

# lab12

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## Load package and data

```
library(BiocManager)
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 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
```

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

```
head(counts)
```

	SRR1039508	SRR1039509	SRR1039512	SRR1039513	SRR1039516
ENSG000000000003	723	486	904	445	1170
ENSG000000000005	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		
ENSG000000000003	1097	806	604		
ENSG000000000005	0	0	0		
ENSG00000000419	781	417	509		
ENSG00000000457	447	330	324		
ENSG00000000460	94	102	74		
ENSG00000000938	0	0	0		

```
Q1.
```

```
print(paste(nrow(counts), 'genes'))
```

```
[1] "38694 genes"
```

Q2.

```
print(paste(nrow(metadata[metadata$dex=='control',]), 'control cell lines'))
```

```
[1] "4 control cell lines"
```

## Toy differential gene expression

```
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
```

Q3.

Change the hardcoded “4” to be the number of genes.

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

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

Q4.

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

```
head(treated.mean)
```

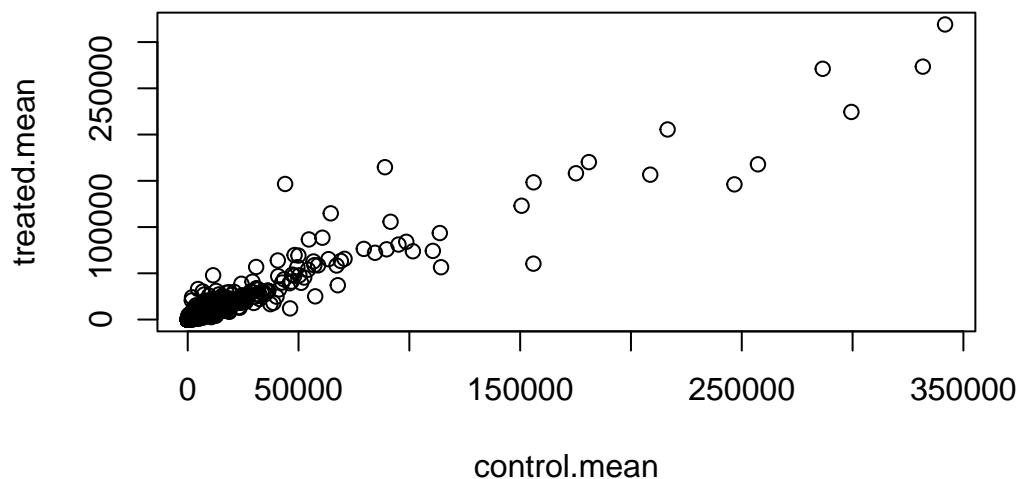
```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460  
658.00 0.00 546.00 316.50 78.75  
ENSG00000000938  
0.00
```

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

```
control.mean treated.mean  
23005324 22196524
```

