Class13

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1. Background

The data for this hands-on session comes from a published RNA-seq experiment where airway smooth muscle cells were treated with dexamethasone, a synthetic glucocorticoid steroid with anti-inflammatory effects (Himes et al. 2014).

2. Bioconductor setup

```
library(BiocManager)
library(DESeq2)
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics

Loading required package: generics


Attaching package: 'generics'

The following objects are masked from 'package:base':

    as.difftime, as.factor, as.ordered, intersect, is.element, setdiff,
    setequal, union


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, is.unsorted, lapply, Map, mapply, match, mget,
    order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
    rbind, Reduce, rownames, sapply, saveRDS, table, tapply, 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: Seqinfo

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
```

3. Import countData and colData

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

```
head(counts)
```

```
                SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516
ENSG00000000003        723        486        904        445       1170
ENSG00000000005          0          0          0          0          0
ENSG00000000419        467        523        616        371        582
ENSG00000000457        347        258        364        237        318
ENSG00000000460         96         81         73         66        118
ENSG00000000938          0          0          1          0          2
                SRR1039517 SRR1039520 SRR1039521
ENSG00000000003       1097        806        604
ENSG00000000005          0          0          0
ENSG00000000419        781        417        509
ENSG00000000457        447        330        324
ENSG00000000460         94        102         74
ENSG00000000938          0          0          0
```

Q1. How many genes are in this dataset?

```
nrow(counts)
```

```
[1] 38694
```

38694

Q2. How many 'control' cell lines do we have?

```
control <- metadata[metadata[,"dex"]=="control",]
control.counts <- counts[ ,control$id]
control.mean <- rowSums( control.counts )/4
head(control.mean)
```

```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         900.75            0.00          520.50          339.75           97.25
ENSG00000000938
           0.75
```

4. Toy differential gene expression

Q3. How would you make the above code in either approach more robust? Is there a function that could help here?

```
control <- metadata[metadata$dex == "control", ]
control.counts <- counts[, control$id]
control.mean <- rowMeans(control.counts)
head(control.mean)
```

```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         900.75            0.00          520.50          339.75           97.25
ENSG00000000938
           0.75
```

Q4. Follow the same procedure for the treated samples (i.e. calculate the mean per gene across drug treated samples and assign to a labeled vector called treated.mean)

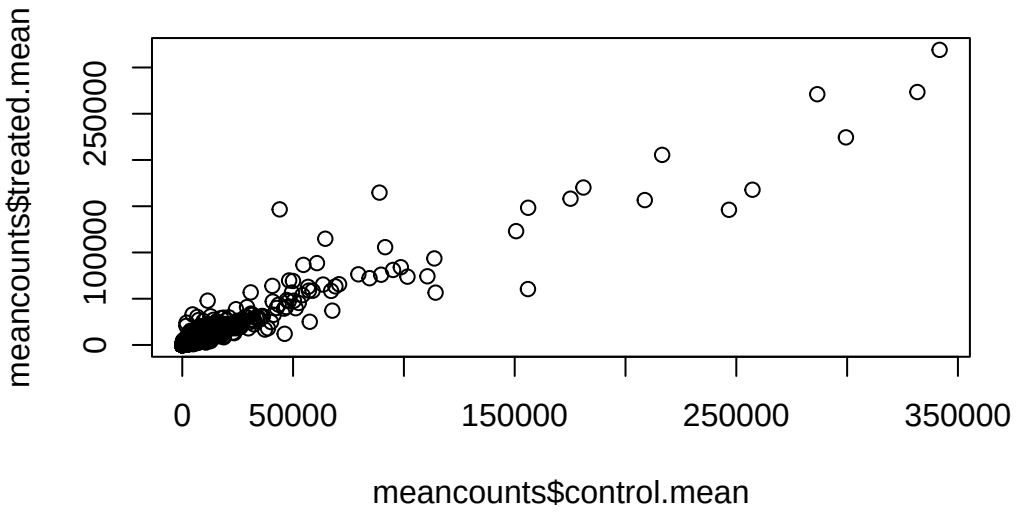
```
treated <- metadata[metadata[,"dex"]=="treated",]
treated.counts <- counts[ ,treated$id]
treated.mean <- rowSums( treated.counts )/4
head(treated.mean)
```

```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         658.00            0.00          546.00          316.50           78.75
ENSG00000000938
           0.00
```

```
meancounts <- data.frame(control.mean, treated.mean)
```

Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

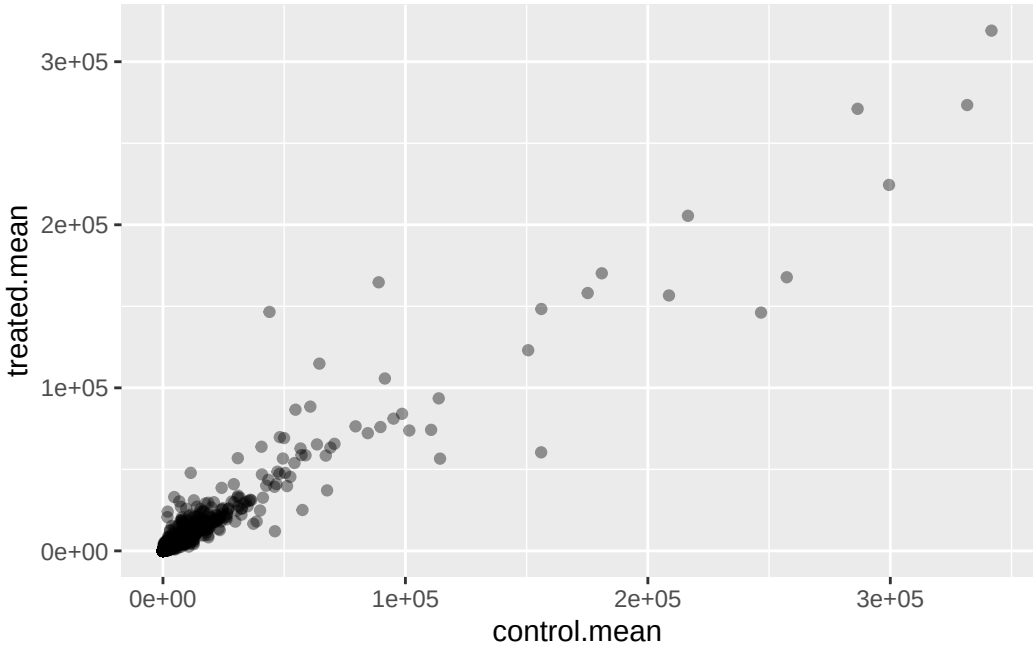
```
plot(meancounts$control.mean,
     meancounts$treated.mean)
```



