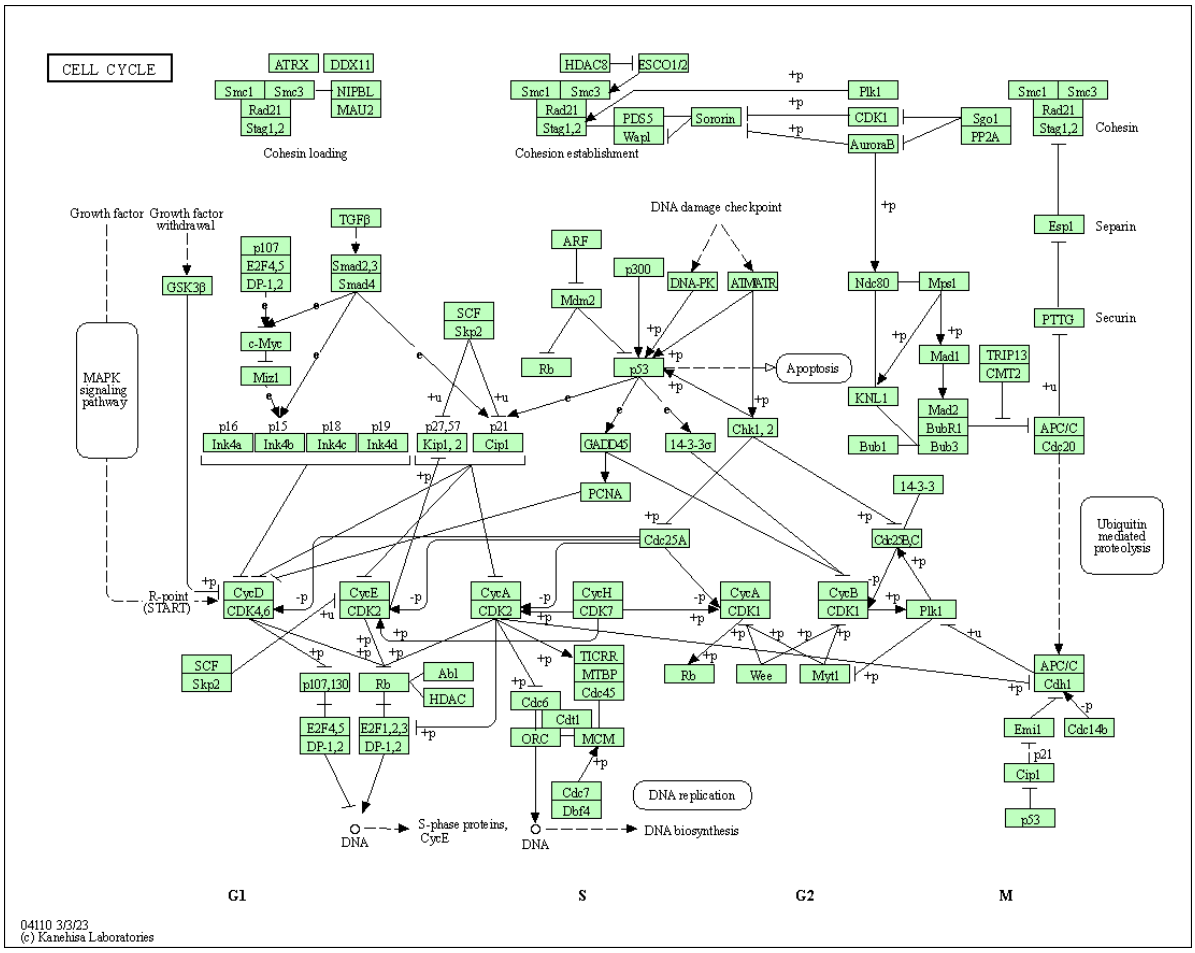


Figure 1: Cell cycle pathway from KEGG

```
                                           set.size exp1
GO:0000002 mitochondrial genome maintenance        0   NA
GO:0000003 reproduction                            0   NA
GO:0000012 single strand break repair              0   NA
GO:0000018 regulation of DNA recombination         0   NA
GO:0000019 regulation of mitotic recombination     0   NA
GO:0000022 mitotic spindle elongation              0   NA

$less
                                           p.geomean stat.mean p.val q.val
GO:0000002 mitochondrial genome maintenance        NA       NaN    NA    NA
GO:0000003 reproduction                            NA       NaN    NA    NA
GO:0000012 single strand break repair              NA       NaN    NA    NA
GO:0000018 regulation of DNA recombination         NA       NaN    NA    NA
GO:0000019 regulation of mitotic recombination     NA       NaN    NA    NA
GO:0000022 mitotic spindle elongation              NA       NaN    NA    NA
                                           set.size exp1
GO:0000002 mitochondrial genome maintenance        0   NA
GO:0000003 reproduction                            0   NA
GO:0000012 single strand break repair              0   NA
GO:0000018 regulation of DNA recombination         0   NA
GO:0000019 regulation of mitotic recombination     0   NA
GO:0000022 mitotic spindle elongation              0   NA

$stats
                                           stat.mean exp1
GO:0000002 mitochondrial genome maintenance       NaN   NA
GO:0000003 reproduction                           NaN   NA
GO:0000012 single strand break repair             NaN   NA
GO:0000018 regulation of DNA recombination        NaN   NA
GO:0000019 regulation of mitotic recombination    NaN   NA
GO:0000022 mitotic spindle elongation             NaN   NA
```