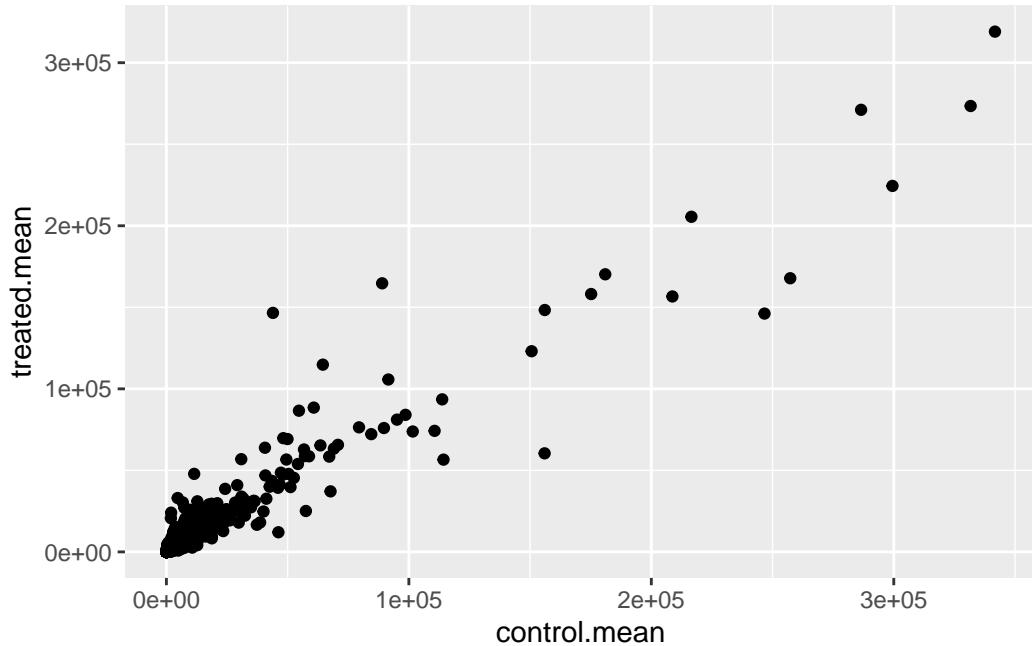
Q5.

```
plot(meancounts)
```



Q5(b)

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

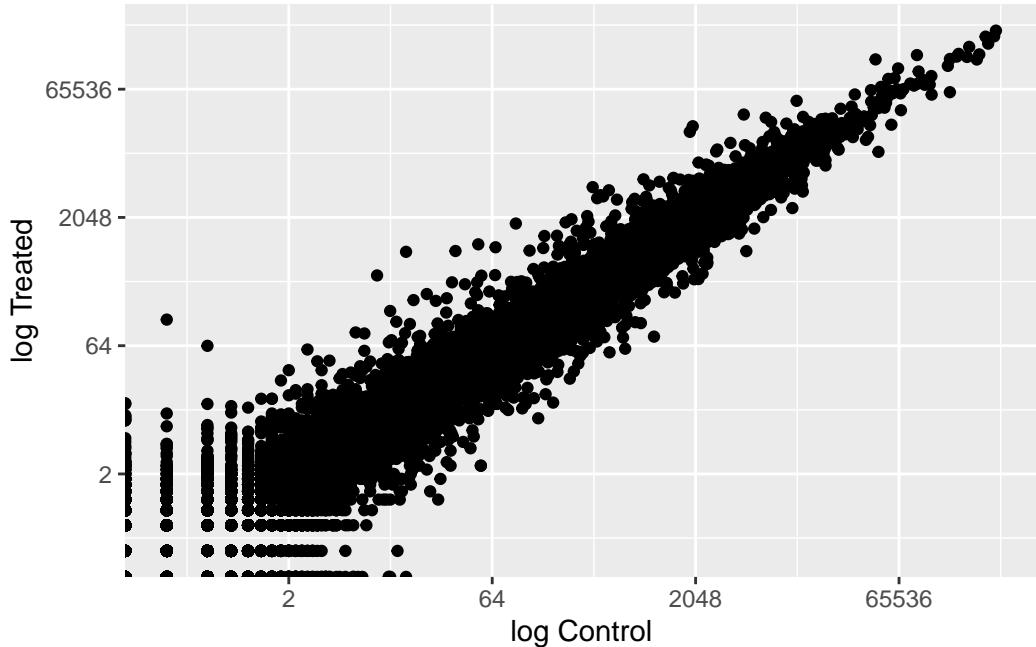


Q6.

```
ggplot(meancounts, aes(x=control.mean, y=treated.mean))+
  geom_point() +
  scale_x_continuous(trans="log2") +
  scale_y_continuous(trans="log2") +
  labs(x='log Control', y= 'log Treated')
```

Warning: Transformation introduced infinite values in continuous x-axis

Warning: Transformation introduced infinite values in continuous y-axis



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

	control.mean	treated.mean	log2fc
ENSG000000000003	900.75	658.00	-0.45303916
ENSG000000000005	0.00	0.00	NaN
ENSG000000000419	520.50	546.00	0.06900279
ENSG000000000457	339.75	316.50	-0.10226805
ENSG000000000460	97.25	78.75	-0.30441833
ENSG000000000938	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
ENSG000000000003	900.75	658.00	-0.45303916
ENSG000000000419	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.

`arr.ind` decides whether to return the array indices if `x` is an array. We want it to be TRUE as we want to know which genes and samples have zero counts. We are going to delete all genes that have zero count so we'll need the row indices. Calling `unique()` will ensure each row will only be counted once even if for one gene there're two samples are zero.