Q5 (b).You could also use the ggplot2 package to make this figure producing the plot below. What geom_?() function would you use for this plot?

we would use geome_scatter

```
library(ggplot2)
ggplot(meancounts, aes(x = control.mean, y = treated.mean)) +
  geom_point(alpha=0.4)
```



Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

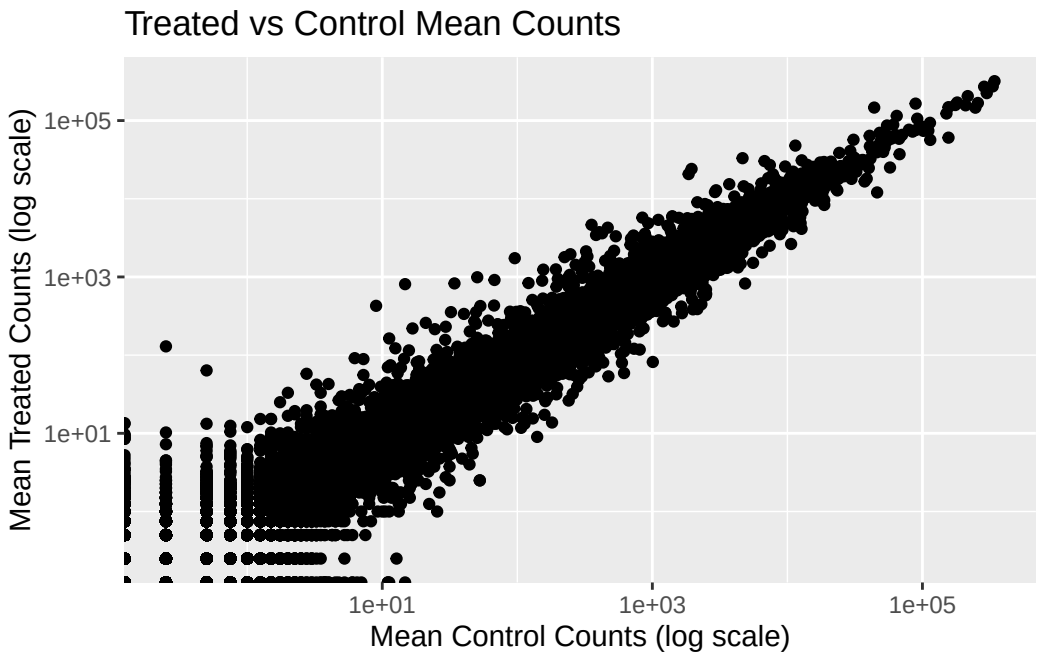
scale_x_log10() and scale_y_log10() allows us to do this

```
library(ggplot2)

ggplot(meancounts, aes(x = control.mean, y = treated.mean)) +
  geom_point() +
  scale_x_log10() +
  scale_y_log10() +
  labs(
    x = "Mean Control Counts (log scale)",
    y = "Mean Treated Counts (log scale)",
    title = "Treated vs Control Mean Counts"
  )
```

```
Warning in scale_x_log10(): log-10 transformation introduced infinite values.
```

```
Warning in scale_y_log10(): log-10 transformation introduced infinite values.
```




```
meancounts$log2fc <- log2(meancounts[,"treated.mean"]/meancounts[,"control.mean"])
head(meancounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000005         0.00         0.00         NaN
ENSG00000000419       520.50       546.00  0.06900279
ENSG00000000457       339.75       316.50 -0.10226805
ENSG00000000460        97.25        78.75 -0.30441833
ENSG00000000938         0.75         0.00        -Inf
```

```
zero.vals <- which(meancounts[,1:2]==0, arr.ind=TRUE)

to.rm <- unique(zero.vals[,1])
mycounts <- meancounts[-to.rm,]
head(mycounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000419       520.50       546.00  0.06900279
ENSG00000000457       339.75       316.50 -0.10226805
ENSG00000000460        97.25        78.75 -0.30441833
ENSG00000000971      5219.00      6687.50  0.35769358
ENSG00000001036      2327.00      1785.75 -0.38194109
```

> Q7. What is the purpose of the arr.ind argument in the which() function call above? Why would we then take the first column of the output and need to call the unique() function?

The arr.ind argument makes which() give us a row and columns that allows us to see both gene and which sample satisfy the condition. We can then take the first column of the output to get only the genes. Because the same gene can meet the condition in multiple samples, it can appear multiple times so we use unique() so it would only be counted once.

```
up.ind <- mycounts$log2fc > 2
down.ind <- mycounts$log2fc < (-2)
#up.ind
#down.ind
```

> Q8. Using the up.ind vector above can you determine how many up regulated genes we have at the greater than 2 fc level?

```
sum(up.ind)
```

```
[1] 250
```

250

> Q9. Using the down.ind vector above can you determine how many down regulated genes we have at the greater than 2 fc level?

```
sum(down.ind)
```

```
[1] 367
```

367

> Q10. Do you trust these results? Why or why not?