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

```
                                           p.geomean stat.mean p.val q.val
GO:0000002 mitochondrial genome maintenance        NA       NaN    NA    NA
GO:0000003 reproduction                            NA       NaN    NA    NA
GO:0000012 single strand break repair              NA       NaN    NA    NA
GO:0000018 regulation of DNA recombination         NA       NaN    NA    NA
GO:0000019 regulation of mitotic recombination     NA       NaN    NA    NA
```

```
GO:0000022 mitotic spindle elongation                 NA       NaN    NA    NA
                                                 set.size exp1
GO:0000002 mitochondrial genome maintenance             0   NA
GO:0000003 reproduction                                 0   NA
GO:0000012 single strand break repair                   0   NA
GO:0000018 regulation of DNA recombination              0   NA
GO:0000019 regulation of mitotic recombination          0   NA
GO:0000022 mitotic spindle elongation                   0   NA
```

Reactome Analysis

```
#Pick all genes with fold change greater than 2

#Checks two things at once:

indices <- (abs(res_df$log2FoldChange) > 2) & (abs(res_df$padj) < 0.05)

my_genes <- res_df$symbol[indices]
```

```
cat(head(my_genes), sep = "\n")
```

```
HES4
HES2
DRAXIN
CDA
RUNX3
AUNIP
```

```
write.table(my_genes, file="my_genes.txt", quote = FALSE, row.names = FALSE, col.names = F
```

Added the file to

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

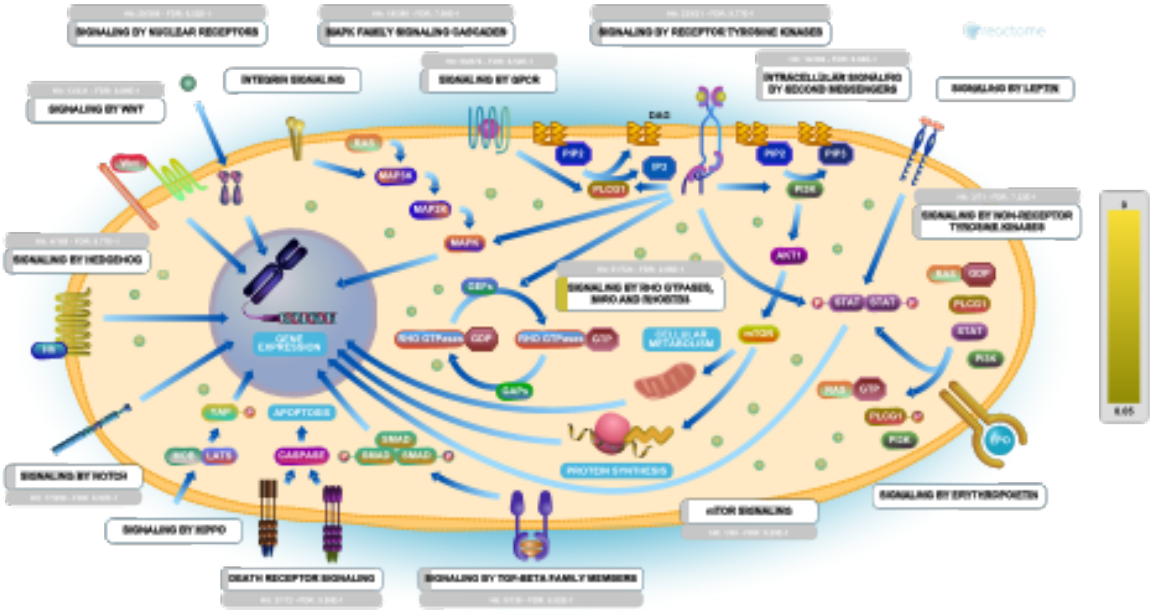


Figure 2: Cell cycle pathway from KEGG