Q8. 250 genes (code see below)

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

nrow(mycounts[up.ind,])
```

[1] 250

Q9. 367

```
nrow(mycounts[down.ind,])
```

[1] 367

Q10.

We shouldn't trust these results, as they are purely based on log-fold change. The difference can be large without being statistically significant. We need to perform statistical test to determine the statistical significance of the difference.

## DESeq2 analysis

```
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
```

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

	baseMean	log2FoldChange	lfcSE	stat	pvalue
ENSG00000000003	747.1941954	-0.35070302	0.1682457	-2.0844697	0.03711747
ENSG00000000005	0.0000000	NA	NA	NA	NA
ENSG00000000419	520.1341601	0.20610777	0.1010592	2.0394752	0.04140263
ENSG00000000457	322.6648439	0.02452695	0.1451451	0.1689823	0.86581056
ENSG00000000460	87.6826252	-0.14714205	0.2570073	-0.5725210	0.56696907
ENSG00000000938	0.3191666	-1.73228897	3.4936010	-0.4958463	0.62000288

	padj
ENSG00000000003	0.1630348
ENSG00000000005	NA
ENSG00000000419	0.1760317
ENSG00000000457	0.9616942
ENSG00000000460	0.8158486
ENSG00000000938	NA

```
summary(res)
```

baseMean	log2FoldChange	lfcSE	stat
Min. : 0.0	Min. :-6.030	Min. : 0.057	Min. :-15.894
1st Qu.: 0.0	1st Qu.:-0.425	1st Qu.: 0.174	1st Qu.: -0.643
Median : 1.1	Median :-0.009	Median : 0.445	Median : -0.027
Mean : 570.2	Mean :-0.011	Mean : 1.136	Mean : 0.045
3rd Qu.: 201.8	3rd Qu.: 0.306	3rd Qu.: 1.848	3rd Qu.: 0.593
Max. :329280.4	Max. : 8.906	Max. : 3.534	Max. : 18.422
	NA's :13436	NA's :13436	NA's :13436
pvalue	padj		
Min. :0.000	Min. :0.000		
1st Qu.:0.168	1st Qu.:0.203		
Median :0.533	Median :0.606		
Mean :0.495	Mean :0.539		
3rd Qu.:0.800	3rd Qu.:0.866		
Max. :1.000	Max. :1.000		
NA's :13578	NA's :23549		

```
res05 <- results(dds, alpha=0.05)
summary(res05)
```

```
out of 25258 with nonzero total read count
adjusted p-value < 0.05
LFC > 0 (up)      : 1236, 4.9%
LFC < 0 (down)    : 933, 3.7%
outliers [1]       : 142, 0.56%
low counts [2]     : 9033, 36%
(mean count < 6)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

## 1. Adding annotation data

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

```
columns(org.Hs.eg.db)
```

```

[1] "ACNUM"          "ALIAS"           "ENSEMBL"         "ENSEMLPROT"      "ENSEMLTRANS"
[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"

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)

  baseMean log2FoldChange      lfcSE      stat      pvalue
ENSG000000000003 747.1941954 -0.35070302 0.1682457 -2.0844697 0.03711747
ENSG000000000005  0.0000000      NA        NA        NA        NA
ENSG00000000419   520.1341601  0.20610777 0.1010592  2.0394752 0.04140263
ENSG00000000457   322.6648439  0.02452695 0.1451451  0.1689823 0.86581056
ENSG00000000460   87.6826252  -0.14714205 0.2570073 -0.5725210 0.56696907
ENSG00000000938   0.3191666  -1.73228897 3.4936010 -0.4958463 0.62000288
  padj     symbol
ENSG000000000003 0.1630348 TSPAN6
ENSG000000000005  NA        TNMD
ENSG00000000419  0.1760317 DPM1
ENSG00000000457  0.9616942 SCYL3
ENSG00000000460  0.8158486 C1orf112
ENSG00000000938  NA        FGR