No we cant trust these results yet because they're based only on fold change and we dont know if they are statistically significant. We haven't calculated p-values, so the results might be misleading.

5. Setting up for DESeq

```
{message=FALSE}
library(DESeq2)
citation("DESeq2")
```

```
To cite package 'DESeq2' in publications use:

  Love, M.I., Huber, W., Anders, S. Moderated estimation of fold change
  and dispersion for RNA-seq data with DESeq2 Genome Biology 15(12):550
  (2014)

A BibTeX entry for LaTeX users is

  @Article{,
    title = {Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2
    author = {Michael I. Love and Wolfgang Huber and Simon Anders},
    year = {2014},
    journal = {Genome Biology},
    doi = {10.1186/s13059-014-0550-8},
    volume = {15},
    issue = {12},
    pages = {550},
  }
```

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

```
converting counts to integer mode

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

```
dds
```

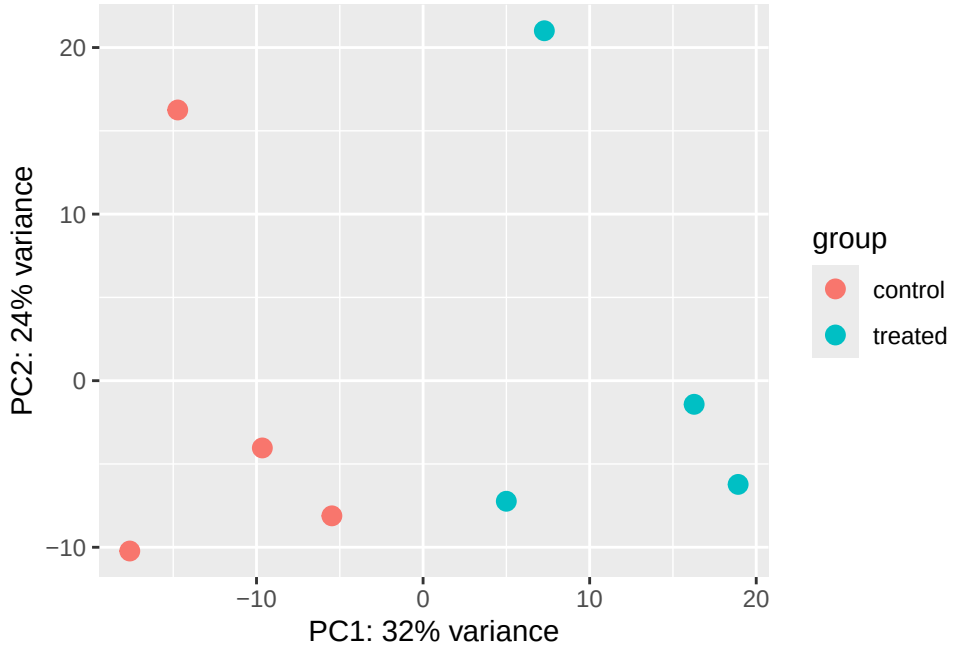
```
class: DESeqDataSet
dim: 38694 8
metadata(1): version
```

```
assays(1): counts
rownames(38694): ENSG00000000003 ENSG00000000005 ... ENSG00000283120
  ENSG00000283123
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(4): id dex celltype geo_id
```

6. Principal Component Analysis (PCA)

```
vsd <- vst(dds, blind = FALSE)
plotPCA(vsd, intgroup = c("dex"))
```

```
using ntop=500 top features by variance
```




```
pcaData <- plotPCA(vsd, intgroup=c("dex"), returnData=TRUE)
```

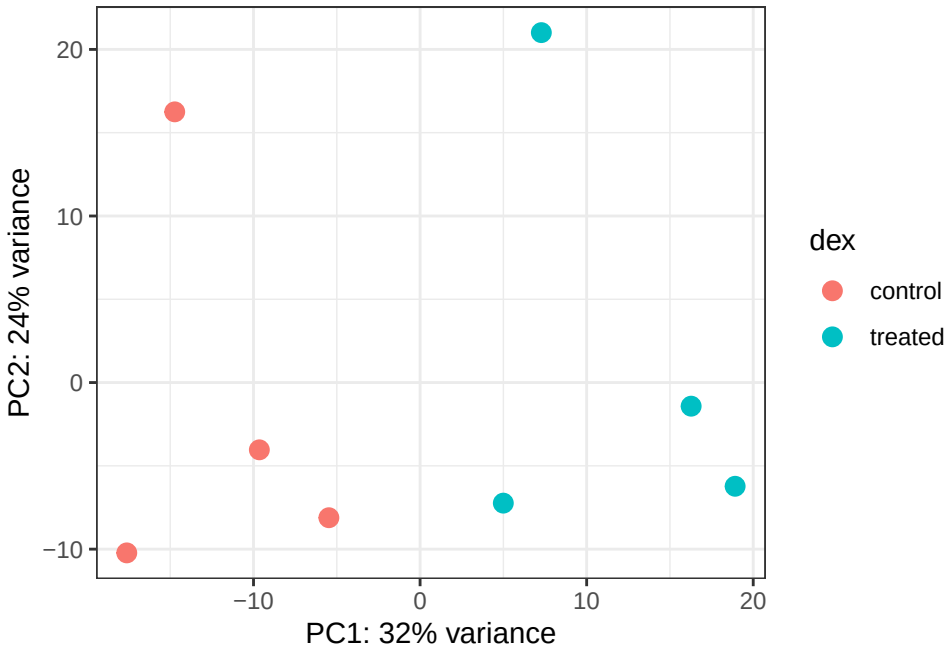
```
using ntop=500 top features by variance
```

```
head(pcaData)
```

```
                 PC1        PC2   group       name         id     dex celltype
SRR1039508 -17.607922 -10.225252 control SRR1039508 SRR1039508 control   N61311
SRR1039509   4.996738  -7.238117 treated SRR1039509 SRR1039509 treated   N61311
SRR1039512  -5.474456  -8.113993 control SRR1039512 SRR1039512 control  N052611
SRR1039513  18.912974  -6.226041 treated SRR1039513 SRR1039513 treated  N052611
SRR1039516 -14.729173  16.252000 control SRR1039516 SRR1039516 control  N080611
SRR1039517   7.279863  21.008034 treated SRR1039517 SRR1039517 treated  N080611
               geo_id sizeFactor
SRR1039508 GSM1275862  1.0193796
SRR1039509 GSM1275863  0.9005653
SRR1039512 GSM1275866  1.1784239
SRR1039513 GSM1275867  0.6709854
SRR1039516 GSM1275870  1.1731984
SRR1039517 GSM1275871  1.3929361
```

```
percentVar <- round(100 * attr(pcaData, "percentVar"))
```

```
library(ggplot2)
ggplot(pcaData) +
  aes(x = PC1, y = PC2, color = dex) +
  geom_point(size =3) +
  xlab(paste0("PC1: ", percentVar[1], "% variance")) +
  ylab(paste0("PC2: ", percentVar[2], "% variance")) +
  coord_fixed() +
  theme_bw()
```



7. DESeq analysis

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```
results(dds)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 38694 rows and 6 columns
                 baseMean log2FoldChange     lfcSE      stat    pvalue
                <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003  747.1942      -0.350703  0.168242 -2.084514 0.0371134
ENSG00000000005    0.0000             NA        NA        NA        NA
ENSG00000000419  520.1342       0.206107  0.101042  2.039828 0.0413675
ENSG00000000457  322.6648       0.024527  0.145134  0.168996 0.8658000
ENSG00000000460   87.6826      -0.147143  0.256995 -0.572550 0.5669497
...                   ...            ...       ...       ...       ...
ENSG00000283115  0.000000             NA        NA        NA        NA
ENSG00000283116  0.000000             NA        NA        NA        NA
ENSG00000283119  0.000000             NA        NA        NA        NA
ENSG00000283120  0.974916       -0.66825   1.69441 -0.394385  0.693297
ENSG00000283123  0.000000             NA        NA        NA        NA
                     padj
                <numeric>
ENSG00000000003  0.163017
ENSG00000000005        NA
ENSG00000000419  0.175937
ENSG00000000457  0.961682
ENSG00000000460  0.815805
...                   ...
ENSG00000283115        NA
ENSG00000283116        NA
ENSG00000283119        NA
ENSG00000283120        NA
ENSG00000283123        NA
```

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

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 38694 rows and 6 columns
                 baseMean log2FoldChange     lfcSE      stat    pvalue
                <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003  747.1942      -0.350703  0.168242 -2.084514 0.0371134
ENSG00000000005    0.0000             NA        NA        NA        NA
ENSG00000000419  520.1342       0.206107  0.101042  2.039828 0.0413675
ENSG00000000457  322.6648       0.024527  0.145134  0.168996 0.8658000
```

```
ENSG00000000460   87.6826      -0.147143  0.256995 -0.572550 0.5669497
...                   ...            ...       ...       ...       ...
ENSG00000283115  0.000000             NA        NA        NA        NA
ENSG00000283116  0.000000             NA        NA        NA        NA
ENSG00000283119  0.000000             NA        NA        NA        NA
ENSG00000283120  0.974916       -0.66825   1.69441 -0.394385  0.693297
ENSG00000283123  0.000000             NA        NA        NA        NA
                     padj
                <numeric>
ENSG00000000003  0.163017
ENSG00000000005        NA
ENSG00000000419  0.175937
ENSG00000000457  0.961682
ENSG00000000460  0.815805
...                   ...
ENSG00000283115        NA
ENSG00000283116        NA
ENSG00000283119        NA
ENSG00000283120        NA
ENSG00000283123        NA
```

```
summary(res)
```

```
out of 25258 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 1564, 6.2%
LFC < 0 (down)     : 1188, 4.7%
outliers [1]       : 142, 0.56%
low counts [2]     : 9971, 39%
(mean count < 10)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

8. Adding annotation data

We need to add missing annotation data to our main `res` results object

this includes the common gene symbol

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 6 columns
                  baseMean log2FoldChange     lfcSE      stat    pvalue
                 <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003 747.194195      -0.350703  0.168242 -2.084514 0.0371134
ENSG00000000005   0.000000             NA        NA        NA        NA
ENSG00000000419 520.134160       0.206107  0.101042  2.039828 0.0413675
ENSG00000000457 322.664844       0.024527  0.145134  0.168996 0.8658000
ENSG00000000460  87.682625      -0.147143  0.256995 -0.572550 0.5669497
ENSG00000000938   0.319167      -1.732289  3.493601 -0.495846 0.6200029
                     padj
                <numeric>
ENSG00000000003  0.163017
ENSG00000000005        NA
ENSG00000000419  0.175937
ENSG00000000457  0.961682
ENSG00000000460  0.815805
ENSG00000000938        NA
```

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

Lets see what data bases we can use for translation/mapping

```
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"
```

We can use the map IDs function now to translate

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     keytype="ENSEMBL",        # The format of our genenames
                     column="SYMBOL",          # The new format we want to add
                     multiVals="first")
```