```

Q11.

```

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

```

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

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

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

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

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

head(res)

  baseMean log2FoldChange      lfcSE      stat     pvalue
ENSG000000000003 747.1941954 -0.35070302 0.1682457 -2.0844697 0.03711747
ENSG000000000005  0.0000000    NA         NA         NA         NA
ENSG000000000419 520.1341601  0.20610777 0.1010592  2.0394752 0.04140263
ENSG000000000457 322.6648439  0.02452695 0.1451451  0.1689823 0.86581056
ENSG000000000460 87.6826252   -0.14714205 0.2570073 -0.5725210 0.56696907
ENSG000000000938 0.3191666   -1.73228897 3.4936010 -0.4958463 0.62000288
                  padj     symbol entrez     uniprot
ENSG000000000003 0.1630348   TSPAN6    7105 AOA024RC10
ENSG000000000005  NA        TNMD     64102 Q9H2S6
ENSG000000000419 0.1760317   DPM1     8813  O60762
ENSG000000000457 0.9616942   SCYL3    57147  Q8IZE3
ENSG000000000460 0.8158486   C1orf112 55732 AOA024R922
ENSG000000000938  NA        FGR      2268  P09769
                                         genename
ENSG000000000003
ENSG000000000005
ENSG000000000419 dolichyl-phosphate mannosyltransferase subunit 1, catalytic
```

```

ENSG00000000457          SCY1 like pseudokinase 3
ENSG00000000460          chromosome 1 open reading frame 112
ENSG00000000938          FGR proto-oncogene, Src family tyrosine kinase

```

```

# arrange and view the results by the adjusted p-value
ord <- order( res$padj )
#View(res[ord,])
head(res[ord,])

```

	baseMean	log2FoldChange	lfcSE	stat	pvalue
ENSG00000152583	954.7709	4.368359	0.23712679	18.42204	8.744898e-76
ENSG00000179094	743.2527	2.863889	0.17556931	16.31201	8.107836e-60
ENSG00000116584	2277.9135	-1.034701	0.06509844	-15.89440	6.928546e-57
ENSG00000189221	2383.7537	3.341544	0.21240579	15.73189	9.144326e-56
ENSG00000120129	3440.7038	2.965211	0.20369513	14.55710	5.264243e-48
ENSG00000148175	13493.9204	1.427168	0.10038904	14.21638	7.251278e-46
	padj	symbol	entrez	uniprot	
ENSG00000152583	1.324415e-71	SPARCL1	8404	AOA024RDE1	
ENSG00000179094	6.139658e-56	PER1	5187	015534	
ENSG00000116584	3.497761e-53	ARHGEF2	9181	Q92974	
ENSG00000189221	3.462270e-52	MAOA	4128	P21397	
ENSG00000120129	1.594539e-44	DUSP1	1843	B4DU40	
ENSG00000148175	1.830344e-42	STOM	2040	F8VSL7	
				genename	
ENSG00000152583				SPARC like 1	
ENSG00000179094			period circadian regulator 1		
ENSG00000116584	Rho/Rac guanine nucleotide exchange factor 2				
ENSG00000189221			monoamine oxidase A		
ENSG00000120129		dual specificity phosphatase 1			
ENSG00000148175			stomatin		

write and save it to csv

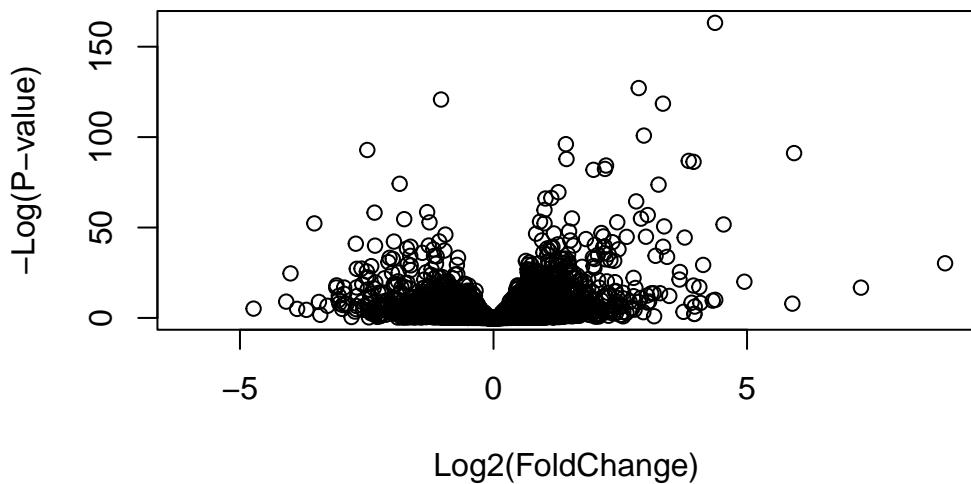
```