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

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 7 columns
                  baseMean log2FoldChange     lfcSE      stat    pvalue
                 <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003 747.194195      -0.350703  0.168242 -2.084514 0.0371134
ENSG00000000005   0.000000             NA        NA        NA        NA
ENSG00000000419 520.134160       0.206107  0.101042  2.039828 0.0413675
ENSG00000000457 322.664844       0.024527  0.145134  0.168996 0.8658000
ENSG00000000460  87.682625      -0.147143  0.256995 -0.572550 0.5669497
ENSG00000000938   0.319167      -1.732289  3.493601 -0.495846 0.6200029
                     padj      symbol
                <numeric> <character>
ENSG00000000003  0.163017      TSPAN6
ENSG00000000005        NA        TNMD
ENSG00000000419  0.175937        DPM1
ENSG00000000457  0.961682       SCYL3
ENSG00000000460  0.815805       FIRRM
ENSG00000000938        NA         FGR
```

Q11. Run the mapIds() function two more times to add the Entrez ID and UniProt accession and GENENAME as new columns called res$entrez, res$uniprot and res$genename.

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

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

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

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

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 9 columns
                  baseMean log2FoldChange     lfcSE      stat    pvalue
                 <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003 747.194195      -0.350703  0.168242 -2.084514 0.0371134
ENSG00000000005   0.000000             NA        NA        NA        NA
ENSG00000000419 520.134160       0.206107  0.101042  2.039828 0.0413675
ENSG00000000457 322.664844       0.024527  0.145134  0.168996 0.8658000
ENSG00000000460  87.682625      -0.147143  0.256995 -0.572550 0.5669497
ENSG00000000938   0.319167      -1.732289  3.493601 -0.495846 0.6200029
                     padj      symbol      entrez              genename
                <numeric> <character> <character>           <character>
ENSG00000000003  0.163017      TSPAN6        7105          tetraspanin 6
ENSG00000000005        NA        TNMD       64102            tenomodulin
ENSG00000000419  0.175937        DPM1        8813 dolichyl-phosphate m..
ENSG00000000457  0.961682       SCYL3       57147 SCY1 like pseudokina..
ENSG00000000460  0.815805       FIRRM       55732 FIGNL1 interacting r..
ENSG00000000938        NA         FGR        2268 FGR proto-oncogene, ..
```

```
ord <- order( res$padj )
#View(res[ord,])
head(res[ord,])
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 9 columns
                 baseMean log2FoldChange     lfcSE      stat      pvalue
                <numeric>      <numeric> <numeric> <numeric>   <numeric>
ENSG00000152583   954.771        4.36836 0.2371306   18.4217 8.79214e-76
```

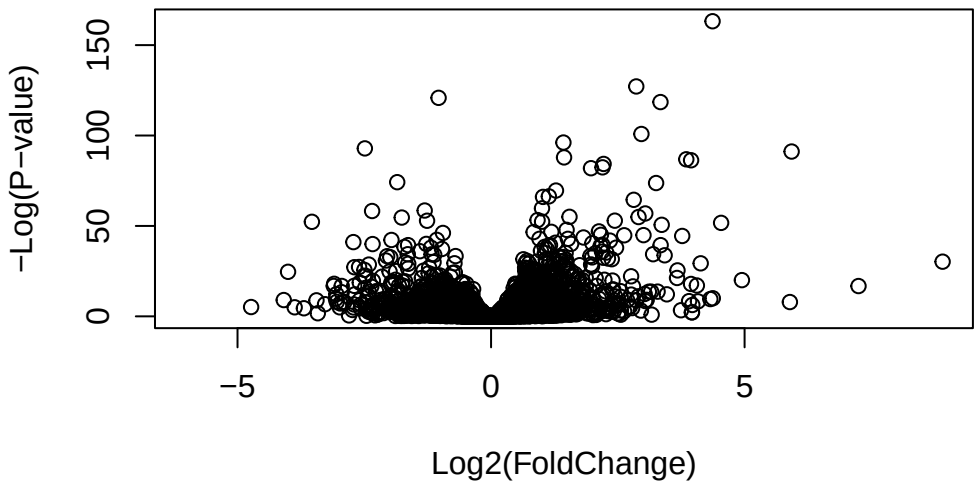
```
ENSG00000179094   743.253        2.86389 0.1755659   16.3123 8.06568e-60
ENSG00000116584  2277.913       -1.03470 0.0650826  -15.8983 6.51317e-57
ENSG00000189221  2383.754        3.34154 0.2124091   15.7316 9.17960e-56
ENSG00000120129  3440.704        2.96521 0.2036978   14.5569 5.27883e-48
ENSG00000148175 13493.920        1.42717 0.1003811   14.2175 7.13625e-46
                      padj      symbol      entrez              genename
                 <numeric> <character> <character>           <character>
ENSG00000152583 1.33157e-71     SPARCL1        8404           SPARC like 1
ENSG00000179094 6.10774e-56        PER1        5187 period circadian reg..
ENSG00000116584 3.28806e-53     ARHGEF2        9181 Rho/Rac guanine nucl..
ENSG00000189221 3.47563e-52        MAOA        4128    monoamine oxidase A
ENSG00000120129 1.59896e-44       DUSP1        1843 dual specificity pho..
ENSG00000148175 1.80131e-42        STOM        2040               stomatin
```

Save annotated results to a CSV file

```
write.csv(res[ord,], "deseq_results.csv")
```

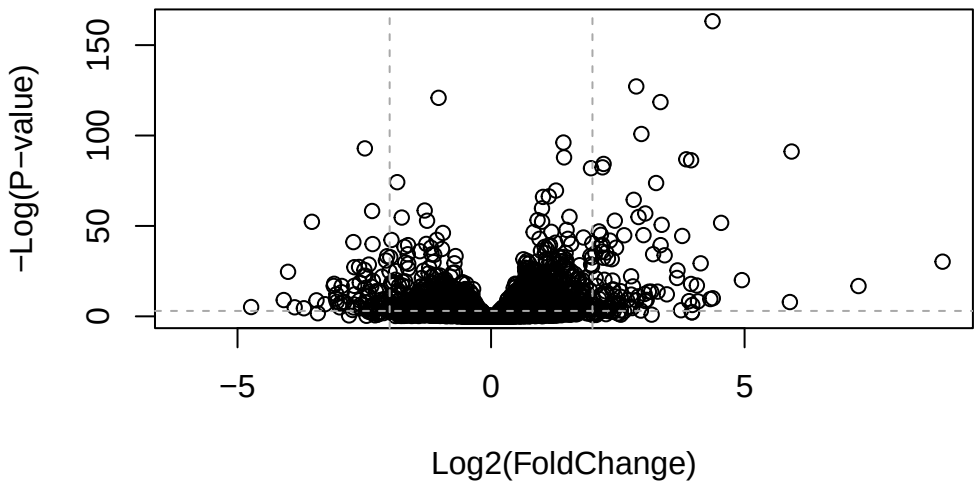
9. Data Visualization

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




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

# Add some cut-off lines
abline(v=c(-2,2), col="darkgray", lty=2)
abline(h=-log(0.05), col="darkgray", lty=2)
```

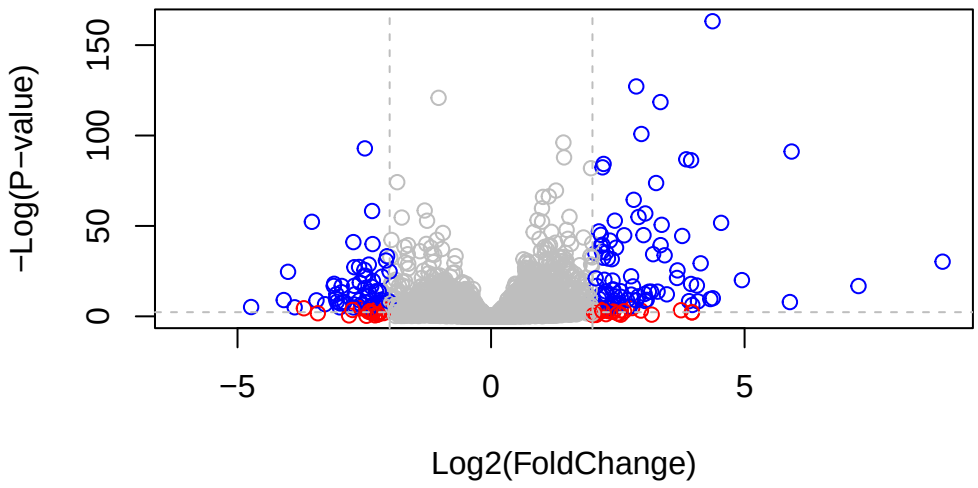



```
# Setup our custom point color vector
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "red"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

# Volcano plot with custom colors
plot( res$log2FoldChange,  -log(res$padj),
 col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )

# Cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", lty=2)
```




```
library(EnhancedVolcano)
```

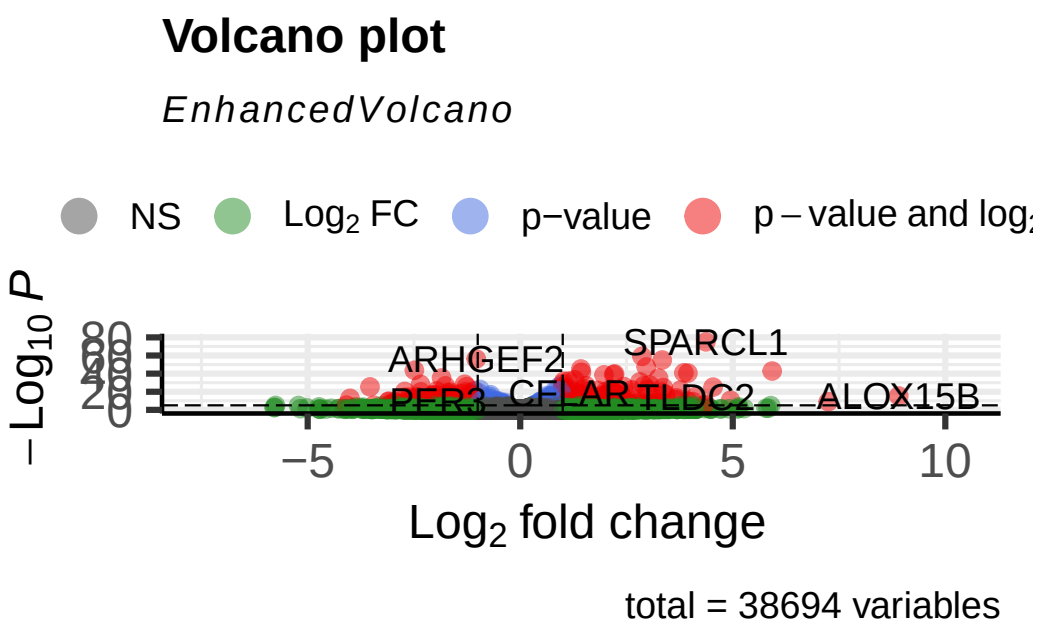
```
Loading required package: ggrepel
```

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

EnhancedVolcano(x,
    lab = x$symbol,
    x = 'log2FoldChange',
    y = 'pvalue')
```

```
Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
i Please use `linewidth` instead.
i The deprecated feature was likely used in the EnhancedVolcano package.
  Please report the issue to the authors.
```

```
Warning: The `size` argument of `element_line()` is deprecated as of ggplot2 3.4.0.
i Please use the `linewidth` argument instead.
i The deprecated feature was likely used in the EnhancedVolcano package.
  Please report the issue to the authors.
```



10. Pathway analysis

What Known Biological Pathways do our differential expressed genes overlap with (i.e play a role in)?