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

```

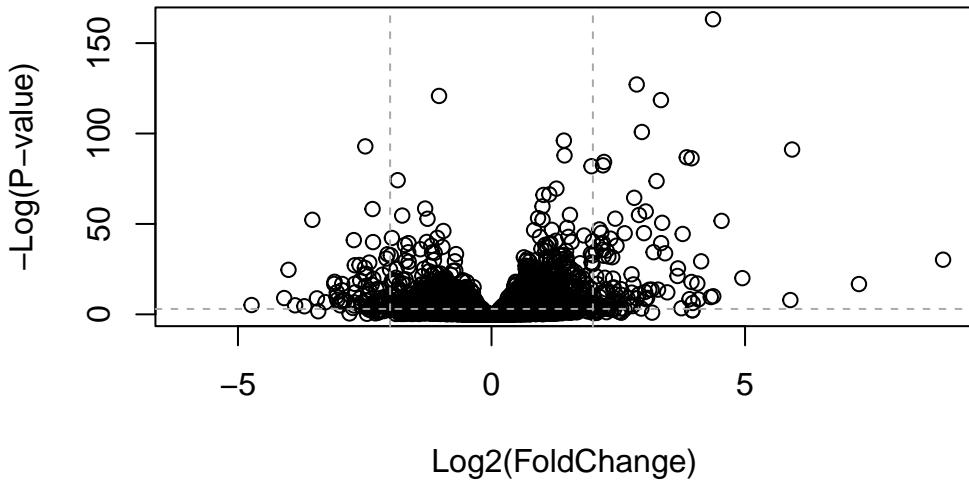
## Data Visualization

```
# volcano plot
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)
```



```

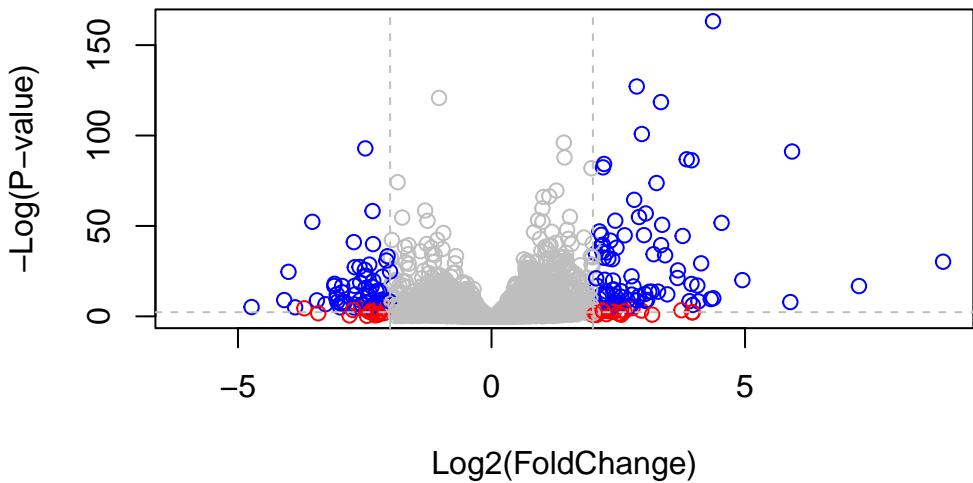
# Setup our custom point color vector
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