There are lots of conductor packages that do this analysis.

we will use the oldest one of these called **gage**

```
{message=FALSE}
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).
##############################################################################
```

```
library(gage)
```

```
library(gageData)

data(kegg.sets.hs)

head(kegg.sets.hs, 2)
```

```
$`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"
```

The main `gage()` function that does the work wants a simple vector as input.

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

```
       7105       64102        8813       57147       55732        2268
-0.35070296          NA  0.20610728  0.02452701 -0.14714263 -1.73228897
```

The Kegg data base uses Entrez IDs so we need to provide these vector in out input for **gage**
Get the results

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

```
attributes(keggres)
```

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

look at the first three (less) Pathways

```
head(keggres$less, 3)
```

```
                                      p.geomean stat.mean        p.val
hsa05332 Graft-versus-host disease 0.0004250607 -3.473335 0.0004250607
hsa04940 Type I diabetes mellitus  0.0017820379 -3.002350 0.0017820379
hsa05310 Asthma                    0.0020046180 -3.009045 0.0020046180
                                        q.val set.size         exp1
hsa05332 Graft-versus-host disease 0.09053792       40 0.0004250607
hsa04940 Type I diabetes mellitus  0.14232788       42 0.0017820379
hsa05310 Asthma                    0.14232788       29 0.0020046180
```

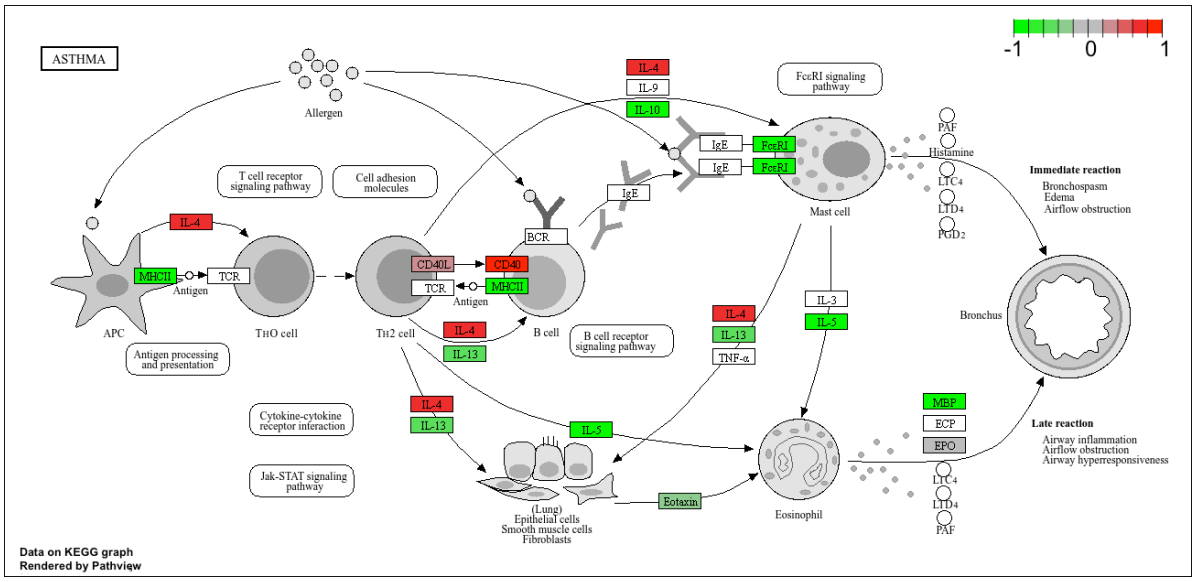
We can use the path view function package to render a figure of any of these pathways along with annotation for our DEGs

```
pathview(gene.data=foldchanges, pathway.id="hsa05310")
```

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

Info: Working in directory /Users/kylewittkop/Desktop/UCSD Winter 2026/BIMM 143/Class13

Info: Writing image file hsa05310.pathview.png
```



Lets do Graph vs host disease and type 1 diabetes

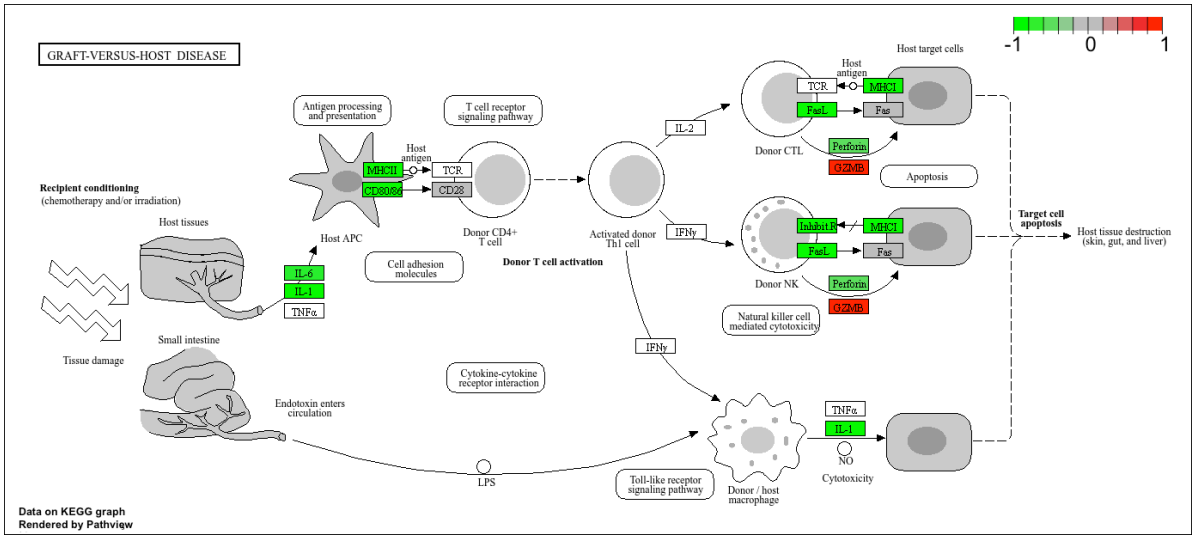
Graft-versus-host disease