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)
```

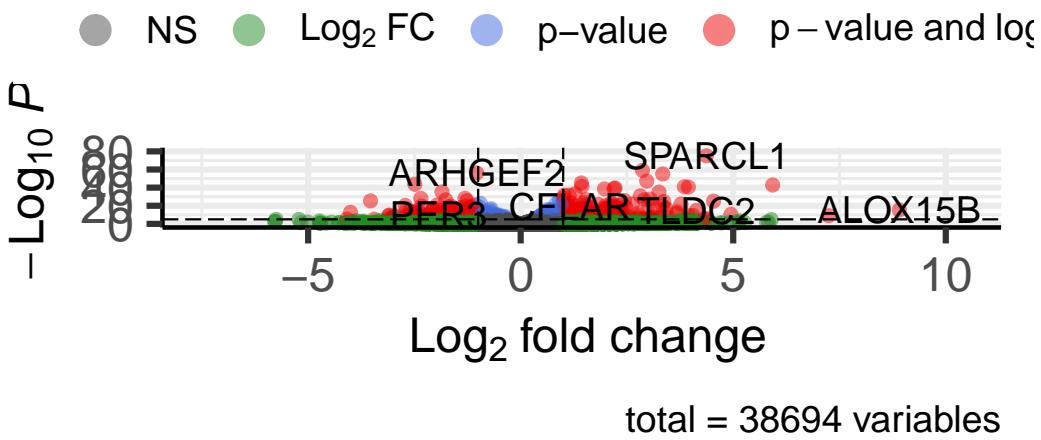
```
Loading required package: ggrepel
```

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

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

## Volcano plot

### *Enhanced Volcano*



## Pathway analysis

Now we can load the packages and setup the KEGG data-sets we need. The gageData package has pre-compiled databases mapping genes to KEGG pathways and GO terms for common organisms. kegg.sets.hs is a named list of 229 elements. Each element is a character vector of member gene Entrez IDs for a single KEGG pathway.

```
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)

# Examine the first 2 pathways in this kegg set for humans
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"

```

gage() function requires a **named** vector of fold changes.

```

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

```

7105	64102	8813	57147	55732	2268
-0.35070302	NA	0.20610777	0.02452695	-0.14714205	-1.73228897

```

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

attributes(keggres)

```

```

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

```

```
# Look at the first three down (less) pathways
head(keggres$less, 3)

          p.geomean stat.mean      p.val
hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
hsa04940 Type I diabetes mellitus 0.0017820293 -3.002352 0.0017820293
hsa05310 Asthma                 0.0020045888 -3.009050 0.0020045888
                                         q.val set.size      exp1
hsa05332 Graft-versus-host disease 0.09053483        40 0.0004250461
hsa04940 Type I diabetes mellitus 0.14232581        42 0.0017820293
hsa05310 Asthma                 0.14232581        29 0.0020045888
```

Visualize the pathway

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

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

Info: Working in directory /Users/ritawan/Documents/Win22/BIMM143/class12

Info: Writing image file hsa05310.pathview.png

# A different PDF based output of the same data
pathview(gene.data=foldchanges, pathway.id="hsa05310", kegg.native=FALSE)

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

```
Info: Working in directory /Users/ritawan/Documents/Win22/BIMM143/class12

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

Q12.

```
# get the id of the top 2 downregulated pathways

# Look at the first three down (less) pathways
head(keggres$less, 2)
```

```

          p.geomean stat.mean      p.val
hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
hsa04940 Type I diabetes mellitus  0.0017820293 -3.002352 0.0017820293
                           q.val set.size      exp1
hsa05332 Graft-versus-host disease 0.09053483        40 0.0004250461
hsa04940 Type I diabetes mellitus  0.14232581        42 0.0017820293

pathview(gene.data=foldchanges, pathway.id=c('hsa04940', 'hsa05332'))

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

Info: Working in directory /Users/ritawan/Documents/Win22/BIMM143/class12

Info: Writing image file hsa04940.pathview.png

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

Info: Working in directory /Users/ritawan/Documents/Win22/BIMM143/class12

Info: Writing image file hsa05332.pathview.png

sessionInfo()

R version 4.2.2 (2022-10-31)
Platform: aarch64-apple-darwin20 (64-bit)
Running under: macOS Monterey 12.5

Matrix products: default
BLAS:    /Library/Frameworks/R.framework/Versions/4.2-arm64/Resources/lib/libRblas.0.dylib
LAPACK:  /Library/Frameworks/R.framework/Versions/4.2-arm64/Resources/lib/libRlapack.dylib

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

attached base packages:
[1] stats4   stats    graphics grDevices utils     datasets methods
[8] base
```

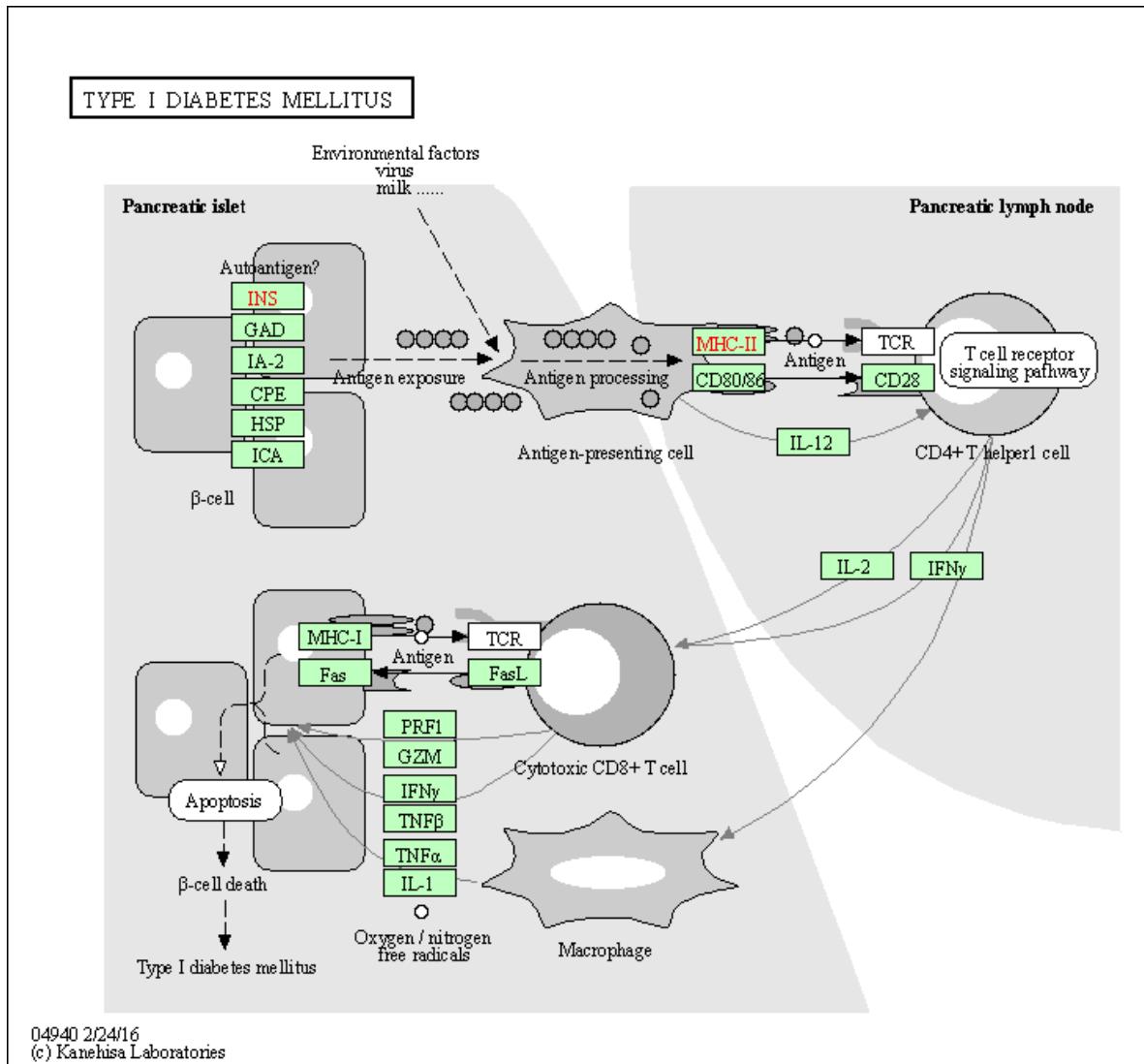


Figure 1: hsa04940

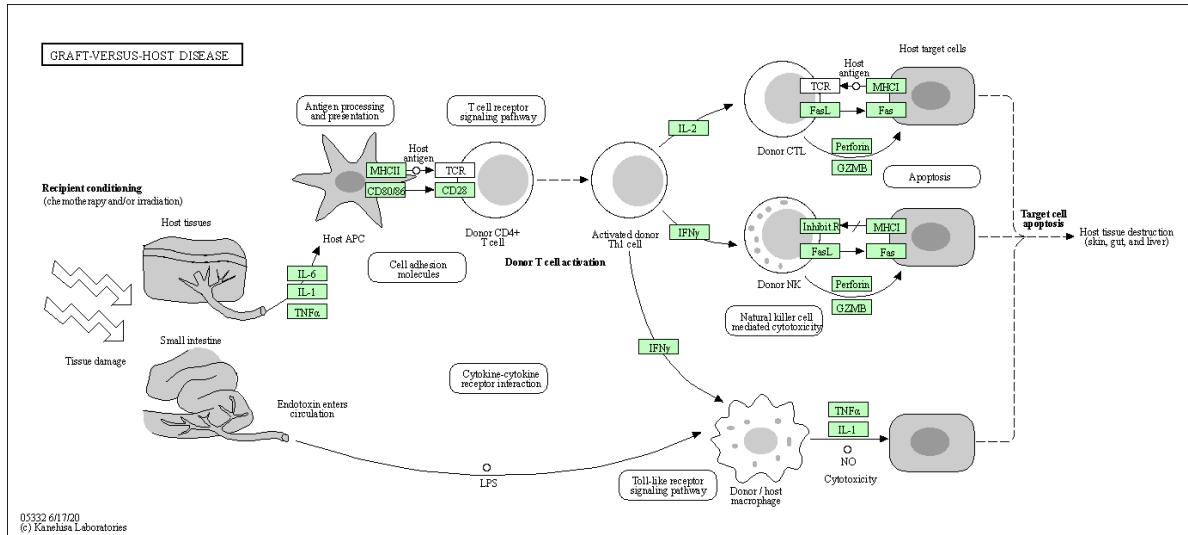


Figure 2: hsa05332

other attached packages:

```
[1] gageData_2.36.0          gage_2.48.0
[3] pathview_1.38.0          EnhancedVolcano_1.16.0
[5] ggrepel_0.9.3            org.Hs.eg.db_3.16.0
[7] AnnotationDbi_1.60.0     ggplot2_3.4.1
[9] DESeq2_1.38.3            SummarizedExperiment_1.28.0
[11] Biobase_2.58.0          MatrixGenerics_1.10.0
[13] matrixStats_0.63.0       GenomicRanges_1.50.2
[15] GenomeInfoDb_1.34.9      IRanges_2.32.0
[17] S4Vectors_0.36.1         BiocGenerics_0.44.0
[19] BiocManager_1.30.19
```

loaded via a namespace (and not attached):

```
[1] httr_1.4.4                bit64_4.0.5           jsonlite_1.8.4
[4] blob_1.2.3                GenomeInfoDbData_1.2.9 yaml_2.3.7
[7] pillar_1.8.1              RSQLite_2.2.20        lattice_0.20-45
[10] glue_1.6.2                digest_0.6.31         RColorBrewer_1.1-3
[13] XVector_0.38.0           colorspace_2.1-0      htmltools_0.5.4
[16] Matrix_1.5-3              XML_3.99-0.13        pkgconfig_2.0.3