```
pathview(gene.data=foldchanges, pathway.id="hsa05332")
```

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

```
Info: Working in directory /Users/kylewittkop/Desktop/UCSD Winter 2026/BIMM 143/Class13
```

```
Info: Writing image file hsa05332.pathview.png
```



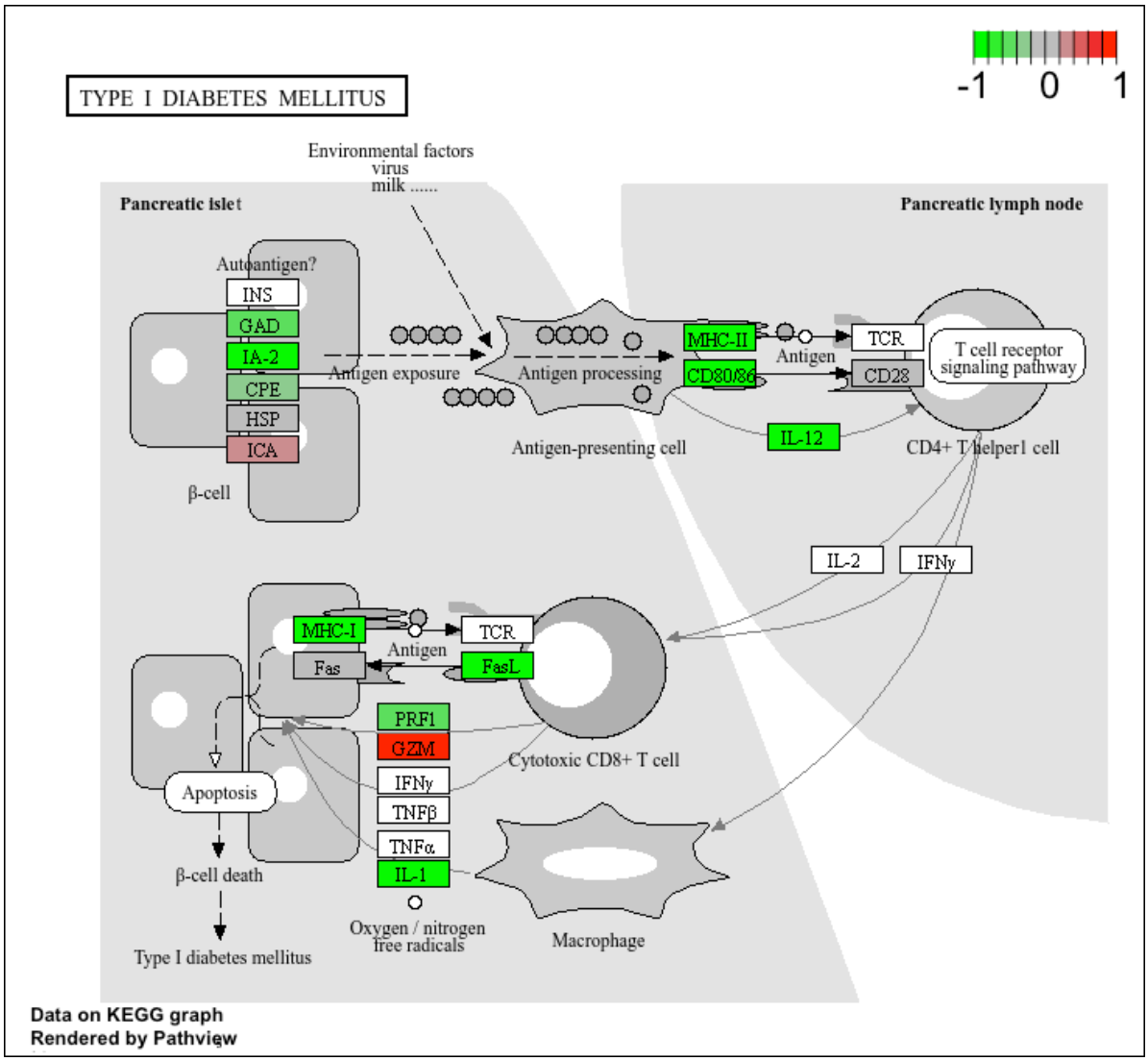
Type I diabetes mellitus

```
pathview(gene.data=foldchanges, pathway.id="hsa04940")
```

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

```
Info: Working in directory /Users/kylewittkop/Desktop/UCSD Winter 2026/BIMM 143/Class13
```

```
Info: Writing image file hsa04940.pathview.png
```


TYPE I DIABETES MELLITUS
-1
0
1
Environmental factors
virus
milk .....
Pancreatic islet
Pancreatic lymph node
Autoantigen?
INS
GAD
IA-2
CPE
HSP
ICA
β-cell
Antigen exposure
Antigen processing
MHC-II
CD80/86
Antigen
TCR
CD28
T cell receptor signaling pathway
IL-12
Antigen-presenting cell
CD4+ T helper1 cell
IL-2
IFNγ
MHC-I
Antigen
TCR
Fas
FasL
PRF1
GZM
IFNγ
TNFβ
TNFα
IL-1
Cytotoxic CD8+ T cell
Apoptosis
β-cell death
Type I diabetes mellitus
Oxygen / nitrogen
free radicals
Macrophage
Data on KEGG graph
Rendered by Pathview