[19] zlibbioc_1.44.0           GO.db_3.16.0         xtable_1.8-4
[22] scales_1.2.1              BiocParallel_1.32.5   tibble_3.1.8
[25] annotate_1.76.0           KEGGREST_1.38.0      generics_0.1.3
[28] farver_2.1.1              cachem_1.0.6          withr_2.5.0
[31] cli_3.6.0                 magrittr_2.0.3         crayon_1.5.2
[34] KEGGgraph_1.58.3          memoise_2.0.1         evaluate_0.20
```

```
[37] fansi_1.0.4           graph_1.76.0          tools_4.2.2
[40] lifecycle_1.0.3       munsell_0.5.0         locfit_1.5-9.7
[43] DelayedArray_0.24.0   Biostrings_2.66.0      compiler_4.2.2
[46] rlang_1.0.6            grid_4.2.2           RCurl_1.98-1.10
[49] rstudioapi_0.14        bitops_1.0-7          labeling_0.4.2
[52] rmarkdown_2.20          gtable_0.3.1          codetools_0.2-19
[55] DBI_1.1.3              R6_2.5.1             knitr_1.42
[58] dplyr_1.1.0             fastmap_1.1.0        bit_4.0.5
[61] utf8_1.2.3              Rgraphviz_2.42.0     parallel_4.2.2
[64] Rcpp_1.0.10             vctrs_0.5.2          geneplotter_1.76.0
[67] png_0.1-8               tidyselect_1.2.0     xfun_0.37
```